METABOLOMICS
SOCIETY

METABOLOMICS 2018

JUNE 24-28 SEATTLE, WASHINGTON

14th Annual Conference of the Metabolomics Society

AGENDA AT A GLANCE

Workshops | Plenary Sessions | Session Groups | Poster Sessions

SUNDAY, JUNE 24

	Tahoma 1/2	Tahoma 3	Tahoma 4
11:00 a.m. – 6:30 p.m.	REGISTRATION OPEN		
12:00 p.m. – 2:00 p.m.	**W1:** QA and QC for Untargeted Metabolomics	**W2:** EMN – Experimental Design in Metabolomics	**W3:** Metabolomics Data Analysis with XCMS and Galaxy
2:15 p.m. – 4:15 p.m.	**W4:** Targeted MS and Skyline	**W5:** EMN – Intro to Mass Spectrometry	**W6:** Advanced Multivariate Statistics
4:30 p.m. – 6:30 p.m.	**W7:** Stable Isotope Approaches in Metabolomics	**W8:** EMN – Principles of Multivariate Data Analysis	**W9:** Mining the Metabolome – Annotation Strategies
7:00 p.m. – 8:30 p.m.	MANA Core Facilities Forum	MANA Early-Career Focus Group 7 p.m. – 9 p.m.	

MONDAY, JUNE 25

	Tahoma 1/2	Tahoma 3	Tahoma 4
7:30 a.m. – 7:00 p.m.	REGISTRATION / INFO DESK OPEN		
8:30 a.m. – 10:15 a.m.	**W10:** Improving Metabolite ID Approaches – Confidence and CASMI	**W11:** Systems Biology Workflows for Data Analysis and Integration	**W12:** Introduction to Data Workflows – Metaboanalyst 4.0
10:30 a.m. – 12:15 p.m.	**W13:** Visualizing Metabolic Data: Introduction to Visual Analytics	**W14:** Metabolomics and Precision Medicine	**W15:** EMN – Professional Career Development
	LUNCH BREAK – ON YOUR OWN		
1:30 p.m. – 3:00 p.m.	**Welcome and Plenary Session 1** – Elaine Holmes – Hall 4E		
3:00 p.m. – 3:30 p.m.	BREAK		
3:30 p.m. – 5:15 p.m.	**1.** Rare Diseases and Novel Therapies	**2.** Food & Nutrition I	**3.** Big Data: Databases & Cloud Computing
5:15 p.m. – 6:45 p.m.	**Welcome Reception and Poster Session 1** – Exhibit Hall		
7:00 p.m. – 8:00 p.m.	Metabolomics Society Town Hall Meeting		
8:15 p.m. – 9:00 p.m.	**MANA Networking Reception** – Sky Bridge		

TUESDAY, JUNE 26

	Tahoma 1/2	Tahoma 3	Tahoma 4
7:45 a.m. – 7:00 p.m.	REGISTRATION / INFO DESK OPEN		
8:30 a.m. – 9:30 a.m.	**Plenary Session 2 –** Robert Gerszten – Hall 4E		
9:30 a.m. – 10:15 a.m.	BREAK – EXHIBIT HALL		
10:15 a.m. – 12:00 p.m.	**4.** Plant Metabolomics I	**5.** Neurology & Psychiatry	**6.** Multiomics
12:00 p.m. – 1:30 p.m.	LUNCH – EXHIBIT HALL – PLATINUM SPONSOR PRESENTATIONS		
12:20 p.m. – 1:20 p.m.		Sponsor Pres: **LECO Corporation**	Sponsor Pres: **Bruker**
1:30 p.m. – 3:00 p.m.	**7.** Metabolic Networks	**8.** Pediatrics	**9.** Microbiome & Metabolomics I
3:00 p.m. – 3:30 p.m.	BREAK – EXHIBIT HALL		
3:30 p.m. – 5:15 p.m.	**10.** Obesity & Metabolic Syndrome	**11.** Ecology & Environmental Metabolomics	**12.** New Methods & Technologies I
5:15 p.m. – 6:45 p.m.	**Poster Session 2** – Exhibit Hall		
7:00 p.m. – 8:30 p.m.	EMN Reception	Pacific Rim Networking Reception – 7 p.m – 8 p.m. – Sky Bridge	

WEDNESDAY, JUNE 27

	Tahoma 1/2	Tahoma 3	Tahoma 4
8:00 a.m. – 7:00 p.m.	REGISTRATION / INFO DESK OPEN		
8:30 a.m. – 9:30 a.m.	**Plenary Session 3** – Jean-Luc Wolfender – Hall 4E		
9:30 a.m. – 10:15 a.m.	BREAK – EXHIBIT HALL		
10:15 a.m. – 12:00 p.m.	**13.** Informatics & Statistics for Metabolomics	**14.** Pharmacometabolomics	**15.** Metabolite ID Methods
12:00 p.m. – 1:30 p.m.	LUNCH – EXHIBIT HALL – PLATINUM SPONSOR PRESENTATIONS		
12:20 p.m. – 1:20 p.m.	Sponsor Pres: **Agilent Technologies**	Sponsor Pres: **Waters Corporation**	
1:30 p.m. – 3:00 p.m.	**16.** Cancer	**17.** Internal Medicine	**18.** Agriculture
3:00 p.m. – 3:30 p.m.	BREAK – EXHIBIT HALL		
3:30 p.m. – 5:15 p.m.	**19.** Food & Nutrition II	**20.** Metabolomics & Model Organisms	**21.** New Methods & Technologies II
5:15 p.m. – 6:45 p.m.	**Poster Session 3** – Exhibit Hall		
7:30 p.m. – 11:00 p.m.	**Conference Dinner Museum of Pop Culture (MoPOP)**		

THURSDAY, JUNE 28

	Tahoma 1/2	Tahoma 3	Tahoma 4
8:15 a.m. – 3:30 p.m.	REGISTRATION / INFO DESK OPEN		
8:45 a.m. – 9:45 a.m.	**Plenary Session 4** – Guowang Xu – Hall 4E		
9:45 a.m. – 10:30 a.m.	**Poster Session 4** – Exhibit Hall		
10:30 a.m. – 12:00 p.m.	**22.** Diabetes, Diet and Glucose Intolerance	**23.** Biospecimens, Sample Prep and Quality	**24.** Plant Metabolomics II
12:00 p.m. – 1:30 p.m.	LUNCH – EXHIBIT HALL – PLATINUM SPONSOR PRESENTATIONS		
12:20 p.m. – 1:20 p.m.	Sponsor Pres: **Thermo Fisher Scientific**		
1:30 p.m. – 3:00 p.m.	**25.** Wellness and Aging	**26.** Microbiome & Metabolomics II	**27.** New Methods & Technologies III
	Gather in Plenary Session Room for Closing		
3:15 p.m. – 4:45 p.m.	**Plenary Session 5 and Closing** – Leroy Hood – Hall 4E		

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Oral Abstracts

PLENARY SESSION 1

The Role of Metabolic Profiling in 21st Century Medicine

Monday June 25, 2:00 p.m. – 3:00 p.m.
Presenting Author – Elaine Holmes, Imperial College London, UK

Metabolic phenotyping has emerged as an enabling tool for exploring, characterizing, and understanding the dynamic interactions between our genes and environment (diet, lifestyle, microbiomes), and their phenotypic expression across diverse human populations. The chemical consequences of these interactions are directly observable by application of modern spectroscopic profiling technologies (predominantly LC-MS, GC-MS and NMR spectroscopy) applied to biofluids, tissues and cell cultures. The metabolic coverage and capacity for mapping complex and dynamic interactions afforded by phenotyping methods enhances classical clinical chemistry approaches to medicine, offering diagnostic, prognostic, and mechanistic insight to human disease (risk, progression, and recovery), treatment (stratification and response), nutrition, and xenobiotic exposure. Clinical studies have shown that inter-individual differences in either host or microbial metabolism can impact on patient responses to therapeutics or surgical interventions (pharmacometabonomics) and that individuals' baseline or pretreatment profiles can be used prognostically to predict drug metabolism, efficacy or toxicity. This technology allows the construction of a framework for stratifying patients and improving clinical care with respect to clinical outcomes and reduced expenditure. Numerous analytical platforms for metabolic profiling exist and an even greater number of methodologies and tool boxes for processing, modeling and interpreting the data. Methods for characterizing the metabolic consequences of biological processes will be discussed with particular emphasis on accommodating extraneous variation and optimizing biomarker recovery in population studies. Finally, a series of metabolic phenotyping pipelines and tools for predicting response to interventions at the individual level will be outlined and several clinical and laboratory-based examples provided.

PLENARY SESSION 2

Mining the Blood for New Cardiometabolic Hormones

Tuesday June 26, 8:30 a.m. – 9:30 a.m.
Presenting Author – Robert Gerszten, Beth Israel Deaconess Medical Center, USA

In large population based studies, we have identified metabolites and proteins that presage the onset of overt cardiometabolic disease by over a decade. A key question is whether these circulating factors are purely disease markers or whether a subset of them participate in a functional manner to contribute to disease pathogenesis. To address this question, we have integrated genetic information with metabolomics to understand the genetic underpinnings of the human metabolome, and to identify relevant pathways. We then return to the bench to test hypotheses in cell and animal based models of human cardiovascular disease. This presentation will highlight the "retrotranslational" research integrating metabolomics, proteomic and genetics – all with a foundation in clinical medicine.

PLENARY SESSION 3

Contextualized Metabolomics Transforms Pharmacognosy – A Paradigm Shift in Natural Product Research

Wednesday June 27, 8:30 a.m. – 9:30 a.m.
Presenting Author – Jean-Luc Wolfender, University of Geneva, Switzerland

The rapid innovations in metabolite profiling, bioassays and chemometrics lead to a paradigm shift in natural product (NP) research. Indeed, having at hand partial/full structure information of possibly all secondary metabolites and an estimation of their levels in plants, provides a way to perform pharmacognostic investigations from a new and holistic perspective. The increasing amount of accurate metabolome data that can be acquired on massive sample sets, notably through data dependent HRMS/MS, allows mapping natural extracts at an unprecedented precision level. In this context, data contextualization is however still a lagging process. For this, we investigated methods that could provide enhanced annotation confidence level through multiple scores integrating taxonomy information and molecular network (MN) structural consistency as well as other orthogonal analytical data. Benchmarking of such approaches is currently assessed by profiling mixtures of herbs with well-studied composition. We also investigate the best way to integrate extracts bioactivity data in MN and shortcut bioactivity guided isolation for an efficient targeted identification of bioactive NPs. To this end, accurate chromatography gradient methods at various scales have been developed for MS-targeted purification of biomarkers and their full de novo structure identification by NMR. Different recent applications of our metabolomics/phytochemical investigations will illustrate these aspects and also highlight their efficiency for the study of the dynamic production of biomarkers in plant and microorganism in ecologically relevant problematics. Evaluation of what is readily implemented and is still required in NP research will be made, notably in terms of contextualisation of the data.

PLENARY SESSION 4

Functional metabolomics: from discovery of differential metabolites to unraveling of biological functions

Thursday 8:45 a.m. – 9:45 a.m.
Presenting Author – Guowang Xu, Chinese Academy of Sciences, China
Co-Authors – Xinyu Liu, Lina Zhou, Zhichao Wang, Xinjie Zhao, Chunxiu Hu, Xin Lu

Metabolomics is a science for investigating the whole metabolome in a given biological system under specific conditions. Over last 10 years, metabolomics has become a powerful tool in studying disease metabolic marker, therapeutic mechanism of drugs, gene function and pathogenesis of diseases. Generally, metabolomics consists of 5 steps including study design, sample collection, metabolic profiling analysis, data handling and biological explanation, the discovery of small molecules related to investigatied biological questions is the key of metabolomics studies. Mass spectrometry (MS) and NMR are usually used to collect metabolome data which can be employed to classify the different groups and define the differential metabolites which are the bridge of understanding diverse biological processes and associated events. However, until now most of studies are of a descriptive nature, and the missing link between the differential metabolites and their biological functions has not been well elucidated. In this lecture, we shall report a functional metabolomics platform including comprehensive targeted, nontargeted and pseudotargeted analyses based on one-dimensional/two-dimensional gas or liquid chromatography – MS, stable isotope-labelled cell-based dynamics metabolomics, and molecular biology methods. This platform can be used for not only defining differential metabolites as potential biomarkers, but also unraveling the mechanistic association between functional metabolites and specific biological events. The newest results will be given to show the applications of developed methods in defining biomarkers for the early detection of hepatocellular carcinoma and oncometabolites of liver cancer and glioma. Effects of preanalytical factors on metabolomics results will also be presented.

PLENARY SESSION 5

21st Century Medicine Is Transforming Healthcare

Thursday June 28, 3:15 p.m. – 4:45 p.m.
Presenting Author – Leroy Hood, Institute for Systems Biology, USA

I will discuss our successful effort to introduce P4 healthcare into the current healthcare system with a P4 pilot program on scientific wellness — a longitudinal, high-dimensional data cloud study on each of 108 well patients over 2014. The preliminary results both with regard to data analyses and patient responses from these studies are striking. They point to the emerging discipline of scientific wellness—and the fact that it will catalyze several new thrusts in healthcare: 1) optimizing wellness,
2) identifying the earliest disease transition points for all common diseases and learning how to reverse them and 3) employing the dense, dynamic, personal data cloud approach to study diseases (e.g. cancer, Alzheimer's, multiple sclerosis) and their responses to therapy. Scientific wellness will also pioneer N=1 experiments to deconvolute the staggering complexity of human biology and disease. We started Arivale, a company focused on scientific wellness for the consumer, in 2015 and already have about 4000 individuals enrolled. I will also discuss some preliminary results from the Arivale studies.

Thus, scientific wellness will catalyze a transformation in contemporary healthcare and it will provide eventually millions of dense, dynamic, personal data clouds that will present striking new opportunities for pharma, biotech, nutrition and diagnostic companies to identify biomarkers and drug target candidates. As the cost of the assays for the dense, dynamic, personal data clouds decline dramatically; scientific wellness can eventually be brought to the developing world and to the poor leading to a democratization of healthcare unimaginable even a few years ago.

SESSION 1: RARE DISEASES AND NOVEL THERAPIES	Monday June 25	3:30 p.m. – 5:15 p.m.

1A KEYNOTE 3:30 p.m. – 4:00 p.m.	Metabolic remodeling regulates cellular epigenetic state

PRESENTING AUTHOR: ***Hannele Ruohola-Baker, University of Washington, United States***

CO-AUTHORS: ***Shiri Levy, Jason Miklas, Elisa Clark, Julie Mathieu, Megan Showalter, Daniel Raftery, Oliver Fiehn***

Aberrations in metabolism contribute to a large number of diseases, such as diabetes, obesity, cancer, and cardiovascular diseases. However, the mechanisms leading to these changes in metabolic state is not well understood. We have analyzed regulation of metabolic remodeling and its function in epigenetic control both in early stage embryonic stem cells and during the maturation of cardiomyocytes. We showed that metabolic enzyme NNMT regulates PRC2 dependent H3K27me3 marks in early human pluripotent stages, however, it was not known if this modification was essential for the human pluripotency. We applied computational protein design to engineer a synthetic, novel protein, EED-binder (EB) that binds with picomolar affinity to PRC2 and completely abolishes histone methylation in embryonic stem cells. Importantly, by using this computer designed protein, we show that H3K27me3 marks are essential in primed but not in naïve human pluripotent stem cells. The metabolic enzyme, NNMT thereby regulates the first critical PRC2 activity in embryonic lineage. We have now developed a method to inhibit PRC2 function at specific genetic loci using EBdCas9/gRNA system to precisely determine the critical loci regulated by PRC2.

1B 4:00 p.m. – 4:15 p.m.	Repair of damaged nicotinamide cofactors is crucial to maintain healthy metabolism and brain function

PRESENTING AUTHOR: ***Carole L Linster, University of Luxembourg, Luxembourg Centre for Systems Biomedicine, Luxembourg***

CO-AUTHORS: ***Julia Becker-Kettern, Nicole Paczia, Christian Jaeger, Nicole Van Bergen, John Christodoulou***

NADHX and NADPHX are hydrated and redox inactive forms of the NADH and NADPH cofactors, known to inhibit several dehydrogenases in vitro. A few years ago, we discovered a highly conserved metabolite repair system, composed of an epimerase and a dehydratase, that convert thosedamaged NAD(P)H derivatives back to the normal cofactors. Together with a growing list of other recently identified enzymes, they function to eliminate abnormal metabolites formed spontaneously or by enzymatic mistakes. NAD(P)HX repair deficiency has recently been shown to cause severe neurometabolic disease in children. We found that knocking out NAD(P)HX dehydratase in yeast leads to high NADHX accumulation in quiescent cells with a concomitant depletion of NAD+pools. Transcriptomic and metabolomicanalyses in the yeast model pointed us towards perturbations in serine metabolism, for which we were able to find a mechanistic explanation at the molecular level by subsequent in vitro studies. Human NAD(P)HX dehydratase knockout HAP1 cells also accumulated NADHX, and showed decreased viability as well as higher glucose consumption and lactate production. Ongoing metabolomics analyses in the knockout HAP1 cells indicate additional metabolic perturbations. Fibroblasts derived from NAD(P)HX repair deficient patients showed signs of mitochondrial dysfunction, including decreased levels of certain respiratory chain complexes. Our results pave the way for elucidating the molecular mechanism(s) underlying the acute neurodegeneration leading to early death in NAD(P)HX repair deficient children.

1C 4:15 p.m. – 4:30 p.m.	Structural Isomer Interferences in the Context of Precision Medicine: Efficacy of Galabiosylceramide as a Fabry Disease Biomarker

PRESENTING AUTHOR: ***Michel Boutin, Université de Sherbrooke, Canada***

CO-AUTHORS: ***Iskren Menkovic, Tristan Martineau, Vanessa Vaillancourt-Lavigueur, Amanda Toupin, Christiane Auray-Blais***

Fabry Disease is a monogenic X-linked lysosomal storage disorder caused by decreased alpha-galactosidase A activity.This latter enzyme is involved inthe recycling of cellular sphingolipids. Renal, cerebral, and cardiovascular manifestations of Fabry disease often result in premature death of patients. An untargeted metabolomic study performed in our laboratory with urine from untreated Fabry males and healthy control malesrevealed 22 galabiosylceramide (Ga2)isoforms/analogs as Fabry disease biomarkers. Unfortunately, the sensitivity (true positive/true positive+false negative) of these biomarkers wasunsatisfactory forfemales and maleshaving residual enzyme activity. A comprehensive investigation of these metabolites revealed that these results were influenced by the co-analysis of lactosylceramide (LacCer), a structural isomer of Ga2, which is not a Fabry disease biomarker.Ga2 and LaCer are differentiated only by the conformation of their glycosidic linkage. A normal phase UPLC-MS/MS method was developed to separate Ga2 isoforms/analogs from their isobaric LacCer interferences and a complete validation was performed. Urine samples from 34 untreated Fabry males, 33 treated Fabry males, 34 healthy control males, 54 untreated Fabry females, 19 treated Fabry females, 25 control females were analyzed. The separation of Ga2 from LacCer significantly increasedits sensitivityespecially for untreated Fabry females (from 9.3 to 70.4%). In the context of personalized medicine, this novel analytical methodology will greatly improve the efficiency of Ga2 as a Fabry disease biomarker for the diagnosis and monitoring of the disease. This study also highlighted significantly higher urinary levels of LacCer for females compared to males.

1D 4:30 p.m. – 4:45 p.m. Investigating the Impacts of Endoplasmic Reticulum Stress associated Chondrodysplasias using Metabolomics

PRESENTING AUTHOR: ***Jordan M.A. Wragg, The University of Manchester, United Kingdom***

CO-AUTHORS: ***Katherine A. Hollywood, Drupad K. Trivedi, Royston Goodacre, Raymond P. Boot-Handford***

Longitudinal bone growth via endochondral ossification can be disrupted by increased endoplasmic reticulum (ER) stress and the associated unfolded protein response, observed in cells expressing mutant protein coding extracellular matrix components of cartilage. These cellular stresses cause reduced proliferation and differentiation of these cells, ultimately leading to the clinical phenotype of various forms of dwarfism including the chondrodysplasias. Gaining a biological understanding of these disease mechanisms at the biochemical level is important, as it is possible to reduce cellular ER stress resulting from these genetic mutations pharmacologically and therefore potentially reduce clinical consequences of the chondrodysplasias. Establishing quantitative metabolite profiles for these disease states allows assessment of which areas of metabolism are affected by each given mutation and comparison can then be made between disease states. Initial focus centred on four collagen X mutations that have the downstream consequence of causing the metaphyseal chondrodysplasia type Schmid form of dwarfism as well as a wild-type collagen X construct expressed as a control comparison. Work then expanded to include similar comparisons between the matrilin-3 mutant V194D (causing multiple epiphyseal dysplasia) and its wild type control as well as several cartilage oligomeric matrix protein mutations that lead to a variety of chondrodysplasias. Stable cell lines expressing each construct were generated and metabolic profiles were generated from the cell models by established mass spectrometry techniques. Mummichog was used to quickly generate hypotheses by leveraging the organisation of metabolic networks to predict functional activity directly from feature tables, bypassing the need for individual metabolite identification.

1E 4:45 p.m. – 5:00 p.m. A strategy for comprehensive targeted metabolomics and metabolite identification via LC-QTOF-MS: application to the rare genetic disease Alkaptonuria

PRESENTING AUTHOR: ***Brendan P Norman, Insititue of Ageing & Chronic Disease, University of Liverpool, United Kingdom***

CO-AUTHORS: ***Andrew S Davison, Gordon A Ross, Anna M Milan, Andrew T Hughes, Norman B Roberts, James A Gallagher, Lakshminarayan R Ranganath***

Data processing and identification of 'unknowns' is the bottleneck in metabolomics. To resolve this an approach has been evaluated for comprehensive targeted metabolomics employing three complementary LC-QTOF-MS methods and accurate-mass retention time (AMRT) databases generated in-house from 619 metabolite standards. The strategy was then applied to Alkaptonuria, a rare inborn error of tyrosine metabolism. 619 standards (mw:45-1354 Da) covering a broad range of primary metabolism, including carbohydrates, amino and organic acids and lipids, were analysed by three chromatographic methods coupled to an Agilent 6550 LC-QTOF-MS; two reversed-phase (C18 2.1x100mm 1.8µm and dC18 3.0x100mm 3µm columns, mobile phases (A) water and (B) methanol, containing 5mM ammonium formate and 0.1% formic acid, one normal-phase (BEH amide 3.0x150mm 1.7µm column, mobile phases (A) water and (B) acetonitrile, containing 0.1% formic acid. Standards were analysed at 1.4µg/mL in positive and negative polarity. 561/619 standards(90.6%) were detected (theoretical mass +/-5ppm) across all methods. m/z-RT distributions showed the complementarity of the methods for separating metabolites from different biochemical classes. Analysis of urine samples (diluted 1:3 urine:water) from a patient with Alkaptonuria pre- and post-treatment with nitisinone found that 41.8% of the 619 standards were identified using AMRT targeted feature extraction. The changes to concentrations of homogentisic acid and tyrosine were confirmed as well as other tyrosine-related metabolites previously not reported. A powerful LC-QTOF-MS strategy has been evaluated for profiling metabolites in complex matrices such as urine using high-resolution accurate-mass and retention time. The strategy provides an invaluable phenotyping tool for understanding metabolism in Alkaptonuria.

1F 5:00 p.m. – 5:15 p.m. Metabolite profiling reveals a fundamental rewiring of glutamine metabolism for cell survival in the setting of human mitochondria DNA mutations: a novel insight for therapy of mitochondrial myopathies

PRESENTING AUTHOR: ***Steven Gross, Weill Cornell Medical College, United States***

CO-AUTHORS: ***Qiuying Chen, Kathryne Kirk, Yevgeniya I. Shurubor, Dazhi Zhao, Andrea J. Arreguin, Ifrah Shahi, Federica Valsecchi, Guido Primiano, Elizabeth L. Calder, Valerio Carelli, Travis T. Denton, Flint M.Beal , Giovanni Manfredi, Marilena D'Aurelio***

Human disorders caused by mitochondrial gene mutations result in oxidative phosphorylation (OXPHOS) impairment and typically affect multiple organs, manifesting with severe neurological and myopathic symptoms. Currently, there are neither effective treatments for mitochondrial diseases, nor a recognized therapeutic target. Using untargeted metabolite profiling and untargeted stable isotope tracing approaches, we reveal a previously unappreciated dependence on glutamine-derived α-ketoglutarate (αKG) for energy-generating anaplerotic flux - critical in mitochondrial DNA (mtDNA) mutant cells and humans with disease-associated OXPHOS defects. In cells with partial OXOPHOS inactivation, glutamine-derived glutamate influx into mitochondria occurs in exchange with aspartate efflux and subsequent cytosolic aspartate conversion to alanine and lactate. This unappreciated pathway maintains a continuous glutamine influx into the TCA cycle via a mitochondrial glutamate/aspartate antiporter, sustains substrate-level ATP production, contributes to re-oxidization of glycolytic NADH to NAD and provides a source of cytosolic NADPH for redox maintenance. Stimulating this metabolism by supplementation with αKG enables the survival of mtDNA mutant cells, under otherwise lethal conditions of OXPHOS insufficiency. Strikingly, we demonstrate that glutamine-glutamate-αKG utilization progressively switches its directionality in the TCA Cycle from reductive to oxidative, as mutant cell mitochondrial respiration increases from 0% to 100% of control levels. In accord with cell findings, increased oxidative αKG flux was found in a mouse model of mitochondriopathy where enhanced alanine levels in blood occur concomitantly with accelerated amino acid catabolism. Importantly αKG supplementation normalizes this muscle amino acid imbalance. These findings provide a rationale for αKG supplementation as a potentially generic therapeutic strategy for mitochondrial myopathies.

SESSION 2: FOOD & NUTRITION I	Monday June 25	3:30 p.m. – 5:15 p.m.

2A SESSION KEYNOTE 3:30 p.m. – 4:00 p.m.	Metabolomics approach to characterizing total carbohydrate intake in a controlled feeding study in participants from the Women's Health Initiative (WHI) cohort

PRESENTING AUTHOR: ***Johanna Lampe, Fred Hutchinson Cancer Research Center, United States***

CO-AUTHORS: ***Cheng Zheng, Xiaoling Song, Daniel Raftery, Nagana Gowda, Danijel Djukovic, Ying Huang, Shirley AA Beresford, Lesley F Tinker, Ross L Prentice, Marian L Neuhouser***

Most dietary assessment methods rely on participant self-reported food intake. Objective dietary exposure biomarkers are needed to infer diet-disease associations. Unfortunately, robust biomarkers of macronutrient intake are scarce, particularly for total carbohydrate. We showed recently that a subset of phospholipid fatty acids (PLFA), doubly labeled water-derived energy intake (Ein), and participant-related covariates explained 41.6% [cross-validated (CV) R2] of the variation in total carbohydrate intake (Song, AJCN, 2017). Here, we tested metabolomics in a predictive model of carbohydrate intake. We used an individualized feeding study wherein 153 postmenopausal women from the WHI consumed a 2-week controlled diet that reflected each woman's usual food intake. Fasting blood samples and 24h and spot urines were collected after the 2-week period. Urine was analyzed by 800 MHz NMR. Serum was assayed using a 158-metabolite targeted LC-MS platform and by GC-FID for 41 PLFA. Our linear regression model with metabolites and Ein explained 32.1%, 43.1% and 42.0% (CVR2) of the variation in total carbohydrate intake (g/d) with use of serum alone, 24h urine alone, or 24h urine+serum, respectively. Inclusion of PLFA and/or participant covariates did not improve estimates substantially, except for serum alone—39.2% with 13 PLFA and several covariates. Use of spot versus 24h urines was less effective. In all models, Ein explained ~20% of the variation and a variety of metabolites, particularly in urine, each contributed modestly to the model. This work suggests that a metabolomics-based biomarker for carbohydrate intake may be attainable with 24h urine metabolites being important contributors to the estimate.

2B 4:00 p.m. – 4:15 p.m.	Objective biomarkers of usual diet: a metabolomics analysis of weighed food intake

PRESENTING AUTHOR: ***Mary Christine Playdon, National Cancer Institute, United States***

CO-AUTHORS: ***Marian L Neuhouser, Johanna W Lampe, Lesley F Tinker, Ross Prentice, Kathleen M Hayden, Linda Van Horn, Joshua Sampson, Rachael Stolzenberg-Solomon, Steven C Moore***

Metabolomics can offer objective measurement of an individual's diet that might improve validity and reproducibility of research findings relating diet and health. Few objective biomarkers are available for use in clinical and population studies. We measured both serum and urinary metabolites after a feeding intervention nested within the Women's Health Initiative Nutrition and Physical Activity Assessment Study (NPAAS). Four-day weighed food records measured the usual diets of 153 women. These were adapted to acknowledge recognized biases and create carefully weighed and recorded individualized 14-day feeding menus. Participants took usual medications and dietary supplements. Fasting serum and 24-hour urine samples were collected post-intervention. Using liquid chromatography tandem mass-spectrometry, we identified 157 urinary and 55 serum metabolites associated with intake of 23 foods, including fruits, vegetables, grains, dairy, meats, alcohol, coffee, and dietary supplements. Forty-eight diet-related metabolites were observed in both serum and urine samples; many replicate observations from prior population studies (e.g. citrus-related stachydrine: urine r=0.80, serum r=0.77; coffee-related quinate: urine r=0.75, serum r=0.76). Eight coffee and citrus-related urinary metabolites had correlations ≥0.80. Metabolic signatures (LASSO regression) were strongly correlated (r>0.40) with coffee, alcohol, citrus, broccoli, dietary supplements. Most diet-related metabolites were food xenobiotic chemicals. Some revealed food preparation methods (e.g. nicotinate from heating coffee beans) and identified biomarkers with potential biological effects (e.g. dopamine-4-sulfate from banana). Our findings of dietary metabolite biomarkers provide a benchmark for magnitudes of association based on objective dietary measures that are applicable to large-scale population studies since they are based on usual dietary intake.

2C 4:15 p.m. – 4:30 p.m.	Foodomics: Why an apple a day keeps the doctor away.

PRESENTING AUTHOR: ***Brian Christopher Tooker, University of Colorado Denver, United States***

CO-AUTHORS: ***Yasmeen Nkrumah-Elie, Richard Reisdorph, Minghua Tang, Lauren O'Connor, Sarah Borengasser, Yu Wang, Richard Sayer, Nancy Krebs, Wayne Campbell, Nichole Reisdorph***

Certain dietary patterns have demonstrated numerous health benefits from long-term consumption. However, there is a significant gap in knowledge between what specific elements of these dietary patterns result in both positive and negative health outcomes. To address this gap, we have undertaken a step-wise approach that aims to go beyond traditional association studies. Step one entails the identification of unique molecular signatures from individual foods. Urine and plasma are then examined post-consumption of these foods to determine if these molecular signatures are present. Finally, our group is working to correlate these molecular signatures as biomarkers for health outcomes. To identify unique molecular signatures for individual foods, our group performed untargeted LC-MS on a subset of thirteen individual foods encompassing fruits, vegetables and animal protein sources. These thirteen individual foods yielded 10,948 annotated compounds. Compounds from each individual food were compared across all foods to identify unique molecular signatures specific to individual foods. The urine of study participants from a well characterized clinical nutrition study were then examined to determine presence of these signatures. Of the 10,948 food derived compounds, 4,237 were detected in the urine of study participants. More than 81% of the most abundant urinary compounds were food-derived. For example, of the 1,321 compounds unique to blueberries, 83 were found in the urine of 4 study participants (n=8) post-consumption. Quasi-kinetics showed the degradation of these blueberry metabolites over time. Our group is currently associating these molecular signatures with health outcomes including blood pressure and microbiome composition.

2D 4:30 p.m. – 4:45 p.m.	Metabolic profiling of high egg consumption and the associated lower risk of type 2 diabetes in middle-aged Finnish men

PRESENTING AUTHOR: ***Stefania Noerman, University of Eastern Finland, Indonesia***

CO-AUTHORS: ***Olli Kärkkäinen, Anton Mattson, Jussi Paananen, Marko Lehtonen, Tarja Nurmi, Tomi-Pekka Tuomainen, Sari Voutilainen, Jyrki K Virtanen, Kati Hanhineva***

High egg intake has traditionally been discouraged because the high cholesterol content may impair glucose metabolism and promote inflammation, thereby potentially increasing the risk of type 2 diabetes (T2D). However, in middle-aged men from the prospective, population-based Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) in eastern Finland, higher egg intake was previously associated with a lower risk of T2D. This study aimed to examine potential biomarkers linking egg intake with the lower T2D risk using non-targeted HPLC-MS/MS-based metabolic profiling. We analyzed 239 baseline serum samples from the KIHD in 4 groups: 1) subjects with high egg consumption (mean intake 1 egg/d) who remained free of T2D during the mean follow-up of 19.3 years (controls), 2) controls with low egg intake (about 2 eggs/wk), 3) subjects who developed T2D during the follow-up (cases) with high egg intake, and 4) T2D cases with low egg intake. Higher egg intake was positively associated with indolelactic acid and two (lyso)phosphatidylcholines ((lyso)PCs) containing 21:2 fatty acids, and negatively with unsaturated acylcarnitines, lysophosphatidylethanolamines, (lyso)PCs, and γ-glutamylated-branched-chain amino acids. Those who developed T2D had higher levels of tyrosine and PCs containing saturated and medium-chain fatty acids, whereas controls had predominantly choline, phosphocholine, glycerophosphocholine, plasmalogens, and (lyso)PCs containing odd- and long-chain polyunsaturated fatty acids. The distinguished metabolic profiles between subjects with varying egg intake and incidence of T2D may imply the potential of the metabolites involved in (phospho)lipids and amino acids metabolisms to partly explain the inverse association between egg intake and T2D risk in this population.

2E 4:45 p.m. – 5:00 p.m.	From 50 um to 5,000 people: lipidomics studies of diet induced non-alcoholic fatty liver disease

PRESENTING AUTHOR: ***Julian L. Griffin, University of Cambridge, United Kingdom***

CO-AUTHORS: ***Francis W.B. Sanders, Animesh Acharjee, Celia Walker, Luke Marney, Lee D. Roberts, Xinzhu Wang, Yajing Chu, Fumiaki Imamura, Benjamin Jenkins, Jack Case, Sumantra Ray, Diana Kuh, Rebecca Hardy, Michael Allison, Nita Forouhi, Michele Vacca, Andrew J. Murray, Nick Wareham, Albert Koulman, Zoe Hall.***

Diet is a major contributor to metabolic disease risk, but there is controversy as to whether increased incidences of diseases such as non-alcoholic fatty liver disease (NAFLD) arise from consumption of saturated fats or free sugars. To investigate how diet influences NAFLD we conducted direct infusion mass spectrometry of lipids in plasma to study the association between the lipidome and hepatic steatosis assessed by ultrasound in volunteers from the UK-based Fenland Study (n=1507), and relate associations with food intake. A cluster of triacylglycerols (TAGs) containing saturated and monounsaturated fatty acids with 16-18 carbons were associated with hepatic steatosis, higher consumption of carbohydrate and variations in the gene for protein phosphatase 1, regulatory subunit 3b (PPP1R3B), which regulates partly glycogen synthesis. This cluster of TAGs was also detected in another cohort, the National Survey of Health and Development UK (n=1701). DNL was measured using stable isotope techniques in hyperphagic ob/ob mice and in humans following high carbohydrate meals, demonstrating the rate of DNL correlates with increased synthesis of this cluster of TAGs. To further characterise hepatic steatosis we have also examined liver tissue directly using mass spectrometry imaging, demonstrating that the development of disease is associated with loss of zonation in the liver, along with lipid remodelling of arachidonic acid containing phosphatidylcholines that contribute to liver inflammation. In conclusion, a sub-set of TAGs are associated with hepatic steatosis, even when correcting for confounding factors. We suggest that hepatic steatosis risk is in part driven by increased DNL following carbohydrate rich meals.

2F 5:00 p.m. – 5:15 p.m.	Lipidomic profile of human cerebrospinal fluid after a triglyceride infusion

PRESENTING AUTHOR: ***Angela J Hanson, University of Washington, United States***

CO-AUTHORS: ***Lisa F Bettcher, Dan Raftery, Suzanne Craft, William A Banks***

Background: High-fat diets increase risk for Alzheimer's disease, but little is known about the mechanisms. In addition, little is known about which lipids enter the brain during a high triglyceride (TG) state. We analyzed the lipidomic signature of cerebrospinal fluid (CSF) in older adults that received a TG infusion. Methods: Adults with Alzheimer's risk factors (n=21, age 67.7 ± 8.6) underwent a 2-hour TG or saline infusion (10% safflower oil and 10% soybean oil) followed by CSF collection on different days in crossover design. Lipids were extracted using dichloromethane/methanol across 13 different lipid classes and analyzed using the Lipidyzer platform consisting of an AB Sciex 5500 MS/MS system equipped with a SelexION for differential mobility spectrometry (DMS). Multiple reaction monitoring was used to target and quantify over 1000 lipids in positive and negative ionization modes with and without DMS. Results: We detected around 200 lipids per sample, average CV of 5.2%. The infusion increased diacylglycerols (0.87% vs 1.08%, p=0.022) and lysophosphatidylcholines (LPC) (0.22 vs 0.37%, p=0.002), and reduced dihydroceramides (0.11% vs 0.08%, p=0.006). Analysis using MetaboAnalyst revealed 4 significant hits: FFA 18:2, LPC 18:1, FFA 18:3, and LPC 16:0. Conclusions: We detected statistically significant differences in classes of lipids, and individual lipid species, in older adults that underwent a triglyceride infusion. Further analysis of the lipid profiles with age and other demographics, and in conjunction with metabolomics data may help us understand the brain lipid milieu in older adults at risk for Alzheimer's.

SESSION 3: BIG DATA: DATABASES AND CLOUD COMPUTING	Monday June 25	3:30 p.m. – 5:15 p.m.

3A SESSION KEYNOTE 3:30 p.m. – 4:00 p.m.	The Human Metabolome Database – A Major Update for 2018

PRESENTING AUTHOR: ***David Wishart, University of Alberta, Canada***

CO-AUTHORS: ***Yannick Djoumbou Feunang, Ana Marcu, An Chi Guo, Carin Li, Naama Karu, Zinat Sayeeda, Mark Berjanskii, Sandeep Singhal, David Arndt1, Yonjie Liang, Hasan Badran, Rupa Mandal, Allison Pon, Craig Knox, Michael Wilson, Claudine Manach, Augustin Scalbert***

The Human Metabolome Database (HMDB) is a web-enabled metabolomic database containing comprehensive information about human metabolites along with their biological roles, physiological concentrations, disease associations, chemical reactions, metabolic pathways, and reference spectra. First described in 2007, the HMDB has gone through a number of updates and improvements over the past decade. This year's update, HMDB 4.0, represents the most significant upgrade to the database in its history. The database now contains >115,000 compounds, the number of experimental spectra has grown by almost fourfold and the number of illustrated metabolic pathways has grown by a factor of almost 60. Significant improvements have also been made to the HMDB's chemical taxonomy, chemical ontology, spectral viewing, and spectral/text searching tools. A great deal of brand new data has also been added to HMDB 4.0. This includes more information on metabolite-SNP interactions and more data on the influence of drugs on metabolite levels (pharmacometabolomics). Many other important improvements in the content, the interface, and the performance of the HMDB website have been made and these are described in detail. Overall, the 2018 version of HMDB should greatly enhance its ease of use and the potential applications of metabolomics toward nutrition, biochemistry, clinical chemistry, clinical genetics, medicine, and other fields of biomedical science.

3B 4:00 p.m. – 4:15 p.m.	Mind the gap: mapping mass spectral databases in genome-scale metabolic networks reveals poorly covered areas

PRESENTING AUTHOR: ***Fabien Jourdan, INRA-MetaboHub, France***

CO-AUTHORS: ***Clement Frainay, Emma L. Schymanski, Steffen Neumann, Benjamin Merlet, Reza M Salek, Oscar Yanes***

The use of mass spectrometry-based metabolomics to study human, plant and microbial biochemistry and their interactions with the environment largely depends on the ability to annotate metabolite structures by matching mass spectral features of the measured metabolites to curated spectra of reference standards. While reference databases for metabolomics now provide information for hundreds of thousands of compounds, barely 5% of these known small molecules have experimental data from pure standards. Remarkably, it is still unknown how well existing mass spectral libraries cover the biochemical landscape of prokaryotic and eukaryotic organisms. To address this issue, we have investigated the coverage of 38 genome-scale metabolic networks by public and commercial mass spectral databases, and found that on average only 40% of nodes in metabolic networks could be mapped by mass spectral information from standards. Next, we deciphered computationally which parts of the human metabolic network are poorly covered by mass spectral libraries, revealing gaps in the eicosanoids, vitamins and bile acid metabolism. Finally, our network topology analysis based on the betweenness centrality of metabolites revealed the top 20 most important metabolites that, if added to MS databases, may facilitate human metabolome characterization in the future.

3C 4:15 p.m. – 4:30 p.m.	MetaboLights; An Open Source Metabolomics Resource

PRESENTING AUTHOR: ***Keeva Cochrane, EMBl-EBI, United Kingdom***

CO-AUTHORS: ***Keeva Cochrane, Venkata Chandrasekhar Nainala, Kenneth Haug, Namrata Kale, Kalai Vanii Jayaseelan, Jose Ramon Macias, Pablo Moreno, Rachel Spicer, Mark Williams & Claire O'Donovan***

EMBL-EBI has established the MetaboLights database as one of the most successful international metabolomics repositories. Recommended by a number of leading journals including Nature, PLOS and Metabolomics. MetaboLights hosts a wealth of cross-species, cross-technique, open access experimental research. The services unique manual curation maintains quality, provides helpful support for users and ensures accessibility for secondary analysis of studies. With nearly 3000 species represented, the enriched reference layer displays information outlining the chemical and biological nature of numerous metabolites. Structural and spectral references, network pathways, enzymatic reactions, and associated literature equips the user with a knowledge base to enrich their research. Focusing on adding further value and functionality, EMBL-EBI is currently working on integrating online analysis tools to provide a complete workflow, from data management, processing and analysis through to data publication. A clinically focused service is available through our H2020 PhenoMeNal (Phenome and Metabolome aNalysis) project, an e-infrastructure which provides a secure environment for the handling of sensitive data with Ethical Legal and Social Implications (ELSI) consideration for molecular phenotype data from a range of clinical and analytical sources. MetaboLights strives to become the model resource for metabolomics and therefore is eager to develop and integrate with others where possible. As such, MetaboLights works closely with companies and Phenome Centres to ensure easy integration of data derived from commercially available kits into the database. MetaboLights actively collaborates in the development of tools that allow convenient discovery of metabolomics and multi-omics research such as MetabolomeXchange and OmicsDI.

SESSION 3: BIG DATA: DATABASES AND CLOUD COMPUTING	Monday June 25	3:30 p.m. – 5:15 p.m.

3D 4:30 p.m. – 4:45 p.m.	Generating a comprehensive blood exposome database using computational text mining of data from the National Center for Biotechnology Information

PRESENTING AUTHOR: ***Dinesh Kumar Barupal, NIH West Coast Metabolomics Center, University of California Davis, United States***

CO-AUTHORS: ***Oliver Fiehn***

Which compounds have been detected in blood? To this end, we have processed data from the National Center for Biotechnology Information (NCBI) in the R programming environment using ElasticSearch tools to develop a consolidated database of unique compound structures linked to blood. All 28 million PubMed abstracts were indexed in an ElasticSearch database. 2.7 million blood-related papers were subjected to ElasticSearch's aggregation function and gave 27,004 unique chemical structures. PubChem CID- MeSH term, CID-PubMed ID, CID-synonyms mappings and a subset of PubChem entries were obtained from the NCBI server based on 'biologically relevant structure' depositors, including 1,632 unique compounds retrieved from PubMed Central supplementary files related to 2,348 blood metabolomics papers. Linking PubChem entries to PubMed files yielded 125,600 unique structures to be associated with blood-related studies. We then queried synonyms for all 6.8 million PubChem 'relevant structure' compounds against PubMed abstracts and yielded around 50,000 compounds associated with blood. When testing the compound overlap across the different approaches, we found that PubChem CID - PMID mapping provided the most comprehensive overview. Manually curated human metabolite databases such as HMDB underestimate the number of compounds present in human blood. Our new human exposome compound database is available at http://exposome.fiehnlab.ucdavis.edu, for example, to predict MS/MS spectra in untargeted exposome studies using MS-FINDER or CFM-ID software.

3E 4:45 p.m. – 5:00 p.m.	Metabolite profiling and annotation with interoperable and scalable microservices

PRESENTING AUTHOR: ***Steffen Neumann, Leibniz Institute of Plant Biochemistry, Germany***

CO-AUTHORS: ***Payam Emami Khoonsari, Pablo Moreno, Marco Capuccini, Namrata Kale, Anders Larsson, Steffen Neumann, Christoph Ruttkies, Christoph Steinbeck, Kim Kultima, Ola Spjuth, PhenoMeNal consortium***

Metabolomics as a high-throughput molecular phenotyping technique is growing across all biomedical domains. The data processing and analysis is often performed with many programs using conventional computing solutions and little standardisation for interoperable and reproducible research. With increasing data size this becomes intractable for desktop computers. Cloud computing allows to instantiate on-demand resources (virtual servers, networks, storage), users only pay for the time the resources are used. Microservices can run in clouds that can dynamically grow or shrink, enabling applications to be scaled. We developed a robust and performant data analysis infrastructure that integrates all necessary components. The software tools are encapsulated as Docker containers. To automate the instantiation of this cloud-portable microservice-based system, the PhenoMeNal consortium developed a Virtual Research Environment (https://portal.phenomenal-h2020.eu/) to deploy on some of the largest public cloud providers, including Amazon Web Services, Microsoft Azure, Google Cloud Platform and OpenStack-based private clouds. Using Galaxy (https://galaxyproject.org/) as interface for individual tools, users can share workflows and analysis histories. Jupyter and Luigi are also supported. Kubernetes (https://kubernetes.io/) is used for container orchestration in the cloud. Mass spectrometry data processing often requires a combination of different tools. Our workflow combines noise reduction and filtering (OpenMS), quantification, alignment and matching (XCMS), filtering features based on blank and dilution series samples (R), feature annotation (CAMERA), statistics (ANOVA, PLS-DA) and identification (MetFrag). Other workflows support NMR and fluxomics data analyses. We achieved a complete integration of the major software suites resulting in the first turn-key workflow for mass-spectrometry-based metabolomics with Microservices.

3F 5:00 p.m. – 5:15 p.m.	Omics Big Data and Analytics at Google Cloud

PRESENTING AUTHOR: ***Jonathan Sheffi, Google Cloud, United States***

Big data and advanced analytics have completely altered the landscape in fundamental ways for many areas of science and business. For genomic medicine, bid data and machine learning approaches are helping to create individual strategies for patient care from diagnostics to therapeutic decision making. The incorporation and integration of additional sources of information such as electronic health records or other omics data such as proteomics and metabolomics will make improve accuracy and predictive power using advanced machine learning approaches. In addition to genomics, the field of proteomics has started to take advantage of the power of big data and advanced analytics, which should provide a useful guide for metabolomics researchers. In this talk I will describe how Google Cloud enables scientists to change the way they perform research and collaborate with one another. This presentation will highlight the ways in which Google Cloud is accelerating life sciences research and finding new ways to innovate using several recent successful examples. In particular, I will focus on how Pipelines API, standard tools like GATK, optimized machine learning tools like DeepVariant, and big data tools such as BigQuery make answering these big data research questions straightforward, fast and efficient. The talk will also aim to show how metabolomics researchers can think about leveraging big data analytics in their workflows.

SESSION 4: PLANT METABOLOMICS I	Tuesday June 26	10:15 a.m. – 12:00 p.m.

4A KEYNOTE 10:15 a.m. – 10:45 a.m.	Spatially resolved metabolomics to identify novel salinity tolerance mechanisms in barley roots

PRESENTING AUTHOR: ***Ute Roessner, The University of Melbourne, Australia***

CO-AUTHORS: ***W.H. Ho, C.B. Hill, T. Rupasinghe, S. Natera, D.L.S. Lopez, D. Yu, B.A. Boughton, U. Roessner***

Barley is an important cereal crop and suffers yield loss under salinity. Little is understood of salinity perception and responses in roots, which involve complex changes at the physiological, metabolic, molecular, transcriptional, and genetic level. We develop new tools to unravel how plants respond to the perception of salinity. Evidence is accumulating that lipid signalling is an integral part of complex regulatory networks upon salinity by modifications of membrane lipids, which through the activity of phospholipases, lipid kinases and phosphatases that produce different classes of lipid and lipid-derived messengers. These provide spatial and temporal regulatory functions crucial for cell survival, growth and for an appropriate response of the plant to environmental stimuli. Initial analyses indicate that different tissue types within the root respond differently to salt stress in tolerant and sensitive cultivars. Here we study the root responses to salinity using a combination of RNA-sequencing and targeted metabolite and lipid analyses of three key sections of barley roots. In addition, we are using MALDI-FT-MS based imaging technologies to monitor spatial distributions of metabolites and lipids across root sections of salt-treated tolerant and sensitive barley genotypes. Transcriptomics results are now being integrated with spatial biochemical data, enhancing our understanding of system-wide and tissue-specific responses of roots to salinity stress. Given the lack of fundamental knowledge of the genes and proteins involved in signalling and lipid metabolism under salinity stress, and the enormous potential for biotechnological application in this area, our results provide insight into novel mechanisms responsible for barley salt tolerance.

4B 10:45 a.m. – 11:00 a.m.	Multi-platform metabolomics to unravel the priming effects of seaweed extracts on maize (Zea mays) under drought stress

PRESENTING AUTHOR: ***Fidele Tugizimana, University of Johannesburg; International R&D Division, Omnia, South Africa***

CO-AUTHORS: ***Johan Huyser, Lungile Sitole, Ian Dubery, Paul Steenkamp***

Drought, one of the major abiotic stresses, decreases plant water status, leading to a metabolic reprogramming that negatively affects plant growth and yield. Seaweed extracts, as plant biostimulants, have shown growth-promoting effects and ameliorating effect on crop tolerance to abiotic and biotic stresses. However, there are still grey areas in describing holistically molecular mechanisms underlying these benefits of the seaweed extracts on plants under environmental stresses. In this study, a multi-platform metabolomic approach was applied to unravel molecular 'stamps' that define the effects of seaweed extracts on greenhouse-grown maize (Zea mays) under drought conditions. Metabolites were methanol extracted from leaves tissues and analysed on both UHPLC-HDMS and 1H NMR platforms. The results showed that the application of seaweed extracts induced alterations in different pathways of primary and secondary metabolism such as phenylpropanoid, flavonoid biosynthesis, fatty acid metabolism, amino acids and phytohormones pathways. Metabolic network analysis allowed the characterisation of key metabolic 'hubs' that define the observed metabolic reprogramming. These changes were found to be related to enhanced drought resistance traits. Thus, our results suggest that seaweed extracts act as a priming agent, enhancing plant resistance to abiotic stresses. Unravelling key characteristics of the biochemistry underlying the effects of seaweed extracts on maize plants under drought stress provided valuable insights with potential applications. Furthermore, the study contributes to ongoing efforts towards a comprehensive understanding of the defence priming phenomenon, which is a sustainable alternative strategy that can provide avenues for plant protection against environmental stresses.

4C 11:00 a.m. – 11:15 a.m.	Multi-metabolomics by liquid chromatography-tandem mass spectrometry and imaging mass spectrometry for phytochemical genomics on the saponin metabolism in Asparagus officinalis

PRESENTING AUTHOR: ***Ryo Nakabayashi, RIKEN CSRS, Japan***

CO-AUTHORS: ***Tetsuya Mori, Feng Qiu, Rai Amit, Kei Hashimoto, Takashi Nirasawa, Hiroshi Sudo, Kiminori Toyooka, Lloyd W. Sumner, Kazuki Saito***

It is considered that saponins have important roles for symbiotic interactions between plants and other organisms. Asparagus officinalis, one of staple crops in the world, accumulates furostan- and spirostan-type of saponins in aerial and underground parts. Here, we perform an integrated analysis of metabolomics and transcriptomics to identify molecules and biosynthetic genes regarding the saponin metabolism. Asparagus seedlings were grown on agar in plant boxes for 4 weeks and were harvested for aerial and root parts. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) with database references and the computational tool PlantMAT chemically assigned known and unknown saponins in the aerial and root parts. With this approach, components of unknown saponins (e.g., aglycon and sugar) were efficiently characterized. The chemical assignments were performed according to the guideline of the Metabolomics Standards Initiative. To reveal the localization of the saponins in the roots, imaging mass spectrometry (IMS) by matrix assisted laser desorption/ionization was performed on cross sections sprayed with 2,5-dihydroxybenzoic acid in 50% MeOH including 0.2% trifluoroacetic acid (30 mg/ml). Collating results of the LC-MS/MS and the IMS analyses showed several patterns of the localization of the saponins. Transcriptomics by RNA-sequencing was performed in the both samples. On the basis of the accumulation patterns of the saponins, gene expression patterns will be analyzed to narrow down biosynthetic genes to form aglycons and sugar moieties in the future.


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SESSION 4: PLANT METABOLOMICS I	Tuesday June 26	10:15 a.m. – 12:00 p.m.

4D 11:15 a.m. – 11:30 a.m.	Toxic beauties: metabolite mapping shows different strategies in the deployment of chemical defence metabolites in floral tissues

PRESENTING AUTHOR: ***Edita Ritmejeryte, The University of Melbourne, Australia***

CO-AUTHORS: ***Berin A Boughton, Michael J Bayly, Rebecca E Miller***

Floral chemical defence strategies remain little investigated, despite the significance of flowers to plant fitness and the sizeable investment of resources in floral structures. Cyanogenic glycosides are nitrogen containing plant secondary metabolites that deter herbivores by releasing hydrogen cyanide upon tissue damage, but also play a role in nitrogen and glucose transport. Broadly consistent with the optimal allocation theory of plant defence are high concentrations of cyanogenic glycosides in flowers in the Proteaceae, a family with a high frequency of floral cyanogenesis. Little is known about the distribution of defence metabolites within flowers; at what scale is there strategic allocation of cyanogenic glycosides in flowers, and what does their localisation reveal about their function? Here we use flowers of different morphology from across the Proteaceae phylogeny to quantitatively compare the distribution of defence metabolites in floral tissues throughout development. Spatial mapping of cyanogenic glycosides was performed by MALDI MS imaging of cryosectioned floral tissues, using a method developed to prevent the hydrolysis of these labile glycosides. At least four distinct patterns in the distribution of cyanogenic glycosides within floral tissues were identified across 14 species. In one genus, cyanogenic glycosides were restricted to pollen and surface stigmatic tissues, whereas in several other genera pistillate tissues were most defended. Metabolite mapping identified differential localisation of different cyanogenic glycosides, with the diglycoside proteacin co-localized with sucrose in vascular tissues, and the monoglycoside dhurrin distributed evenly across floral tissues. Floral defence strategies, cyanogenic glycoside distributions and functions will be discussed.

4E 11:30 a.m. – 11:45 a.m.	Biomarker Identification in Stinking Smut (Tilletia caries) infection of Wheat

PRESENTING AUTHOR: ***Rebecca Weed, Washington State University, United States***

CO-AUTHORS: ***Kyryll Savchenko, Leandro Lessin, Lori Carris, David R. Gang***

Stinking smut (Tilletia caries), is one of the most important pathogens of wheat with a worldwide distribution. This fungus overwinters in the soil, infects newly germinated seeds and remains undetected until the crop is ready to harvest, when the fungus has replaced normal seeds with intact seed coats full of teliospores. If biomarkers could be identified at early wheat growth stages, then the crop could be destroyed prior to full infection and proliferation of the teliospores. We investigated the differences in the metabolome of wheat infected with T. caries at 3 different stages of plant development; booting, flowering and spike senescence. Five different tissue types were analyzed at each stage: root, upper leaf, upper stem, base stem, and spike. The samples were compared via metabolomic profiling of infected and non-infected wheat using three different extraction methods to isolate a range of compounds from very polar to non-polar. Samples were analyzed using a Q-IMS-TOF HDMS. A primary metabolite extraction method was also completed with the samples and analyzed using GCxGC-TOF. The total results suggest that there are potential biomarkersvary in type and intensity across developmental growth stages of wheat and stage of infection.

SESSION 5: NEUROLOGY & PSYCHIATRY	Tuesday June 26	10:15 a.m. – 12:00 p.m.

5A SESSION KEYNOTE 10:15 a.m. – 10:45 a.m.	Metabolic profiles reveal gender differences in APP/PS1 transgenic Alzheimer's disease mice

PRESENTING AUTHOR: ***Yulan Wang, Wuhan Institute of Physics and Mathematics, China***

CO-AUTHORS: ***Junfang Wu, Hehua Lei, Huiru Tang***

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive impairment. Currently, research has focused attention on the central nervous system; the involvement/contribution of the peripheral biofluid/organ in AD has largely been overlooked. We investigated metabolic responses of both biofluids and liver tissues using an APP/PS1 double mutant transgenic mouse model with NMR spectroscopy as well as analysis from serum biochemistry and histological staining. Quantitative targeted analysis was also performed on biofluids/organs using mass spectrometry technique. We found clear gender differences in AD transgenic mice when compared with their wild type counterparts. Female AD mice displayed more intensive responses, which highlighted by higher levels of lipids, 3-hydroxybutyrate and nucleotides, together with lower levels of glucose. These observations indicate that AD induces oxidative stress and impairs cellular energy metabolism in peripheral organs. Disturbances in AD male mice were milder with depletion of monounsaturated fatty acids. We also observed a higher activity of delta-6-desaturate and suppressed activity of delta-5-desaturate in female mice, whereas inhibited stearoyl-CoA-desaturase in male mice suggested that AD induced by the double mutant genes results in different fatty acids catabolism depending on gender. In addition, we also observed gender differences in regulatory bile acid metabolism in APP/PS1 transgenic AD mice. Our observation is broadly consistent with epidemiological investigation showing high morbidity AD cases in women, suggesting that a gender-specific scheme for AD treatment in men and women may be required.

5B 10:45 a.m. – 11:00 a.m. **Targeted plasma lipidomic analysis to better understand and predict Alzheimer's Disease**

PRESENTING AUTHOR: ***Peter J Meikle, Baker Heart and Diabetes Institute, Australia***

CO-AUTHORS: ***Kevin Huynh, W L Florence Lim, Pratishtha Chatterjee3 Naltalie A Mellett, Corey Giles, Kaushala S Jayawardana, Ralph N Martins***

Introduction: Alzheimer's disease (AD) is the predominant form of dementia in older people. Early detection and treatment is required to address the growing disease burden. Lipid metabolism is tightly coupled to AD onset and progression, although the details are poorly defined. To explore the lipid biology underpinning AD pathology and to determine the potential of lipid biomarkers, we performed plasma lipidomic profiling (582 lipid species across 30 classes and subclasses) on the Australian Imaging, Biomarkers and Lifestyle flagship study of aging (AIBL) cohort. Method: Baseline and up to four longitudinal samples from subjects in the AIBL cohort (747 healthy control, 126 mild cognitive impairment (MCI) and 202 AD) were analysed. Associations between lipid species and outcomes were examined using logistic and linear regression. Multivariable models were created to predict incident AD in an age, gender and BMI matched subset (68 AD converters and 136 controls). Bootstrap resampling (2,500 repeats) was used to correct for over-fitting and optimism in model performance. Results and Discussion: Lipid species were associated with prevalent AD (275) and incident AD (55), including ceramides, ether phospholipids and acylcarnitines. The addition of 10 lipid species to a base model of age and ApoE status increased the C-statistic (0.66 (95%CI 0.52-0.79) to 0.82 (95%CI 0.71-0.93)) and had a net reclassification index (NRI) of 1.32 (95%CI 0.86-1.73). These data identify metabolic pathways associated with AD, show promise for lipid biomarkers and highlight potential therapeutic targets to aid in developing early diagnostics and effective treatments for AD.

5C 11:00 a.m. – 11:15 a.m. **Increased trans-sulfuration and glucose metabolism define a distinct sporadic ALS subtype**

PRESENTING AUTHOR: ***Qiuying Chen, Weill Cornell Medical Ciollege, United States***

CO-AUTHORS: ***Davinder Sandhu, Csaba Konrad, Dipa Roychoudhury, Kirsten Bredvik, Hibiki Kawamata, Elizabeth L. Calder, Lorenz Studer, Steven. M. Fischer, Giovanni Manfredi, Steven. S. Gross***

Sporadic amyotrophic lateral sclerosis (sALS) is a rapidly progressive motor neuron disease that results in paralysis and death. There is no cure for sALS. The genetic and molecular bases are unknown, and we hypothesized that sALS cases can have multiple origins. In attempt to discover disease-associated biomarkers for potential stratification of sALS cases, we performed untargeted metabolite profiling analysis on 77 patient-derived skin fibroblast lines and 45 age- and sex-matched healthy controls. We found that >25% of sALS cases share distinct metabolic perturbations, typified by upregulated trans-sulfuration, where methionine-derived homocysteine is preferentially channeled to cysteine synthesis for increased glutathione (GSH) production. Untargeted stable isotope tracing of [13C]-glucose revealed that in this sALS subset, trans-sulfuration pathway activation was concomitant with accelerated glucose flux into the TCA cycle, along with increased conversion to glutamate, GSH, alanine, aspartate, acylcarnitines and nucleotide phosphates. A support vector machine model, based soly on four metabolites, linked to the trans-sulfuration pathway, taurine, 2-hydroxbutyrate, creatinine and propionylcarnitine, successfully distinguished this sALS subset from controls with an accuracy of 97.5%. Importantly, we uncovered this altered trans-sulfuration pathway metabotype in plasma of these sALS cases and found potentially gene transcripts that contribute to the metabotype, reflecting systemic alterations in cysteine metabolism as a convenient readout for sALS case stratification. Together, our findings indicate that sALS can be stratified into distinct metabotypes and highlight the potential for personalized therapy for sALS, where alterations in trans-sulfuration/GSH metabolism identify a subset of sALS patients that may selectively benefit from antioxidant supplementation therapies.

5D 11:15 a.m. – 11:30 a.m. **Characterising the metabolic profile of ALS: results from the Euro-Motor study cohort**

PRESENTING AUTHOR: ***Alexandros P Siskos, Imperial College London, Department of Surgery and Cancer, United Kingdom***

CO-AUTHORS: ***Gabriel N Valbuena, James Rooney, Federico Casale, Fabrizio D'Ovidio, Orla Hardiman, Adriano Chio, Ettore Beghi, Giancarlo Logroscino, Jan Veldink, Leonard van den Berg, Hector Keun***

Amyotrophic Lateral Sclerosis (ALS) is a devastating disease with no cure available and an incidence of 2 to 3 cases per 100,000 individuals in Europe. The aim of the FP7 Euro-MOTOR study is to identify novel causes of ALS using a comprehensive systems biology approach, including a population-based metabolomics study. The Euro-Motor metabolomics cohort consists of 1649 samples from the Netherlands, Italy and Ireland, of which 726 case-control pairs matched by centre, age and gender, and also a further 159 cases unmatched. We have carried out metabolomic analysis of serum samples using the AbsoluteIDQTM p180 platform (Biocrates Life Scieneces AG), which allows the targeted analysis of amino acids, biogenic amines, acylcarnitines, sphingolipids and glycerophospholipids. The presence of ALS has a clear impact on the serum metabolome, across three different European populations Netherlands, Ireland, and Italy. Major differences were identified in the overall profile of amino acids, carnitines, and creatinine likely reflecting changes in muscle metabolism and perturbation of β-oxidation activity in patients. The results suggest differences also to the lipid profile with emphasis to di-acyl-phosphocholines with PUFA lipids, consistent with perturbation of lipid metabolism or nutritional contributions to lipid composition. Based on the identified metabolomic signature of ALS, metabolite-associated survival models were constructed. Moreover, metabolic multivariate models were constructed in order to predict disease stage, outcome, and also disease progression based on functional ALSFRS-R scores. Overall, these results provide insights to understanding disease mechanism and progression with the aim to improve patient management and quality of life.

SESSION 5: NEUROLOGY & PSYCHIATRY	Tuesday June 26	10:15 a.m. – 12:00 p.m.

5E 11:30 a.m. – 11:45 a.m.	Circulating endocannabinoids are dysregulated with respect to central CB1-receptor availability in male patients with first episode psychosis.

PRESENTING AUTHOR: ***Alex M Dickens, University of Turku/Turku Centre for Biotechnology, Finland***

CO-AUTHORS: ***Laurikainen H, Borgan F, Rönkkö T, , Lindeman T, Howes O, Hietala J, Hyötyläinen T, Orešič M***

There is an established link between psychosis and metabolic abnormalities such as altered glucose metabolism and dyslipidemia. However, the mechanism by which these metabolic changes occur remains unclear. Although antipsychotic drugs can contribute to the metabolic changes there is evidence that the alterations precede antipsychotic treatment. Metabolomics approaches have been used to quantify the metabolic changes occurring in first episode psychosis. Identifying dysregulated metabolism, could be used to predict the patients at risk of developing metabolic co-morbidities. Using a quantitative liquid-chromatography triple-quadrupole mass spectrometry assay, nine endogenous endocannabinoids or related structures were measured from serum obtained from first episode psychosis patients (FEP, n=8) and healthy controls (HC, n=10). After serum sampling brain CB1R availability was quantified in the same individuals using positron emission tomography (PET) and specific cannabinoid-1 receptor (CB1R) tracer [18F]FMPEP-d2. Circulating levels of arachidonic acid (p=0.02) and oleyl ethanolamide (p=0.04) were reduced in the FEP individuals. In order to compare the levels of circulating endocannabinoids to the brain CB1R availability PLS regression modelling was used. In HC there was strong association of arachindonoyl glycerol (1+2), stearoyl ethanolamide and palmitoyl ethanolamide with the CB1R availability in the grey matter of the hippocampus (R2CV=0.51) which was lost in the FEP patients (R2CV=0.10). The dysregulation of circulating endocannabinoids in the circulation compared to CB1R following a FEP highlights a possible mechanism by which metabolic co-morbidities occur in psychosis. Despite the small number of patients in this study there is a clear dysregulation of the endocannabinoid system in patients with FEP.

SESSION 6: MULTIOMICS	Tuesday June 26	10:15 a.m. – 12:00 p.m.

6A SESSION KEYNOTE 10:15 a.m. – 10:45 a.m.	A multi-omic investigation of the role of the APOE genotype in Alzheimer's disease

PRESENTING AUTHOR: ***Erin Baker, Pacific Northwest National Laboratory, United States***

CO-AUTHORS: ***Erin S. Baker, Xueyun Zheng, Kristin E. Burnum-Johnson, Carrie D Nicora, Kelly G. Stratton, Jennifer E. Kyle, Kent Bloodsworth, Catriona A. Mclean, 3, Richard D. Smith, Blaine Roberts***

Alzheimer's disease (AD) is the most common neurodegenerative disease, affecting 47 million people worldwide. To date there is no cure for AD and its cause remains unclear, however, both age and the apolipoprotein E (APOE) gene have been linked to its occurrence. Among the APOE ε2, ε3 and ε4 genes, the ε4 carriers have a greater chance of developing AD, while ε2 carriers are less likely to have AD. Thus, studying the molecular changes that occur due to the different APOE genotypes may provide important insights into the mechanisms that protect ε2 carriers or enhance the risk of ε4 carriers. In this study, brain tissue samples from the frontal cortex and cerebellum were obtained from 62 postmortem patients having four different APOE genotypes (ε2/ε3, ε3/ε3, ε3/ε4 and ε4/ε4). Statistical differences in both the lipidome and proteome were noted for the samples from different brain regions of each patient group. Lipidomic profiling showed that many phosphatidylcholines (PC) decreased in the AD patients for all genotypes in both the frontal cortex and cerebellum locations. Most lysophosphatidylcholines (LPC) also decreased in the AD patients for all genotypes. Interestingly, the LPC changes had genotype specific profiles and distinct expression depending on the brain region investigated. Certain phosphatidylserines were also found to decrease in both brain locations for all genotypes except for the cerebellum of ε3/ε4 carriers, indicating a genotype specific lipid metabolism. Statistical analyses for the proteomic analyses are ongoing and will also be presented at the meeting.

6B 10:45 a.m. – 11:00 a.m.	Deciphering the Role of Human Arylamine N-acetyltransferase 1 (NAT1) in Breast Cancer Cell Metabolism Using a Systems Biology Approach

PRESENTING AUTHOR: ***Samantha M. Carlisle, University of Louisville, United States***

CO-AUTHORS: ***Patrick J. Trainor, Mark A. Doll, Marcus W. Stepp, Carolyn M. Klinge, David W. Hein***

Human arylamine N-acetyltransferase 1 (NAT1) is a xenobiotic metabolizing enzyme found in almost all tissues. Recently, NAT1 also has been shown to hydrolyze acetyl-coenzyme A (acetyl-CoA). NAT1 expression varies inter-individually and is elevated in several cancers including estrogen receptor positive breast tumors, but the mechanism by which NAT1 affects cancer risk and progression remains unclear. In 2018, breast cancer is expected to account for 30% of all new cancer cases in US women. Small molecule inhibitors of NAT1 are under investigation for breast cancer prevention and defining NAT1's role in cellular metabolism is important to interpret these studies. We conducted metabolomics, transcriptomics, and bioenergetics analyses of MDA-MB-231 breast cancer cell lines constructed to have increased, decreased, and knockout NAT1 activity. The global metabolomic and transcriptomic profile of each cell line were shown to be distinctive in unbiased multivariable analyses. Integrated analyses of metabolomic and transcriptomic data showed amino acid, lipid, and fatty acid metabolism pathways were significantly enriched following manipulation of NAT1. Bioenergetics results indicated knockout of NAT1 may enhance adaptation to stress by increasing mitochondrial plasticity in response to energy demand. Analysis of the relationships between metabolite abundance and NAT1 N-acetylation activity suggest N-acetylasparagine, N-acetylputrescine, and saccharopine may be involved in reactions catalyzed by NAT1. Overall, NAT1 knockout had a much greater impact on cellular metabolism than overexpression of NAT1. These data suggest NAT1 plays a role in the regulation of cellular metabolism that may be important in carcinogenesis. Partially supported by USPHS grants T32-ES011564, R01-DK053220, P20-GM103436, and P20-GM106396.

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6C 11:00 a.m. – 11:15 a.m.	Omics in aquaculture: understanding the mechanisms of crustacean molt

PRESENTING AUTHOR: ***Elena Legrand, IMET - NIST, United States***

CO-AUTHORS: ***Elena Legrand, J. Sook Chung , Tracey Schock***

Crustacean growth requires numerous molting events to complete their life cycle from hatching to adulthood. In decapod crustaceans, two different types of hormones orchestrate this biological process: the inhibitory neuropeptides produced in eyestalk ganglia and ecdysteroids secreted by Y-organ. Importantly, the ecdysteroids in hemolymph are closely related to molt stage: low at intermolt and high at premolt. The exact mechanism that triggers the initial increase of ecdysteroids at early premolt is still unknown. This project aims to gain a better understanding of the molting process in the blue crab, Callinectes sapidus, an ecological and economically important species, by using transcriptomic and metabolomic analyses. Specifically, we aimed to identify the initial changes occurring between the intermolt animals and those entering the early premolt stage. To this end, the following experiments were set out to determine if there are differences between: 1) intermolt and premolt stages of prepubertal females and 2) intermolt of intact adult females and early premolt induced by a bilateral eyestalk ablated. Based on the transcriptomic and qPCR analysis, expression levels of Spook, Cyp49, and HR4 genes in the Y-organ are positively related to the circulating ecdysteroid concentrations. The NMR metabolomic profiles of the hemolymph shows significant differences between intermolt and premolt stages. The role of the most important features in the molting process will be discussed. Together, transcriptomic and metabolomics approachesoffer a better understanding of a crucial growth mechanism that may provide impact in the aquaculture industry and elsewhere.

6D 11:15 a.m. – 11:30 a.m.	The UK-PBC Nested Cohort Study. Metabolic Profiling for the elucidation of the molecular mechanisms involved in the UDCA response.

PRESENTING AUTHOR: ***Alexandros Pechlivanis, Imperial College London, Greece***

CO-AUTHORS: ***Alexandros Pechlivanis, Julie McDonald, Sofina Begun, Simon Taylor-Robinson, Julian Marchesi, George Mells, David Jones, Elaine Holmes***

Background: Primary biliary cholangitis (PBC) is a chronic, cholestatic liver disease that occurs in 1 in 1000 women above the age of 40 years. It is characterised by autoimmune destruction of the small, interlobular bile ducts. In patients with active disease, PBC may progress to end-stage liver disease with attendant need for liver transplantation (LT), rendering PBC a leading indication for LT in Europe and North America. The main medication licensed for treatment of PBC is ursodeoxycholic acid (UDCA). UDCA response is an important prognostic variable. Survival in UDCA responders is comparable to that of the general population, whereas LT-free survival in UDCA non-responders is substantially reduced. Likewise younger age-at-presentation and male sex are independent risk factors for UDCA non-response – and therefore progressive liver disease. Risk-stratification based on UDCA response is essential for management of PBC. However, there are no proven second-line therapies for UDCA non-responders at high risk of progressive liver disease. Furthermore, lack of data on mechanisms involved in UDCA response and robust measures to identify high-risk patients without 12 months of failed treatment are key points for the development of second-line therapies. Methodology and Approach: Serum (450), urine (590) and faecal (610) samples were analysed using NMR, ultra-performance liquid chromatography mass spectrometry (UPLC-MS) and microbiome 16S sequence. Statistical integration and analysis of these multiomic datasets is providing us with metabolic and mechanistic information for UDCA response in patients with PBC, leading to an earlier and more-accurate stratification based on disease-risk and treatment-responsiveness.

6E 11:30 a.m. – 11:45 a.m.	Describing metabolic reprogramming in a model for collective vs individual cancer invasion

PRESENTING AUTHOR: ***Maxwell Colonna, University of Georgia, United States***

CO-AUTHORS: ***Shaying Zhao, Arthur S. Edison***

Breast cancer is the 2nd deadliest cancer in the United States, affecting 1 in every 8 women throughout their lifetime. The primary manifestation of cancer progression is invasion into the surrounding tissue, and eventually, metastasis. Cancer cell invasion can be broadly categorized into two distinct modes: collective and individual. Collective invasions are characterized by tumor cells that are “sticky,” forming tight cell-cell junctions typical of epithelial cells, and infiltrating surrounding tissue as a solid mass. Individual invasions, on the other hand, are typically comprised of “loose,” mesenchymal-like cells with fewer cell-cell connections as individual cells migrate diffusely into surrounding tissue. The transition from epithelial to mesenchymal-like cells (EMT) is associated with reprogrammed metabolism. It has also been suggested that the causal relationship between metabolism and cell state is mutual. Characterizing these changes in metabolism at the systems level will be essential for understanding how metabolic status and cell state influence each other to determine the mode of cancer invasion. Here we present an experimental model for comparing these changes using divergent MCF-7 breast cancer cell lines with key characteristics of these two cancer states. Using integrated NMR based metabolomics and RNAseq transcriptomics, we identified significant differences in core energy metabolism consistent with previous findings. We also discovered significant differences in branched chain amino acid uptake, which, based on previous reports, may influence mitochondrial biogenesis and function. With this prototypical system, we hope to further identify and model the changes in metabolism that occur between these two cancer states.

SESSION 7: METABOLIC NETWORKS	Tuesday June 26	1:30 p.m. – 3:00 p.m.

7A KEYNOTE 1:30 p.m. – 2:00 p.m.	Metabolomics Activity Screening for Identifying Metabolites that Modulate Phenotype

PRESENTING AUTHOR: ***Gary Siuzdak, The Scripps Research Institute, United States***

Metabolomics is broadly acknowledged to be the omics discipline that is closest to the phenotype. Although appreciated for its role in biomarker discovery programs, metabolomics can also be used to identify metabolites that could alter a cell's or an organism's phenotype. Metabolomics activity screening (MAS) integrates metabolomics data with metabolic pathways and systems biology information, including proteomics and transcriptomics data, to produce a set of endogenous metabolites that can be tested for functionality in altering phenotypes. A growing literature reports the use of metabolites to modulate diverse processes, such as stem cell differentiation, oligodendrocyte maturation, insulin signaling, T-cell survival and macrophage immune responses. This opens up the possibility of identifying and applying metabolites to affect phenotypes. Unlike genes or proteins, metabolites are often readily available, which means that MAS is broadly amenable to high-throughput screening of virtually any biological system. Nature Biotechnology 2018

7B 2:00 p.m. – 2:15 p.m.	In silico study of metabolic rewiring during epithelial-mesenchymal transition

PRESENTING AUTHOR: ***Meztli L Matadamas-Guzman, UNAM-INMEGEN, Mexico***

CO-AUTHORS: ***Osbaldo Resendis-Antonio***

Epithelial to mesenchymal transition (EMT) relates to many molecular and cellular alterations that occur when epithelial cells undergo a switch in differentiation and generate mesenchymal-like cells with newly acquired migratory and invasive properties. Non-small cell lung cancer (NSCLC) cell lines have been broadly used to study this phenomenon at different levels, like metabolism. Many studies recognize metabolic rewiring as an essential factor during this biological process, which reports alterations in Krebs cycle, amino acid metabolism, among others. However global alterations in metabolism have been little explored and are valuable to understand this natural process. In order to identify a set of reactions altered in this transition, independently the genetic background, we scrutinized the landscape of modifications in the metabolism of three different NSCLC cell lines. To this end, we integrated metabolomic and transcriptional high throughput data with a mathematical model of central metabolism to predict enzymatic reactions which differ between epithelial and mesenchymal phenotypes. Our results show that the main affected reactions during EMT include Krebs cycle, alanine, glutamate, and glutathione metabolism. In particular, the alteration of glutathione metabolism joined with the differential expression of catalase and PMEPA1 genes, suggest that antioxidant barrier plays an essential role in EMT. Our holistic approach contributes to understanding extensive metabolic alterations needed in EMT, that further our comprehension of metabolism on cancer progression and metastasis.

7C 2:15 p.m. – 2:30 p.m.	Significant Metabolic Pathway Identification Based on Multi-Block Partial Least Squares Analysis

PRESENTING AUTHOR: ***Jiyang Dong, University of Washington, United States***

CO-AUTHORS: ***Lingli Deng, Kian-Kai Cheng, Haiwei Gu, Jiangjiang Zhu, Daniel Raftery***

Metabolic pathways define the series of biochemical reactions that convert molecules or substrates into different, more readily usable metabolites, and have important biological significance in metabolism. The identification of significant pathwaysis still an open problem in the field, and several approaches have been described such as sorting the topological importance of pathways in a metabolic network. However, topological importance is not equivalent to functional importance. A new method based on a multi-block partial least squares (MB-PLS) model is proposed here. In the current method, metabolites are divided into several sets according to their pathway affiliation, e.g. two metabolites are put into a same set if they belong to a same pathway defined by KEGG. Consequently, the concentration matrix is divided into several data blocks according to the metabolite sets, one block for each metabolite set. Then, a MB-PLS model is built on these data blocks. A parameter, named pathway importance in projection (PIP), is defined to evaluate the importance of a given pathway (data block) with respect to the groups' discrimination in the MB-PLS model. Performance of the proposed method was evaluated using a simulation dataset and a real metabolomic dataset from a colorectal cancer study. For the colorectal cancer dataset, the new method underscored five altered metabolic pathways, including glycolysis, arginine and proline metabolism, lysine degradation, glycine and serine metabolism, and fructose and mannose degradation. These results are consistent with published literature on altered cancer metabolism. The proposed method may facilitate the interpretation of metabolic changes in metabolomics.

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SESSION 7: METABOLIC NETWORKS	Tuesday June 26	1:30 p.m. – 3:00 p.m.

7D 2:30 p.m. – 2:45 p.m.	Inferring metabolite interactomes via Bayesian graphical model selection utilizing molecular structure informative priors

PRESENTING AUTHOR: ***Patrick J. Trainor, University of Louisville, United States***

CO-AUTHORS: ***Joshua M. Mitchell, Samantha M. Carlisle, Hunter N.B. Moseley, Shesh N. Rai, Andrew P. DeFilippis***

While the generation of reference genomes facilitates the elucidation of gene-phenome associations, reference models of the metabolome that are specific to organism, sample type (e.g. plasma, serum, urine, cell-culture), and state (including disease), remain uncommon. In studying heart disease in humans, a reference model describing the relationships between metabolites in plasma has not been determined but would have great utility as a reference for comparing acute disease states such as myocardial infarction. We present a methodology for deriving probabilistic models that describe the partial correlation structure of metabolite distributions ("interactomes") from un-targeted data. As determining partial correlation structures requires estimating p*(p-1)/2 parameters for p metabolites, the dimension of the search space for parameter values is immense. Consequently, we have developed a Bayesian methodology for the penalized estimation of model parameters in which the magnitude of penalization is drawn from probability distributions with hyperparameters linked to molecular structure similarity. In our work, structural similarity was determined as the Tanimoto coefficient of algorithmically generated atom colors that capture the local structure around each atom within each structure. A Gibbs sampler (a Markov chain Monte Carlo technique) was implemented for simulating the posterior distribution of model parameters. We have made software for implementing this methodology publicly available via the R package BayesianGLasso. We present simulation studies demonstrating the positive performance of the method in recovering the partial correlation structures utilized to generate simulated data. Finally, we present an interactome model for stable heart disease inferred from non-targeted mass spectrometry data via this methodology.

7E 2:45 p.m. – 3:00 p.m.	Data-driven Differential Network-based Enrichment Analysis (DNEA) for metabolomics and lipidomics data

PRESENTING AUTHOR: ***Alla Karnovsky, University of Michigan, United States***

CO-AUTHORS: ***Jing Ma, Farsad Afshinnia, Janis Wigginton, Sub Pennathur, George Michailidis***

A common approach to interpreting the results of metabolomics and lipidomics experiments is to map and visualize experimentally measured metabolites in the context of known biochemical pathways. A number of tools for performing this type of analysis have been developed including our own tool Metscape (http://metscape.med.umich.edu/). Some of the existing tools have adopted Functional Enrichment Testing methods developed for gene expression data for the analysis of metabolomics data. However, the scope of their application has been limited to known compounds from large, well-annotated pathways, which are often occupied by a small portion of the experimentally measured metabolome. An alternative to knowledge-based data analysis is to infer meaningful associations between metabolites/lipids from experimental data and build data-driven metabolic networks to help generate biological insights. We developed a new Differential Network Enrichment Analysis method (DNEA) that uses joint structural sparsity estimation to build partial correlation networks from the data (for two or more experimental conditions), performs consensus clustering to identify highly connected network components (subnetworks), and uses Network-based Gene Set Analysis (NetGSA) to identify the differentially enriched subnetworks. We will present the applications of DNEA for the analysis of metabolomics and lipidomics data and demonstrate that it allows to identify alterations in both network structure and expression levels of interacting biomolecules that impact disease phenotypes.

SESSION 8: PEDIATRICS	Tuesday June 26	1:30 p.m. – 3:00 p.m.

8A SESSION KEYNOTE 1:30 p.m. – 2:00 p.m.	Persistent alterations in plasma lipid profiles prior to first dietary exposure to gluten associates with progression to celiac disease during early infancy

PRESENTING AUTHOR: ***Matej Oresic, Örebro University, Sweden***

CO-AUTHORS: ***Partho Sen, Cecilia Carlsson, Suvi Virtanen, Tuulia Hyötyläinen, Jorma Toppari, Riitta Veijola, Heikki Hyöty, Jorma Ilonen, Mikael Knip***

Celiac disease (CD) is a chronic enteropathy characterized by an autoimmune reaction in the small intestine in genetically susceptible individuals. It is well known that gluten is the required environmental trigger of clinical CD, but the underlying causes for the autoimmune reaction are yet unknown. In order to elucidate the early events preceding the clinical disease, we applied global lipidomics profiling by UHPLC-MS in 228 plasma samples from the Type 1 Diabetes Prediction and Prevention study (n=23 CD progressors, n=23 controls) in a prospective series of children ranging from first exposure to gluten to recommended gluten free diet after diagnosed with CD (age groups of 0, 3, 4, 6, 18, 24, 36, 48 and 60 months). The lipidomic profiles revealed that children who later progressed to CD had increased triglycerides (TGs) of low carbon number and double bond count and decrease of phosphatidylcholines already at 4 months of age, i.e. prior to first exposure to gluten. The differences were exacerbated with age but were not observed at birth (cord blood). No differences were observed in dietary TGs such as those containing polyunsaturated fatty acids. The specific TGs found elevated in CD progressors may be due to a host response to compromised intake of dietary lipids in the small intestine, leading to the de novo synthesis of specific TGs. Our findings suggest that some of the features of clinical CD such as abnormal lipid absorption may occur already prior to the first exposure to gluten in the diet.

8B 2:00 p.m. – 2:15 p.m. | **Metabolic Signatures of Cystic Fibrosis Identified in Dried Blood Spots from Asymptomatic Neonates: Resolving Diagnostic Dilemmas in Newborn Screening**

PRESENTING AUTHOR: ***Philip Britz-McKibbin, McMaster University, Canada***

CO-AUTHORS: ***Alicia DiBattista, Nathan Macintosh, Monica Lamoureux, Osama Y. Al-Dirbashi, Pranesh Chakraborty***

Cystic fibrosis (CF) is a complex multi-organ disease that is among the most common life-shortening genetic disorders. Newborn screening (NBS) programs for early detection of CF rely on a two-stage immunoreactive trypsinogen and cystic fibrosis transmembrane conductance regulator (CFTR) mutation panel (IRT/DNA) algorithm that is sensitive, but not specific for early detection of CF neonates in the population. For the first time, we report the discovery of a panel of CF-specific metabolites from dried blood spot (DBS) specimens when using multisegment injection-capillary electrophoresis-mass spectrometry (MSI-CE-MS). This retrospective case-control study design identified 32 metabolites that were differentially expressed in asymptomatic and normal birthweight CF neonates without meconium ileus (n=36) as compared to age/sex-matched screen-negative controls (n=44) after a false discovery rate (FDR) adjustment ($q < 0.05$). Importantly, 16 metabolites from DBS extracts ($q < 0.05$, FDR) also allowed for discrimination of true CF neonates from screen-positivecarriers and transient hypertrysinogenemic cases (n=60) who are presumptive CF until confirmedas unaffected with low sweat chloride (< 29 mM). Notably, CF-specific biomarker candidates satisfying a Bonferroni adjustment ($p < 7.25$ E-5) included several amino acids (Thr, Ser, Pro, Tyr) likely reflecting protein maldigestion/malabsorption, ophthalmic acid as an indicator of severe glutathione depletion, as well as an unknown trivalent peptide that was sorrelated with IRT ($\rho = 0.332$, $p = 4.55E-4$). This work aims to improve the specificity of CF screening algorithms using existing MS/MS infrastructurein order to reduce false-positives and unaffected carrier identification due to detection of CFTR mutations of unknown consequence.

8C 2:15 p.m. – 2:30 p.m. | **The Association of Ursodeoxycholate and Other Secondary Bile Acids with Self-Reported Food Allergy and Oral Immunotherapy Outcomes**

PRESENTING AUTHOR: ***Yamini Virkud, Harvard University, United States***

CO-AUTHORS: ***Yamini V. Virkud, Rachel S. Kelly, Sarita U. Patil, Bert Ruiter, Neal Smith, Allie Hickey, Augusto Litonjua, Scott T. Weiss, Wayne Shreffler, Jessica A. Lasky-Su***

Rationale: Metabolomics studies of food allergy in humans remain limited, and there are no studies of metabolic profiles in investigational therapies for food allergy, such as oral immunotherapy (OIT). Secondary bile acids, including ursodeoxycholate, are being studied as a potential treatment for a variety of immune-mediated diseases. Methods: We examined serum metabolomics from a healthy infant cohort recruited for the VDAART prenatal vitamin D trial (N=238, 25 with self-reported food allergy), a Costa Rican pediatric asthma cohort (N=281, 38 with self-reported food allergy), and a clinical interventional peanut OIT trial (N=20) with a post-treatment one-month avoidance period to test longer-lasting efficacy (LE) versus only transient efficacy (TE). We employed logistic regression to identify metabolites associated with food allergy, and longitudinal Bayesian analysis to examine metabolite trends during OIT. Results: In the infant cohort, secondary bile acids represented 4 of the top 8 metabolites increased in food allergy, including ursodeoxycholate (OR:4.51, p=0.0009). In our asthma cohort, we also found an association between food allergy and other secondary bile acids, deoxycholate (p=0.009) and glycolithocholate (p=0.003). Baseline ursodeoxycholate levels were higher among those with TE compared to LE (p=0.002), but converged between the groups over the course of OIT. Conclusions: In contrast with work investigating ursodeoxycholate as a therapy for immune-mediated diseases, we found ursodeoxycholate was increased in food-allergic subjects and the more severe subgroup of food-allergic patients who fail to develop longer-lasting protection on OIT. Future studies may assess the utility of ursodeoxycholate as a biomarker of food allergy and OIT response.

8D 2:30 p.m. – 2:45 p.m. | **Using Metabolomics to Predict Treatment Response in a Pediatric Asthma Population**

PRESENTING AUTHOR: ***Nichole Reisdorph, University of Colorado Anschutz Medical Campus, United States***

CO-AUTHORS: ***Scott Walmsley, Stanley Szefler, Anne Fitzpatrick, Kevin Quinn, Yasmeen Nkrumah-Elie, Rick Reisdorph, Dave Mauger, Dan Jackson, Dominik Reinhold***

Asthma is a complex disease, with multiple phenotypes including allergic, obesity-related, and exercise-induced asthma. Because individuals respond differently to medication, physicians must rely on step-wise treatments, whereby decisions are based solely on an individual's response. This results in an extended period of trial and error, during which time patients are at risk for serious exacerbations. The goal of our research is to determine if small molecules can be used to predict response to asthma medication. We used unbiased metabolomics profiling of urine and plasma from 230 pediatric asthma patients to address this question. The study was designed as a cross-over study where patients were placed on 3 different medications, inhaled corticosteroid (ICS), short acting beta agonists (SABA), or leukotriene receptor antagonist therapy (LTRA), for 16 weeks each. The main clinical outcome tested was: Did the child have a differential response to the drugs and if so, which drug proved superior in relieving symptoms? A variety of informatics strategies, including o-PLS-DA, PCA, hierarchical clustering, and logistic regression were used to analyze data, followed by validation. While analysis of the entire cohort resulted in failed models, a strategy that included Coursened Exact Matching and Gowers clustering to discover balanced patient subsets was successful. Area under the curve (AUC) reached 0.93 for predicting response to SABA when a panel of metabolites was used in conjunction with clinical data. These findings support the use of small molecules to predict response to medication. Future work includea testing these markers in a larger population.

SESSION 8: PEDIATRICS	Tuesday June 26	1:30 p.m. – 3:00 p.m.

8E 2:45 p.m. – 3:00 p.m.	Metabolomics of Childhood Exposure to Perfluorooctanoic Acid: A Cross-Sectional Study

PRESENTING AUTHOR: ***Samantha L Kingsley, Brown University School of Public Health, United States***

CO-AUTHORS: ***Samantha L. Kingsley, Douglas I. Walker, Aimin Chen, Antonia M. Calafat, Bruce P. Lanphear, Kimberly Yolton, Kurt D. Pennell, Joseph M. Braun***

Background: Exposure to perfluorooctanoic acid (PFOA), a synthetic and persistent chemical used in commercial and industrial processes, is associated with reduced birth weight, increased adiposity, dyslipidemia, and liver injury. These diverse toxic effects suggest that PFOA elicits multiple biological responses. Identifying the metabolic changes induced by PFOA exposure could enhance our understanding of potential biological mechanisms. Objective: To identify metabolic changes associated with serum PFOA concentrations in 8-year old children using a metabolome-wide association study (MWAS). Methods: Using venous blood samples collected from 120 8-year old children in Cincinnati, OH, we quantified serum PFOA concentrations and performed untargeted metabolomic profiling by liquid chromatography-high-resolution mass spectrometry. We evaluated metabolic variations associated with PFOA concentrations using linear regression and false discovery rate (FDR) <20%. We identified associated metabolites with the METLIN mass spectral database and metabolic pathway enrichment analysis. Results: At FDR<20%, higher serum PFOA concentrations were associated with 239 detected chemical signals; 130 were positive associations. Initial annotation of the mass spectral data showed that both endogenous metabolites and perfluoroalkyl chemicals were associated with PFOA. Using untargeted metabolomics, we detected PFOA, perfluorooctanesulfonic acid, and additional signals consistent with fluorinated chemicals. Biological alterations associated with PFOA included keratin sulfate degradation and metabolism of purine, caffeine, Vitamin E, linoleate, urea cycle/amino groups, glyoxylate, dicarboxylate, and galactose, consistent with changes to immunological, oxidative stress and catabolism pathways. Conclusions: This is the first study to perform a MWAS with PFOA exposure in children and provides new insights into the biological responses associated with PFOA exposure.

SESSION 9: MICROBIOME & METABOLOMICS I	Tuesday June 26	1:30 p.m. – 3:00 p.m.

9A SESSION KEYNOTE 1:30 p.m. – 2:00 p.m.	Gut Microbiome Liver Brain Axis in Alzheimer Disease

PRESENTING AUTHOR: ***Rima Kaddurah-Daouk, Duke University Medical Center, United States***

CO-AUTHORS: ***Siamak MahmoudianDehkordi, Ph.D, Matthias Arnold, Ph.D., Kwangsik Nho, Ph.D, Shahzad Ahmad, M.S., Wei Jia, Ph.D., Guoxiang Xie, Gregory Louie, M.S., Alexandra Kueider-Paisley, Ph.D., M. Arthur Moseley, Ph.D., J. Will Thompson, Ph.D., Lisa St John Williams, M.E., Jessica D. Tenenbaum, Ph.D., Colette Blach, M.S., Rebecca Baillie, Ph.D., Xianlin Han, Ph.D., Sudeepa Bhattacharyya, Ph.D., Jon B. Toledo, M.D., Simon Schafferer, Ph.D., Sebastian Klein, Therese Koal, Ph.D., Shannon L. Risacher, Ph.D., Mitchel Allan Kling, M.D., Alison Motsinger-Reif, Ph.D., Daniel M. Rotroff, Ph.D., John Jack, Ph.D., Thomas Hankemeier, Ph.D., David A. Bennett, Ph.D., Philip L. De Jager, Ph.D., John Q. Trojanowski, M.D., Ph.D., Leslie M. Shaw, Ph.D., Michael W. Weiner, M.D., P. Murali Doraiswamy, M.B.B.S., Cornelia M. van Duijn, Ph.D., Andrew J. Saykin, Psy.D., Gabi Kastenmüller, Ph.D., Rima Kaddurah-Daouk, Ph.D.***

Increasing evidence suggests a role for the gut microbiome in central nervous system disorders and specific role for the gut-brain axis in neurodegeneration. Bile acids (BA), products of cholesterol metabolism and clearance, are produced in the liver and are further metabolized by gut bacteria. They have major regulatory and signaling functions and seem dysregulated in Alzheimer disease (AD). Serum levels of 15 primary and secondary BAs and their conjugated forms were measured in 1,464 subjects enrolled in the AD Neuroimaging Initiative study. In AD compared to CN, we observed significantly lower serum concentrations of a primary BAs and increased levels of the bacterially produced secondary BAs and their glycine and taurine conjugated forms pointing to gut dysbiosis and changes in bacterial 7 alpha -dehydroxylation activity. Changes in BA profile strongly associated with cognitive decline; brain imaging changes including abeta tau pathology, brain glucose metabolism and atrophy. Key findings replicated in serum and brain samples (600 brain/100 brains) in the Rush Religious Orders and Memory and Aging Project. Several genetic variants in immune response related genes implicated in AD showed associations with altered BA profiles. These findings suggest a possible role of gut liver brain axis in the pathogenesis of AD.

9B 2:00 p.m. – 2:15 p.m.	Metabolic response of a fungal extremophile to growing in microgravity on the International Space Station.

PRESENTING AUTHOR: ***Heino Martin Heyman, Pacific Northwest National Laboratory, United States***

CO-AUTHORS: ***Jennifer E. Kyle, Morgan S. Sobol, Tatsuhiko Hoshino, Fumio Inagaki, Elizabeth Eder, Carrie D. Nicora1, David W. Hoyt, Malak M. Tfaily, Brandi Kiel Reese, Thomas O. Metz***

The aim of our study was the implementation of an untargeted metabolomics approach to investigate the impact that eco-physiological adaptation has on marine fungi isolated from the deep subsurface sediments from the South Pacific Gyre (SPG). Marine fungi have been studied extensively for more than 50 years, but our knowledge of the fungal life within the deep marine subsurface is still lacking, particularly when it comes to metabolism. Fungi, and especially fungi surviving in atypical environments are known to have an ability to utilize non-traditional nutrient resources. They effectively convert non-bioavailable energy sources into accessible organic carbon reserves, mutually benefitting closely associated heterotrophic microbes as well as equipping the fungi with vital defense compounds. In the current study, unique representative isolates from the SPG subsurface were subjected to growth experiments on the International Space Station to better understand how these fungi have adapted to survive in extreme environments. Comprehensive analysis of the genome, lipidome and the metabolome were carried out to obtain a complete picture of the metabolic response of these fungi to survival under abnormal conditions. By utilizing several metabolomics platforms, including NMR (50 metabolites), GC-MS (110 polar metabolites), LC-FTICR MS (3000+ features) and LC-MS/MS (300+ unique lipids), our findings showed clear metabolic changes due to the growth in a microgravity environment, as well as to how these fungi manage their metabolism to survive and even thrive under extreme environmental pressures. Together with the genomic discoveries, we have made significant strides to better understand these fascinating extremophiles.

9C 2:15 p.m. – 2:30 p.m. | **Metabolic Profiling of Circulating Gut Microbial Metabolites and Host Inflammatory Status**

PRESENTING AUTHOR: ***Zdenek Spacil, Masaryk University, Faculty of Science, Czechia***

CO-AUTHORS: ***Zdenek Spacil, Veronika Vidova, Eliska Cechova, Tereza Pavlova, Julie Bienertova-Vasku, Marian Kacerovsky, Jana Klanova, Vojtech Thon***

The gut microbiota produces a number of metabolites interacting with the human host's metabolic pathways and resulting in systemic effects. In the last decade, the gut microbiota has been linked to health conditions, frequently related to inflammatory status. However, little is known about specific microbial metabolites and their concentrations in the bloodstream or other biological materials. Such data is required to explore functions of circulating bacterial metabolites in the host-bacterial interaction and to attribute them to relevant metagenome. We have used untargeted metabolic profiling by HR/AM-MS (Orbitrap Fusion), followed up by targeted SRM-MS/MS to quantify a panel of microbial metabolites (e.g. metabolism of tryptophan, short chain fatty acids or bile acids) in body fluids (i.e. human serum and urine), some conditional to gestation (i.e. umbilical cord blood, amniotic fluid). For instance concentration levels of indole-3-propionic acid (IPA), a well-established marker of colonization, were readily detectable in the bloodstream and amniotic fluid, but virtually absent in urine. This may suggest an active regulation of IPA distribution within human body. In amniotic fluid we have discovered that IPA levels are significantly correlated with a certain type of colonization. Apart from IPA distribution we have explored additional 15 microbial metabolites. To characterize inflammatory status and to investigate immunomodulatory health effects we have developed assays for quantification of acute phase proteins and adaptive immunity effectors. This study combines multi-omics data to explore circulating metabolites of gut microbiota and to elucidate the host-bacterial interaction.

9D 2:30 p.m. – 2:45 p.m. | **Evaluation of Adverse Outcomes of Bone Marrow Transplant in Humans Using NMR-Based Metabolomics and Metagenomics**

PRESENTING AUTHOR: ***Lindsey E Romick-Rosendale, Cincinnati Children's Hospital/University of Cincinnati, United States***

CO-AUTHORS: ***David Haslam, Adam Lane, Kelly E Lake, Miki Watanabe, Stuart Bauer, Bridget Litts, Nathan Luebbering, Christopher E Dandoy, Stella M Davies***

Acute graft-versus-host disease (GVHD) following hematopoietic stem cell transplant (HSCT) is associated with loss of diversity of the gut microbiome; moreover antibiotics increase risk of GVHD. We hypothesized that short chain fatty acids would decline during the course of HSCT, and that this decline would be associated with important changes in the gut microbiota. The loss of intestinal commensals that produce SCFAwere hypothesized to increase dysbiosis and mediate increased risk of GVHD. We tested our hypothesis using weekly stool, blood and urine samples from 42 children receiving HSCT. Nuclear magnetic resonance (NMR)-based metabolomics was used to assign and quantify metabolites. 16S sequencing of stool identified changes in the gut microbiome. Fecal butyrate and propionate levels were significantly decreased at days 7 and 14 compared to baseline fecal SCFA levels, and were associated with higher total exposure to anaerobic antibiotics. Formate levels increased significantly post-HSCT and were associated with higher levels ofEnterobacteriaceaein the gut, likely increasing risk of bacterial translocation. The key SCFAs in the stool at Day 14 after HSCT were consistently reduced in those individuals that went on to develop GVHD. Additionally, a number of host-derived metabolites were found to be significantly altered following transplant, and more specifically in those individuals that went on to develop GVHD. These data demonstrate a mechanism for prior observations that loss of diversity and increased antibiotic use are associated with GVHD and offer potential modifiable targets in metabolic pathways to reduce risk of GVHD and improve survival after HSCT.

9E 2:45 p.m. – 3:00 p.m. | **Novel metabonomic biomarkers of early-life enteric infections and growth: models of environmental enteropathy and field validation**

PRESENTING AUTHOR: ***Natasa Giallourou, Imperial College London, United Kingdom***

CO-AUTHORS: ***Fahmina Fardus-Reid, Jordi Mayneris-Perxachs, Gregory Medlock, Pedro Quintela, David Bolick, Richard Guerrant, Jonathan Swann***

A fifth of the world's population lives under extreme poverty where safe water, food and sanitation are scarce allowing for a vicious cycle of diseases. Early-life malnutrition and enteric infections are the major causes of diarrhoea in children from developing countries, leading to growth shortfalls increasing the risk for cognitive impairment and predispose a child for cardiometabolic diseases later in life. In this study urinary metabolic profiles of children from Peru (n=1058), Tanzania (n=506) and Bangladesh (n=860) participating in the multisite birth cohort study (MAL-ED) were obtained using 1H NMR spectroscopy and were associated with metrics of nutritional status, enteropathogen load and data from biomarkers of intestinal function.Additionally mouse models of the most prevalent enteropathogens in the cohort study have been developed through dietary and microbial modifications, followed by metabolome and microbiome characterisation. Results reveal distinct metabolic phenotypes associated with poor growth measures and with infection status. Choline and tryptophan metabolism were significantly modulated additionally tothe activity of the gut microbiome, which was suggestive of proteolysis in undernourished and infected children. Additionally, energy expenditure appears to be tightly regulated in affected children reflected in shifts of nicotinamide metabolism. The developed mouse models of infection recapitulate features of enteropathy like growth faltering, inflammation, intestinal damage and biochemical changes. Collectively, these results provide insights into the biochemical mechanisms associated with environmental enteropathy and the multitude of developmental and metabolic perturbations observed in affected children. They also present novel enabling tools and models that support the development of interventions for enteropathy.

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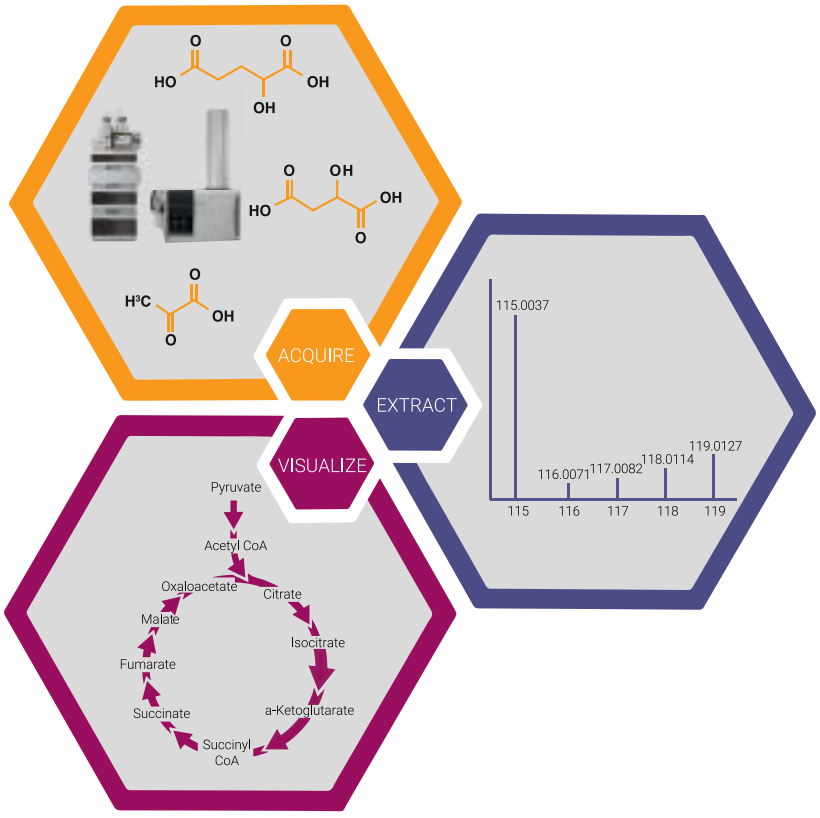

SESSION 10: OBESITY & METABOLIC SYNDROME	Tuesday June 26	3:30 p.m. – 5:15 p.m.

10A 3:30 p.m. – 3:45 p.m.	Chronic palmitate-induced lipotoxicity stimulates phosphatidylcholine remodelling and bioactive eicosanoid synthesis in mouse and human skeletal muscle to regulate inflammatory and stress signalling

PRESENTING AUTHOR: ***Ben D McNally, University of Cambridge, United Kingdom***

CO-AUTHORS: ***Steven A Murfitt, Zoe Hall, Christine Hinz, Jack Garnham, Klaus Witte, Julian L Griffin, Lee D Roberts***

Obesity and type 2 diabetes (T2DM) are increasing burdens on global health. Lipotoxicity-induced endoplasmic reticulum (ER) stress has emerged as a potential mechanism contributing to the pathologies of these diseases, disrupting systemic insulin signalling and lipid homeostasis. Palmitate, the predominant saturated free fatty acid in blood plasma, increases in obesity and T2DM and induces ER stress and insulin resistance in skeletal muscle, a major site of insulin-mediated glucose uptake. We find that chronic treatment of both mouse C2C12 and human primary myotubes with physiologically-relevant 200 μM palmitate induced ER stress, concurrent with perturbations in polyunsaturated fatty acid (PUFA) metabolism. Changes in PUFAs correlated with release of PUFA from phosphatidylcholines (PCs), measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Skeletal muscle from both mouse models of high fat diet-induced obesity and T2DM patients reflect the phospholipid remodelling observed in vitro. Using pharmacological inhibitors, palmitate-induced PC remodelling in skeletal myocytes was found to be dependent on cytosolic phospholipase A2 (cPLA2), an enzyme important for the release of free PUFAs from phospholipids for the synthesis of eicosanoids. A targeted LC-MS/MS method demonstrated an increase in eicosanoid secretion from mouse myotubes in response to chronic palmitate exposure, correlating with an increase in macrophage activation. Inhibition of eicosanoid-generating lipoxygenase (LOX) enzymes highlighted a role for 15-LOX-generated eicosanoids in the control of CCL2 and IL6 expression, and 12-LOX in the suppression of ER stress. In summary, we show that palmitate activates skeletal muscle-derived eicosanoid release, regulating inflammatory and stress signalling, and macrophage activation.

10B 3:45 p.m. – 4:00 p.m.	Turning up the heat – Investigating metabolic fluxes in adipocyte thermogenesis to guide therapeutic intervention

PRESENTING AUTHOR: ***Fynn Niclas Krause, University of Cambridge, United Kingdom***

CO-AUTHORS: ***Graeme Davies, James Dodgson, Christopher Church, Jules Griffin***

Amidst the globally increasing prevalence of obesity, increasing energy expenditure via enhanced thermogenesis in brown and beige adipose tissue offers to be a promising approach for therapeutic intervention. Recently, many diverse browning agents have been reported to enhance metabolic activity including UCP-1 dependent and independent mitochondrial metabolism, protect against weight gain and improve overall metabolic health. However, while many of these agents show major effects in mice, in humans this often poorly translates beyond the gene expression level, and little is known about the ultimate metabolic changes that are being induced. In this project, we aim to gain further understanding of the effect of a diverse panel of browning agents on adipocyte metabolism – do browning agents with different effects on gene expression induce different changes in metabolism, and if so, can this be exploited to tune the therapeutic effects of adipocyte thermogenesis? Here, we used a human adipocyte cell line to screen a diverse selection of 25 published browning agents, including small-molecule drugs, metabolites and peptides, for changes in expressionacross 52 genes involved in adipose tissue metabolism and regulation. Functional characterisation of these cells included extracellular flux analysis, high-content screening and metabolic flux analysis. We used RNAseq data to extract a cell-specific metabolic model from Recon2.2, and are now preparing 13C-assisted metabolic flux analysis to characterise differences in metabolic fluxes. From this, we hope to be able to identify the flux patterns that underline their therapeutic effect, as well as potential additive or synergistic relationships between browning agents.

10C 4:00 p.m. – 4:15 p.m.	Analysis of Serum Changes in Response to a High Fat Diet Challenge Reveals Metabolic Biomarkers of Atherosclerosis

PRESENTING AUTHOR: ***Biswapriya Biswavas Misra, Center for Precision Medicine; Texas Biomedical Research Institute, United States***

CO-AUTHORS: ***Laura A. Cox, Michael Olivier***

Atherosclerotic plaques are characterized by an accumulation of macrophages, lipids, smooth muscle cells, and fibroblasts, and, in advanced stages, necrotic debris within the arterial walls. Dietary habits such as high fat and high cholesterol (HFHC) consumption are known risk factors for the development and progression of atherosclerosis. However, the key metabolic contributors to diet-induced atherosclerosis are far from established. We used a non-human primate (NHP) model of atherosclerosis (baboons, n=60) fed HFHC diet for two years, and compared baseline with challenge diet metabolomic profiles in serum. We analyzed samples using a two-dimensional gas chromatography time-of-flight mass-spectrometer (2D GC-ToF-MS) after derivatization for untargeted metabolomic analysis. In addition, several clinical biomarkers associated with atherosclerosis, fat indices such as liver and visceral fat, and arterial plaques were quantified. The metabolomic analysis using two different derivatization reactions allowed detection of 515 tentatively identified and quantified metabolites belonging to 66 different KEGG-based metabolic pathways. Interestingly, 13 of these HFHC diet fed baboons showed very distinct metabolic phenotypes, strongly correlated with high triglyceride levels, which differed significantly from all other baboons. Levels of metabolites such as palmitic acid, and dietary chemicals were associated with sex. In addition, a two-time point sampling strategy revealed diet-induced metabolic differences after HFHC feeding and revealed individual qualitative and quantitative metabolic differences in a robust manner. This study highlights the feasibility of detecting metabolomics differences in NHPs with diet-induced atherosclerosis, and supports the application of this approach to identify metabolomic signatures associated with atherosclerotic disease progression in NHP and human patients.

10D 4:15 p.m. – 4:30 p.m. **A multi-tissue metabolomics study of metabolic response to bariatric surgery**

PRESENTING AUTHOR: ***Zhanxuan Wu, Food and Bio-based Products, AgResearch Limited, New Zealand***

CO-AUTHORS: ***Karl Fraser, Marlena Kruger, Garth JS Cooper, Ivana R Sequeira, Anne-Thea McGill, Sally D Poppitt***

Bariatric surgery is the most effective treatment for morbid obesity, resulting in significant body weight loss and decreased cardiovascular disease and type 2 diabetes risk. Bariatric surgery-induced alterations of local metabolism in key organ and tissue sites which may underpin these improvements is not known. In this study, a liquid chromatography-mass spectrometry (LC-MS) based multi-tissue global metabolomics analysis was conducted. Subcutaneous abdominal adipose tissue (SAA), subcutaneous thigh adipose tissue (STA) and vastus lateralis (VL, thigh) muscle collected from 28 morbidly obese females at surgery (t1) and at 6-month follow-up (t2) were analysed for polar metabolite and lipid changes. All Partial Least Squares-Discriminant Analysis (PLS-DA) models differed between t1 and t2 ($p<0.05$), except VL muscle lipids. Discriminatory features were selected based on their VIP score denoting contribution to group separation from significant models. A total of 49, 19 and 93 annotated polar metabolites discriminated between t1 and t2 in SAA, STA and VL respectively. Pathway analysis suggested altered metabolism of amino acids, nucleotides, carbohydrates, vitamins and energy in both VL muscle and SAA tissues, whereas amino acid metabolism was the major change in STA tissue. The pattern of lipid changes suggested decreased phospholipids and sphingomyelins and a mixed effect on glycerolipids in SAA, whilst discriminatory lipids in STA were almost exclusively increased after surgery. Collectively, our result suggested that most tissues undergo marked metabolic changes and that different adipose tissue regions have very different metabolic responses. This possibly reflects region specific catabolic rates or mechanisms related to bariatric surgery-induced weight loss.

10E 4:30 p.m. – 4:45 p.m. **Multiplatform Metabolomics Investigation of Anti-Adipogenic Effects on 3T3-L1 Adipocytes by a Potent Diarylheptanoid**

PRESENTING AUTHOR: ***Dan Du, West China Hospital, Sichuan University, China***

CO-AUTHORS: ***Dan Du, Haiwei Gu, Danijel Djukovic, Lisa Bettcher, Meng Gong, Wen Zheng, Liqiang Hu, Daniel Raftery***

Introduction Obesity is fast becoming a serious health problem worldwide. Of the many possible anti-obesity strategies, one interesting approach focuses on blocking adipocyte differentiation and lipid accumulation to counteract the rise in fat storage. However, there is currently no drug available for the treatment of obesity that works by inhibiting adipocyte differentiation. Methods and Results Here we use a broad-based metabolomics approach to interrogate and better understand metabolic changes that occur during adipocyte differentiation. In particular, we focus on changes induced by the anti-adipogenic diarylheptanoid, which was isolated from a traditional Chinese medicine Dioscorea zingiberensis and identified as (3R,5R)-3,5-dihydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)-heptane (1). Targeted aqueous metabolic profiling indicated that a total of 14 metabolites involved in the TCA cycle, glycolysis, amino acid metabolism, and purine catabolism participate in regulating energy metabolism, lipogenesis, and lipolysis in adipocyte differentiation and can be modulated by diarylheptanoid 1. As indicated by lipidomics analysis, diarylheptanoid 1 restored the quantity and degree of unsaturation of long chain free fatty acids, and restored the levels of 171 lipids mainly from 10 lipid species in adipocytes. In addition, carbohydrate metabolism in diarylheptanoid 1 treated adipocytes further demonstrated the delayed differentiation process by flux analysis. Novel Aspect Our results provide valuable information for further understanding the metabolic adjustment in adipocytes subjected to diarylheptanoid 1 treatment. Moreover, this study offers new insight into developing anti-adipogenic leading compounds based on metabolomics.

10F 4:45 p.m. – 5:00 p.m. **Comprehensive Lipidomics Analysis of Plasma Lipid Changes upon Bariatric Surgery using the Lipidyzer Platform: an IMI DIRECT Study**

PRESENTING AUTHOR: ***Mark Haid, Helmholtz Zentrum Muenchen - German Research Center for Environmental Health, Germany***

CO-AUTHORS: ***Cornelia Prehn, Silke Becker, Violeta Raverdy, Dietrich Merkel, Jörg Dojahn, Francois Pattou, Jerzy Adamksi, on behalf of the DIRECT consortium***

Extreme obesity is one of the major risk factors for the development of metabolic disorders like the metabolic syndrome or type 2 diabetes (T2D). For extreme obese patients (BMI > 40 kgm-2), the Roux-en-Y gastric bypass is a well-established method supporting long-term weight reduction and improved health status. It is known that shortly after the bariatric surgery many T2D relevant glycaemic parameters like insulin sensitivity and fasting glucose levels improve significantly and well before weight reduction. This indicates that the effect is not based on weight loss only. In order to investigate how gastric bypass surgery effects levels of plasma lipids, we performed a targeted lipidomics analysis using the Sciex LipidyzerTM platform. We quantified the levels of ~900 molecular lipid species (NEFAs, PCs, PEs, DAGs, TAGs, cholesteryl ester, sphingolipids, ceramides, cerebrosides) in 16 EDTA plasma samples of eight patients that presented full T2D remission as defined by HbA1c levels < 6.5 %. Differential analysis was conducted with plasma collected pre and post Roux-en-Y gastric bypass surgery. Generally, we observed significant reduction of plasma levels for cholesteryl esters, DAGs, TAGs, PCs, and PEs while levels of hexosyl- and lactosylceramides increased significantly. Furthermore, we detected fatty acid chain dependent changes within each lipid class. We applied random forest, partial-least-squares discriminant, and elastic net analyses to reveal those lipids that were most strongly associated with the discrimination between pre- and post-surgery samples. The top 25 metabolites mutually detected by the three methods comprise some TAGs, PCs, and cerebrosides containing nervonic acid.

11A 3:30 p.m. – 3:45 p.m. **Metabolomics of dragonfly nymphs exposed to multiple stressors: The effects of oil sands development and municipal wastewater**

PRESENTING AUTHOR: ***Robert B. Brua, Environment and Climate Change Canada, Canada***

CO-AUTHORS: ***Sarah M. McKenzie, Joseph M. Culp***

The Canadian oil sands (OS) region in northeastern Alberta is one of the largest oil deposits in the world. The Athabasca River, which flows through this region, receives heavy metals and polycyclic aromatic compounds from natural erosion of oil-containing sands as well as from industrial oil extractionfrom bitumen. In addition, spatial co-occurrence with OS development is the discharge of treated municipal sewage effluent (MSE) from Fort McMurray into the Athabasca River, which further complicates ecological bioassessment in this region. Simultaneous exposure to nutrients and contaminants can produce subsidy-stress gradients that have the potential to mask chronic, low level contaminant effects. The objective of this in situ experiment was to use NMR-based metabolomics of caged, transplanted dragonfly nymphs to investigate the multiple stressor effects of MSE and OS activities on the health of the Athabasca River. We transferred caged dragonfly nymphs from reference areas to five other areas to create six distinct exposure areas of the Athabasca River: 1) reference area outside OS; 2) reference area outside OS, but exposed to MSE; 3) reference area inside OS; 4) an area within OS exposed to MSE, but out of OS development; 5) an area within OS development; and 6) an area far downstream of OS development. There was high survival of dragonfly nymphs among all sites. Metabolomic results will be presented and discussed in relation to exposure to environmental stressors. These results will help elucidate the sublethal effects of multiple environmental stressors on the ecosystem health of the Athabasca River.

11B 3:45 p.m. – 4:00 p.m. **Sex, diatoms, and biofilms: bacterial modulation of sexual reproduction of the marine diatom Seminavis robusta**

PRESENTING AUTHOR: ***Emilio Cirri, Friedrich Schiller University Jena, Germany***

CO-AUTHORS: ***Sam De Decker, Katerina Pargana, Wim Vyverman, Georg Pohnert***

Diatoms are important microalgae that shape biofilm communities. Signal molecules mediate their life cycle and mating, but only recently sex pheromones of diatoms were identified in the benthic diatom Seminavis robusta. Sexual inducing pheromones (SIP) arrest the mitotic cell cycle, while a proline-derived diketopiperazine (diproline) drives the chemoattraction of the mating partners. Since such a pheromone based communication is a potential target for competing or pathogenic organisms, we asked if co-occurring species might interfere with the diatoms´ chemical communication and affect diatoms´ reproductive success. Therefore, we studied the pheromone chemistry and behavioral response of S. robusta both in presence and absence of bacteria. Bioassays with different naturally co-occurring bacteria, quantitative target analysis to check the production and degradation of the pheromone diproline, and an untargeted metabolomics and transcriptomics approach were used to characterize this interaction. We found that different bacteria have different effects on the mating success of S. robusta, as well as on the concentration of diproline, which is always higher in axenic conditions. Comparative untargeted metabolomics allowed us to survey the diatom chemistry, while transcriptomics highlighted up- and downregulated biochemical pathways in presence of bacteria. Combining these two techniques, we found that bacteria triggered oxylipins production in diatoms as a reaction to stress conditions. We also found up- and downregulated compounds characterizing different treatments.

11C 4:00 p.m. – 4:15 p.m. **Identifying metabolic alterations associated with coral growth anomalies in Porites compressa using 1H NMR metabolomics**

PRESENTING AUTHOR: ***Erik Roland Andersson, University of Charleston, United States***

CO-AUTHORS: ***Paul Anderson, Thierry M. Work, Cheryl M. Woodley, Russell D. Day, and Tracey B. Schock***

Coral growth anomalies (GAs) are a coral disease characterized by localized irregular growth of the coral skeleton and soft tissues, resulting in an abnormal protuberant mass on a coral colony. Although GAs have been characterized in multiple coral species, the mechanisms responsible for the disruption of the skeletal morphology and associated impacts to coral soft tissues remain unknown. Paired fragments comprising lesion (GA) and healthy Porites compressa were collected (n=15) from Coconut Island (Oahu, Hawaii) where prevalence of GAs is high. A modified Bligh and Dyer extraction method demonstrated reproducible 1H NMR spectra for P. compressa, and principal component analysis revealed observable distinctions between GA and healthy samples. Complementary heteronuclear single quantum correlation (HSQC) data allowed for the identification of 26 metabolites – nine of which were significant for discriminating between GA and healthy samples. Interestingly, betaine was elevated and dimethylglycine was depressed in GA relative to healthy samples. These findings indicate a disruption of choline metabolism and possibly a reduced potential for DNA methylation in GA tissues. These results support the classification of coral GAs as true tumors. Ultimately, the current study provides a baseline for the use of metabolomics to study coral diseases, and identifies a specific metabolic pathway altered in GAs which should be the target of future studies in order to further investigate its role in GA formation.

11D **4:15 p.m. – 4:30 p.m.**	**In utero and lactational exposure to BDE-47 promotes obesity development in mouse offspring fed a high-fat diet: impaired lipid metabolism and intestinal dysbiosis**

PRESENTING AUTHOR: ***Wentao Zhu, China Agricultural University, China***

In this study, we investigated the effects of in utero and lactational exposure to BDE-47 on the progression of obesity and metabolic dysfunction in a diet-induced obesity model. Pregnant ICR mice were treated via oral gavage with low doses of BDE-47 (0, 0.002, and 0.2 mg/kg body weight) from gestational day 6 to postnatal day 21. After weaning, male offspring were fed an AIN93-based normal diet (ND) or high-fat diet (HFD: 60% calories from fat) for 14 weeks. We examined body weight, liver weight, histopathology, blood biochemistry, gene expression, and serum metabolic changes. A combination of 16S rRNA gene sequencing and 1H NMR-based metabolomics was conducted to examine the effects of BDE-47 on the gut microbiome. Results showed that in utero and lactational exposure to BDE-47 caused a worsening of HFD-induced obesity, hepatic steatosis, and injury; impaired glucose homeostasis and metabolic dysfunction, and mRNA levels of genes involved in lipid metabolism were significantly altered in the BDE-47-treated HFD group. The gut microbiome were perturbed by BDE-47, causing diversity reduction, compositional alteration, and metabolic changes. These changes were more pronounced for BDE-47-treated HFD mice. All these results indicate that early life exposure to low doses of BDE-47 can promote obesity and the development of metabolic dysfunction.

11E 4:30 p.m. – 4:45 p.m.	**Metabolite and lipid profiling of blow samples from Arctic beluga whales**

PRESENTING AUTHOR: ***Pim Leonards, VU University, Netherlands***

CO-AUTHORS: ***Pim Leonards, Christian Lydersen, Kit M. Kovacs, Jenny Bytingsvik***

Some environmental contaminants are of particular concern due to their negative impacts on animal health. For instance, high levels of certain persistent organic pollutants (POPs) have been linked to adverse health effects in whales However, it has been challenging to study contaminant effects in large whales without sacrificing the animals. The difficulties associated with sampling blood has limited our knowledge of whale physiology and toxicological health effects among other things. A novel technique that is in its development phase is sampling of exhaled air/respiratory vapour often referred to as “whale blow”. Assessing whale health, including levels of environmental contaminants, by combining whale blow sampling and metabolomics is a promising new field due to its non-invasive nature. In the current study, we explored various analytical methods to detect metabolites including lipids in the blow of beluga whales (Delphinapterus leucas) from the Arctic (Svalbard). Blow samples were collected in the field using a nylon mesh stretched over a petri dish; blood was also sampled and surrounding water. Volatile metabolites (SPME-GC-TOF), polar (HILIC-QTOF), and apolar (LC C18-QTOF, GC-FID, GCxGC) metabolites in the blow, blood, and water samples using a targeted (e.g. amino acids, neurotransmitters, (acyl)carnitines, oxylipins, fatty acids) and non-targeted metabolomics approach were studied. The blows contained more than 200 metabolites and the pattern highly differed from the surrounding water. More than 50 fatty acids dominated by C16:0, C16:1, C18:1n9, C20:1n9, C22:0, C22:6n3, C23:00 were found, oxylipids, acylcarnitines, carbohydrates, and many additional compounds that were also present in blood were detected.

11F 4:45 p.m. – 5:00 p.m.	**Exposome-Explorer: Collection of new data on biomarkers of exposure and their associations with disease risk**

PRESENTING AUTHOR: ***Augustin Scalbert, International Agency for Research on Cancer (IARC), France***

CO-AUTHORS: ***Neveu V., Nicolas G., Knaze V., Scalbert A.***

Introduction: Exposome-Explorer (http://exposome-explorer.iarc.fr) provides detailed information on over 692 biomarkers of dietary and pollutant exposures, with over 10,000 concentration values as measured in various populations for biomonitoring or for studying associations with diseases in epidemiological studies. New information is being added to the database on candidate biomarkers of exposure as identified in cross-sectional studies and on associations of exposure biomarkers with cancer risk in population studies. Methods: Detailed information on exposure biomarkers measured in population studies is systematically collected from peer-reviewed publications. Data is manually extracted from the scientific literature and inserted in Exposome-Explorer using the annotation interface. The database and the web interfaces are developed in MySQL and Ruby on Rails. Results: Publications of interest have been identified and data are being inserted into the database. Candidate biomarker as identified in recent metabolomic studies are described with structural identification level as well as a short description of the methods used for feature selection. Association of dietary biomarkers with cancer risk are described as significant or non-significant, together with the direction of the association. Details on populations, biospecimens, analytical methods and concentrations are also reported. Conclusions: Together with data on concentrations in various populations, on correlations with external measures of exposure, and on reproducibility, the newly collected data will help selecting most promising biomarkers for further validation and identifying most useful biomarkers for future applications in exposome-wide association studies.

SESSION 12: NEW METHODS & TECHNOLOGIES I	Tuesday June 26	3:30 p.m. – 5:15 p.m.

12A 3:30 p.m. – 3:45 p.m.	Laser Assisted Rapid Evaporative Ionisation Mass Spectrometry (LA-REIMS): An Automated Platform for Direct, Sample Preparation Free Metabolomics

PRESENTING AUTHOR: ***Simon JS Cameron, Imperial College London, United Kingdom***

CO-AUTHORS: ***Alvaro Perdones-Montero, Frances Bolt, Adam Burke, Kate Hardiman, Daniel Simon, Richard Schaffer, Tamas Karancsi, Zoltan Takats***

Rapid evaporative ionisation mass spectrometry (REIMS) has seen numerous successful applications in direct-from-sample metabolomics; initially in real-time, intra-operative tissue identification. REIMS works via thermal disintegration of a biomass, generating a vapour containing gas phase ions of metabolites and structural lipids. This is aspirated directly to a mass spectrometer; allowing mass spectral data to be generated within a second of sample heating. Previously, electrical diathermy has been used in REIMS for sample heating, but this limited analytical throughput due to the requirement for sample contact to be made. Here, we report on the creation of an automated, high-throughput robotic platform using a CO2 laser for radiative heating; providing a sample-preparation-free workflow for metabolomic analysis of a diverse range of samples in under ten seconds. The platform was optimised for laser operating power, beam pulsing type and speed, and distance and movement of the laser probe during heating. This presentation will cover the range of samples analysed using this workflow to date. These include microbial speciation directly from agar culture plates, exceeding accuracy rates obtained using electrical diathermy, and in the detection of pathogens from bovine milk samples. It has also been used in the analysis of olive oil samples to determine type and geographical authenticity and as a tool for the rapid screening of microbial transformants used in synthetic biology. The LA-REIMS workflow is also suited to high-throughput metabolic phenotyping of faeces, from both humans and mice, and blood plasma to identify disease biomarkers in cancer and diabetes.

12B 3:45 p.m. – 4:00 p.m.	High Resolution SLIM Traveling Wave Ion Mobility-MS of Arginine Isotopomers.

PRESENTING AUTHOR: ***Roza Wojcik, Pacific Northwest National Laboratory, United States***

CO-AUTHORS: ***Ian K. Webb, Yehia M. Ibrahim, Marshall R. Ligare, Matthew E. Monroe, Christopher D. Chouinard , Erin S. Baker, Richard D. Smith.***

Stable isotope labeling techniques in mass spectrometry-based metabolomics studies rely on high resolution separation techniques to alleviate isotopic and isobaric interferences. High resolution ion mobility (IM) technologies potentially enable separations of isotopomers and isotopologues with either no or minimal differences in collisional cross section. Here we employ a Structures for Lossless Ion Manipulations (SLIM) Serpentine Ultra-long Path with Extended Routing (SUPER) high resolution Traveling Wave Ion Mobility (TWIM) platform coupled with Time-of-Flight mass spectrometry for the separation of arginine isotopomers. The combined platform enables an extension of IM path length by 13 meter increments and application of Compression Ratio Ion Mobility Programming (CRIMP) technology for the IM peak compression. We apply long path length SLIM separations, drift time alignment software tools and CRIMP technology to resolve arginine isotopomers, which are e.g. popular SILAC reagents. In preliminary experiments, we achieve baseline resolution of 'light' arginine and arginine 13C6, 15N4 with 130 meter separations in nitrogen and their partial resolution with up to ~ 500 meter separations in helium. In future experiments we will investigate the potential for separation of isotopomers with the same nominal mass; i.e. differing in the location of the heavy label. We also will describe the impact of SLIM TWIM parameters on the mobilities of arginine isotopomers in different buffer gases and evaluate the contribution of the reduced mass to ion mobility in SLIM TWIM-MS separations.

12C 4:00 p.m. – 4:15 p.m.	SWATHtoMRM: Development of High-Coverage Targeted Metabolomics Method Using SWATH Technology

PRESENTING AUTHOR: ***Zheng-Jiang Zhu, Chinese Academy of Sciences, China***

CO-AUTHORS: ***Haihong Zha, Yuping Cai, Yandong Yin, Xiaotao Shen***

The complexity of metabolome presents a great analytical challenge for quantitative metabolite profiling, and restricts the application of metabolomics in biomarker discovery. LC-MS based targeted metabolomics typically using MRM, which is the gold standard for metabolite quantitation. Nevertheless, the low metabolite coverage is a major bottleneck for targeted metabolomics. Here, we developed a novel high-coverage targeted metabolomics method, namely, SWATHtoMRM with coverage as high as 1,000-2,000 metabolites in one experiment. It mainly includes two parts: untargeted analysis of SWATH-MS data and generation of MRM transitions. For the analysis of SWATH-MS data, there are four procedures: (1) MS1 peak detection and alignment; (2) extraction of MS2 peaks and chromatograms; (3) MS1 & MS2 peak grouping; (4) generation of consensus MS2 spectrum. Then, each product ion in the consensus spectrum was further evaluated to generate the MRM transitions. To demonstrate the broad coverage of our strategy, we sequentially acquired both SWATH-MS and DDA-MS data of the human urine samples. The number of detected metabolites from SWATH-MS data was 66% more than that from DDA-MS data. The quantitative performances of SWATHtoMRM approach was evaluated by quantifying serially diluted human urine samples using SWATHtoMRM, SWATH_MS1, and SWATH_MS2 approaches. SWATHtoMRM approach showed a much better sensitivity and performed significantly better in quantitative reproducibility especially for the lower metabolite concentrations. Finally, we applied SWATHtoMRM approach to discover potential biomarkers for colorectal cancer diagnosis, and 17 metabolites were discovered which have a great potential in clinical application.

12D 4:15 p.m. – 4:30 p.m.	SONAR DESI imaging: a novel method to image precursors and fragments for molecular identification in a single experiment.

PRESENTING AUTHOR: ***Mark Towers, Waters Corporation, United Kingdom***

CO-AUTHORS: ***Lee Gethings, Emmanuelle Claude***

Mass spectrometry imaging (MSI) allows the correlation of spatial and chemical information directly from biological tissues. Desorption Electrospray Ionization (DESI) is an ambient ionization technique that has gained popularity over the past few years due to the ease of sample preparation, the ESI-like spectra, and also the non-destructive nature of the DESI technique. Typically MSI experiments are untargeted and are performed using the full scan MS mode of data acquisition. After mining the MSI data and identifying potential biomarkers, the next step is their identification which is usually performed using a limited number of manually entered MS/MS experiments. Recently a new Data Independent Acquisition (DIA) method called SONARTM has been introduced, utilizing a scanning quadrupole mass filter m/z window in a Q-ToF geometry. In this method, a resolving quadrupole mass filter m/z window is scanned repetitively with precursor and MS/MS data acquired at rapid spectral acquisition rates. The method produces a highly specific and unbiased two-dimensional dataset that can be viewed and processed using a variety of informatics tools. Here, we describe the SONARTM mode of acquisition implemented on a bench top quadrupole - ToF Xevo G2-XS mass spectrometer that has been embedded into a DESI imaging workflow for lipid imaging and identification directly from rat brain tissue sections.

12E 4:30 p.m. – 4:45 p.m.	Accelerated Natural Product Discovery by Molecular Family & Substructure Recognition and Automated Chemical Classification

PRESENTING AUTHOR: ***Justin van der Hooft, Bioinformatics Group - Wageningen University, Netherlands***

CO-AUTHORS: ***Madeleine Ernst, Ricardo da Silva, Mingxun Wang, Kyo Bin Kang, Joe Wandy, Marnix H. Medema, Simon Rogers, Pieter C. Dorrestein***

Microbes and plants produce a gold mine of chemically diverse, high-value molecules like antibiotics. However, chemical structures of many natural products (NPs) remain currently unknown, hampering medicinal applications. A key challenge for natural product discovery is the complexity of metabolomes in natural extracts, from which mass spectrometry data needs to be coupled to chemical structures. Nevertheless, many NPs share molecular substructures and form structurally related molecular families (MFs), which has inspired metabolome mining tools exploiting these biochemical relationships. Here, we introduce a workflow that combines two metabolome mining tools to discover MFs, subfamilies, and subtle structural differences between family members. Where tandem mass spectral Molecular Networking efficiently groups natural products in molecular families, MS2LDA discovers substructures that aid in further recognition of subfamilies and shared modifications. Furthermore, through the combined use of Network Annotation Propagation and ClassyFire, we can automatically perform MF chemical classifications. Especially when unexpected MF classifications are observed, they could represent novel chemical scaffolds, thereby guiding follow-up prioritization efforts towards unknown chemistry. We demonstrate how our integrative workflow discovers dozens of MFs in large-scale metabolomics studies of plant and bacterial extracts. For example, Rhamnaceae plants contained triterpenoid chemistries in which several distinct phenolic acid modifications (e.g., vanillate, protocatechuate) were easily recognized. Furthermore, in marine Streptomyces extracts, amongst others, a previously not annotated tryptophan-based MF and yet unknown actinomycin and suguramide analogues were discovered. Our workflow accelerates NP discovery by MF and substructure annotations on a large scale that will aid in future integration with genome mining workflows.

12F 4:45 p.m. – 5:00 p.m.	Enhancing Spatio-Metabolome Coverage Using MALDI and DESI Mass Spectrometry Imaging

PRESENTING AUTHOR: ***Bindesh Shrestha, Waters Corporation, United States***

CO-AUTHORS: ***Bindesh Shrestha, Anthony J. Midey, Hernando Olivos***

In the last few years, both matrix-assisted laser desorption/ionization (MALDI) and desorption electrospray ionization (DESI) mass spectrometry imaging (MSI) have been developed to image metabolites and lipidsfrom the tissue. DESI often detects a complementary set of molecules to MALDI due to preferential ionization of those species. For instance, DESI detects neurotransmitters, such as serotonin, adenosine, and glutamine directly off mice brain tissue samples. While MALDI can readily visualize molecules such as ATP, ADP, AMP, UDP, etc. Here, we show increased metabolome coverage by using both modalities of imaging on a single mass spectrometer platform. MSI were performed on a quadrupole time of flight mass spectrometer with ion mobility separation equipped with both MALDI and DESI ion modalities (SYNAPT HDMS G2-Si; Waters Corporation, Milford, MA). MSI data were collected and analyzed using High Definition Imaging (HDI) software with normalization (median-tissue ions). Statistical analysis was computed in MetaboAnalyst. Molecular distribution of metabolites and lipids were co-registered with morphological features of H&E stained microscopy images from the same tissue section after MSI. Tentative molecular assignments were obtained from the accurate mass search against of publically curated databases to gauge preferential ionization of specific molecule classes from the tissue. Statistical analysis of ions detected by DESI and MALDI on consecutive sections using similar solvent composition emphasized the complementary characteristics of the two mechanistically different ionization sources. Increased coverage of metabolites and lipids from the same tissue obtained by complementary techniques, DESI and MALDI, painted a more comprehensive picture of the metabolome.

SESSION 13: INFORMATICS & STATISTICS FOR METABOLOMICS	Wednesday June 27	10:15 a.m. – 12:00 p.m.

13A KEYNOTE 10:15 a.m. – 10:45 a.m.	Numerical Representations of Metabolic Systems

PRESENTING AUTHOR: ***Age Klaas Smilde, University of Amsterdam, Netherlands***

In metabolomics we perform measurements. These measurements produce numbers which is not the same as data: data are numbers including their meaning. Data can have different properties depending on how the numbers are measured. One property is measurement scale, which ranges from ratio-scaled data to nominal-scaled data. Another property is comparability across rows and columns of our data table. These different properties will be explained by simple examples from metabolomics data analysis practice. It will also be shown what the repercussions are of those properties for the type of statistical analysis to employ.

13B 10:45 a.m. – 11:00 a.m.	COSMA: A Novel Methodology for High-Throughput Chemical Classification of Unidentified Compounds in Metabolomics via Adaptive MS/MS Spectral Motif Based Searching

PRESENTING AUTHOR: ***Tytus D. Mak, National Institute of Standards and Technology, United States***

CO-AUTHORS: ***Stephen E. Stein***

Mass spectrometry has played a critical role in the rise of metabolomics, enabling high-throughput characterization of small molecules in biological systems at unprecedented levels of sensitivity, resolution, and accuracy. However, this insight has revealed the sheer diversity and breadth of biologically relevant small molecules, which greatly exceeds the scope of mass spectral libraries. Nonetheless, even partial characterization of these unknown analytes can provide critical insight into underlying biological processes. As such, we have developed a novel algorithm, called Class Optimized Spectral Motif Searching Algorithm (COSMA), which recognizes patterns in the MS/MS spectra of classified compounds in an existing spectral library in order to putatively assign classifications to unidentified compounds for which spectral data has been acquired. COSMA adaptively constructs class-specific spectral motifs, which are utilized for interrogating unknown MS/MS spectra. COSMA is designed to accommodate any chemical classification schema and can utilize any spectral library as training data. Untargeted LC-MS/MS data derived from a NIST SRM for human urine (SRM 3667) was analyzed via COSMA. ClassyFire, a classification schema used by HMDB, was utilized to assign classifications to 8238 compounds in the NIST14 MS/MS Library, from which 183069 spectra were utilized to train COSMA. During the first 2/3 of the LC run, 7348 out of 8693 spectra were classified, with acyl carnitines (6.87%), O-glucuronides (6.79%), and N-acetylarylamines (5.32%) constituting the most common classifications. The last 1/3 of the run, with 2961 out of 4467 classified spectra, were dominated by glycerol ethers (32.37%), with 1,2-diacylglycerols (7.82%) as a major subcomponent.

13C 11:00 a.m. – 11:15 a.m.	Standards-free identification of small molecules using multi-feature matching

PRESENTING AUTHOR: ***Jamie R Nuñez, Pacific Northwest National Laboratory, United States***

CO-AUTHORS: ***Dennis Thomas, Sean Colby, Erin Baker, Malak Tfaily, Nikola Tolic, Thomas Metz, Justin Teeguarden, Ryan Renslow***

The gold standard for unambiguous identification of a small molecule in metabolomics is based on comparing two or more orthogonal properties of data from analysis of reference materials to experimental data acquired in the same laboratory with the same analytical methods. This represents a significant limitation since many molecules are not yet represented by standard compounds. To move towards standards-free small molecule identification, we are(i) advancing chemical property predictions (e.g. NMR and IR spectra prediction) through the creation and utilization of a large-scale computational chemistry platform, the In Silico Chemical Library Engine (ISICLE), and (ii) developing multi-feature scoring and matching algorithms. To test our methods, we participated in a blinded analysis of synthetic mixtures as part of a 24-laboratory challenge initiated by the U.S. Environmental Protection Agency (EPA). These mixtures were composed of compounds from the EPA ToxCast library. Accurate mass, isotopic signature, and collisional cross section (CCS) measurements were collected using ion mobility spectrometry–mass spectrometry (IMS-MS) and ultra-high resolution 21-Tesla Fourier transform ion cyclotron resonance–mass spectrometry (FTICR-MS). Each of these properties were also calculated for each compound in the EPA ToxCast library, allowing us to match observed features to library entries. Our percent of true positives for “highly confident” identifications was 71%, and we observed a high number of compounds from the library that were not spiked in but possible through unintentional transformations in the highly concentrated mixtures. Finally, we improved our approach by optimizing scoring parameters, increasing our true positive rate and number of identifications.

SESSION 13: INFORMATICS & STATISTICS FOR METABOLOMICS | Wednesday June 27 | 10:15 a.m. – 12:00 p.m.

13D 11:15 a.m. – 11:30 a.m. — Investigating the accuracy required for the identification of small molecules using calculated NMR chemical shifts

PRESENTING AUTHOR: ***Yasemin Yesiltepe, Washington State University, United States***

CO-AUTHORS: ***Jamie Nunez, Dennis Thomas, Niranjan Govind, Mark Borkum, Nancy Washton, John Cort, Thomas O. Metz, Ryan Renslow***

The majority of metabolites in complex samples have yet to be identified, representing a major challenge in metabolomics. Using currently available experimental methods, characterization of entire metabolomes is infeasible for both technical and economic reasons. For example, measurements are limited to the availability of authentic chemical standards, which, for the majority of known molecules, do not exist. Alternatively, determining nuclear magnetic resonance (NMR) chemical shifts in silico has shown promise in the identification and delineation of metabolite structures, and would thus enable expansion of publicly available databases and libraries. To this end, we developed the In-Silico Chemical Library Engine (ISiCLE), a high-performance cheminformatics workflow designed to predict NMR chemical shifts of any molecule using density functional theory (DFT), a computationally inexpensive yet reliable method for the prediction of carbon and proton NMR chemical shifts of metabolite. We evaluated ISiCLE on over 312 molecules with respect to experimental standards and concluded that the calculated proton and carbon chemical shifts deviate 0.33 and 3.93 ppm, respectively. Furthermore, using the set of small molecules found in the Human Metabolome Database, HMDB we investigated the accuracy required for identification of metabolites. As a result, 90% of these molecules in a pure sample can be successfully identified when errors of 1H and 13C chemical shifts reach 1 ppm and 5 ppm, respectively. Unsurprisingly, in complex mixtures, as the complexity of the mixture increased, greater and greater accuracy of the calculated chemical shifts was required.

13E 11:30 a.m. – 11:45 a.m. — Small Molecule Isotope Resolved Formula Enumerator (SMIRFE): a tool for truly untargeted metabolomics analysis of metabolites represented in Fourier transform mass spectra

PRESENTING AUTHOR: ***Hunter N.B. Moseley, University of Kentucky, United States***

CO-AUTHORS: ***Joshua M. Mitchell, Robert M. Flight***

Fourier-transform mass-spectrometry (FTMS) is often utilized in the detection of small molecules derived from biological samples. What is directly detected in the FTMS spectra are peaks for related sets of isotopologues or molecules that differ only in their isotopic composition for various adducted and charged species corresponding to specific molecules present in a biological sample or introduced by contamination. The sheer complexity of what is detected along with a variety of analytically-introduced variance, error, and artifacts have hindered the systematic analysis of the complex patterns of detected peaks with respect to isotopic content. We have implemented a novel algorithm SMIRFE that detects small biomolecules less than 2000 daltons at a desired statistical confidence and determines their specific elemental molecular formula (EMF) using detected cliques of related isotopologue peaks with compatible isotope-resolved molecular formulae (IMFs). The current implementation efficiently searches a roughly 200 quintillion (2x1020) IMF space for each peak's m/z, but larger IMF spaces are searchable. We validated the assignment performance using verified assignments from a FTMS spectrum of a biological sample treated with ethylchloroformate, a chemoselection agent. SMIRFE provides 100% untargeted EMF assignment recall for verified metabolite cliques and IMF assignments for 63% of the 2557 characterized peaks in the FTMS spectrum. Furthermore, SMIRFE provides E-value estimates of assignment accuracy, which no other available metabolite assignment tool provides. SMIRFE has none of the limitations of current methods that can only detect known metabolites in a database and enables a truly untargeted metabolomics analysis that is beyond simple biomarker detection.

SESSION 14: PHARMACOMETABOLOMICS | Wednesday June 27 | 10:15 a.m. – 12:00 p.m.

14A SESSION KEYNOTE 10:15 a.m. – 10:45 a.m. — The invisible impact of steroid metabolism on global metabolism and personalised medicine

PRESENTING AUTHOR: ***Warwick Dunn, University of Birmingham, United Kingdom***

CO-AUTHORS: ***Riccardo Di Guida, James W. Allwood, Laura Torchen, Jonathan M. Hazlehurst, Laura L. Gathercole, Michael W. O'Reilly, Angela E. Taylor, K N. Manolopoulos, Andrea Dunaif, Jeremy W. Tomlinson, Wiebke Arlt***

Steroid metabolism encompasses the synthesis, degradation and action of androgens, glucocorticoids, estrogens, progestagens and mineralocorticoids in the human body. The influence of steroids on human biological actions including sexual development (androgens and estrogens), pregnancy (progestagens), and glucose/lipid metabolism (glucocorticoids) are well reported. However, the impact of steroid metabolism and their interaction with other hormones (e.g. insulin) on other areas of metabolism including carbohydrate, lipid and protein metabolism are less well defined. In this presentation I will summarise our current knowledge and introduce significant new research including: (1) The interaction of insulin and cortisol and its effect on global metabolism dynamically across the day including a discussion of whether breakfast is metabolically good for us (2) The influence of insulin, androgen metabolism and fed/fasted status on the efficacy of 5-alpha reductase drug treatments (3) The influence of perturbed androgen metabolism on global metabolism in the premenarchal children of Polycystic Ovary Syndrome (PCOS) mothers including the risk of PCOS development in these children. The presentation will demonstrate the wide-ranging effect of steroid metabolites on global metabolism and beyond our current knowledge, the significant metabolic interaction between different steroid hormone classes and insulin and the ability for metabolites to stratify at-risk children in relation to PCOS.

14B 10:45 a.m. – 11:00 a.m.	An automated, high throughput, and personalized metabolomics data analysis pipeline reveals biomarkers of aspirin resistance in blood metabolism

PRESENTING AUTHOR: ***Douglas McCloskey, University of California, San Diego, Denmark***

Metabolomics data is both highly informative and cost effective, and presents an ideal –omics platform for generating personalized diagnostics of human health. However, major bottlenecks to any high throughput metabolomics workflow that renders the acquisition and interpretation of big data sets difficult are data processing and data analysis. This is particularly problematic in clinical settings where speed of data analysis and accuracy and quality of results are paramount for guiding healthcare decisions. This work will describe an automated data processing and analysis pipeline that converts raw data files into actionable insights using state of the art informatics methods. The raw data processing pipeline utilizes advanced peak picking and selection algorithms that outperform current data processing software when applied to complex sample matrices; the data analysis pipeline integrates a combination of statistical and biochemical modeling techniques as well as genomic data to develop personalized models of metabolism. The workflow was applied to the identification of metabolic biomarkers associated with aspirin resistance in human blood metabolism. We find that the workflow is able to uncover biologically meaningful insights about individual patient susceptibility to aspirin administration. The workflow and discovered biomarkers could provide a rapid and personalized diagnostic platform for future clinical work.

14C 11:00 a.m. – 11:15 a.m.	Pre-clinically generated and clinically recapitulated metabolic signatures in plasma and their applications in (cancer) drug development

PRESENTING AUTHOR: ***Dr FI Raynaud, The Institute of Cancer Research, United Kingdom***

CO-AUTHORS: ***Akos Pal, Joo Ern Ang, Yasmin Asad, Ruth Ruddle, Alan T. Henley, Suzanne A. Eccles, Ian Collins, Michelle D. Garrett, Johann De Bono, Udai Banerji, Florence I. Raynaud***

The success of drug candidates in early clinical development is highly dependent on accurate information derived from robust assays measuring pharmacodynamic modulation. Understandably, the availability of human tumour biopsies is limited and the use of surrogate tissues is necessary to assess target engagement. We have quantified plasma metabolites by LC-MS, evaluated them as potential biomarkers and successfully established a workflow from preclinical models to clinical studies as illustrated with different pathway inhibitors (PI3K, MEK etc). We have demonstrated, in a proof-of-concept study, that the plasma metabolomic signature we defined in preclinical models was recapitulated in a Phase I clinical trial. We have also showed similarity between a PI3K and an AKT inhibitor on plasma metabolites compared with a clearly different metabotype generated by a ROCK kinase inhibitor. We identified metabolite markers in BRAF mutant, human melanoma xenograft models where > 70% of the identified metabolites allowed significant prediction of objective responses in patients with BRAF mutant advanced melanoma, treated with the same MEK inhibitor. In addition, we demonstrated that pre-treatment levels of selected plasma metabolites could have significant prognostic value. Our results highlight the potential and flexible application of plasma derived metabolic signatures in (cancer) drug development from target identification to Phase I clinical trials.

14D 11:15 a.m. – 11:30 a.m.	Metabolic response profiles of doxorubicin treatment in breast cancer patients associated with cardiomyopathy

PRESENTING AUTHOR: ***Eirini Kouloura, Imperial College London, United Kingdom***

CO-AUTHORS: ***Pilar Sepulveda, Hector Keun***

Doxorubicin treatment in breast cancer patients is a valuable chemotherapeutic, however causes dose-dependent cardiotoxicity which can progress to heart failure. The present study using a targeted LC-MS metabolomic profiling approach aims to provide insights into metabolic perturbations observed in serum of breast cancer patients treated with doxorubicin regimens. Serum samples were collected from forty eight patients before treatment, after drug infusion at multiple cycles during treatment and follow-up samples several months post treatment were also taken to account for late onset changes. An unsupervised Principal Component Analysis (PCA) was first applied based on serum profiles which revealed a characteristic recovery profile, underlying baseline and follow up samples profile similarity. Tracking the dynamic metabolic changes, ANOVA simultaneous component analysis (ASCA) revealed significant decrease in medium chain ACs levels during chemotherapy treatment. The metabolomic dataset was also correlated to clinical markers of cardiac injury (LVEF, troponin) and interestingly, higher levels in conjugated bile acid were found to be related with high troponin levels. The altered levels of medium chain carnitines in serum denote an inhibition of fatty acid β-oxidation potentially due to doxorubicin induced mitochondrial injury in the heart. Also increase in circulating bile acids is associated with cardiomyopathy in previous studies via suppression of proliferator-activated receptor-c coactivator 1a expression, a key regulator of fatty acid metabolism. Overall, our data support metabolic perturbations in fatty acid oxidation pathway after doxorubicin administration and suggest that medium chain acylcarnitines and conjugated bile acids can be used as metabolite signatures of cardiac toxicity.

SESSION 14: PHARMACOMETABOLOMICS	Wednesday June 27	10:15 a.m. – 12:00 p.m.

14E 11:30 a.m. – 11:45 a.m.	Importance of steroidal drug metabolism in guiding drug development

PRESENTING AUTHOR: ***Mohammad Alyamani, Cleveland Clinic, United States***

CO-AUTHORS: ***Nima Sharifi***

Treatment options including the steroidal drug abiraterone are available to treat patients with prostate cancer. However, despite initial responses, treatment resistance occurs and patients will die from their disease. Abiraterone, a CYP17A1 inhibitor, shares the same A, B ring with endogenous dehydroepiandrosterone, which is a substrates for the enzyme, 3β-hydroxysteroid dehydrogenase (3βHSD) which is required for testosterone and dihydrotestosterone (DHT) synthesis and drive PCa. The common germline variant in HSD3B1(1245C) encodes for a hyperactive (3βHSD1) missense that increases DHT synthesis from extragonadal precursor steroids and is a predictive biomarker of resistance to ADT and sensitivity to non-steroidal CYP17A1 inhibition. Abiraterone, is metabolized by 3βHSD1 to multiple steroidal metabolites, including 3-keto-5α-abiraterone which stimulates the androgen receptor. The HSD3B1 (1245C) variant might therefore increase 3-keto-5α-abiraterone synthesis in patients on abiraterone therapy, possibly limiting clinical benefit. Similarly, we found that galeterone which shares the same Δ5, 3β-hydroxyl-structure with abiraterone, is metabolized by 3βHSD. Therefore metabolism by 3βHSD is a class effect of Δ5, 3β-hydroxyl drugs with important consequences in resultant downstream metabolites and mechanisms of drug response that should be accounted for in preclinical and clinical drug development. We then determined the association between serum 3-keto-5α-abiraterone levels and HSD3B1 genotype in 30 patients treated with abiraterone acetate (AA). Patients who inherit 0, 1 and 2 copies of HSD3B1(1245C) have a stepwise increase in 3-keto-5α-abiraterone. Increased generation of 3-keto-5α-abiraterone in patients with HSD3B1(1245C) might partially negate abiraterone benefits in these patients who otherwise benefit from CYP17A1 inhibition.

SESSION 15: METABOLITE ID METHODS (EXPERIMENTAL)	Wednesday June 27	10:15 a.m. – 12:00 p.m.

15A SESSION KEYNOTE 10:15 a.m. – 10:45 a.m.	Untargeted Analysis of the Exposome in Human Plasma and Urine

PRESENTING AUTHOR: ***Susan Sumner, University of North Carolina at Chapel Hill, United States***

CO-AUTHORS: ***Susan Sumner, Yuanyuan Li, Rodney Snyder, Timothy Fennell***

Metabolomics analysis of urine and plasma using untargeted liquid chromatography mass spectrometry results in thousands of signals associated with endogenous and exogenous metabolites (the Exposome). While the identification of endogenous metabolites has been a considerable challenge in the field of metabolomics, an additional challenge being addressed by the NIEHS-funded Children's Health Exposure Analysis Program (CHEAR) is the assignment of signals derived from environmentally relevant drugs and chemicals and their metabolites. Using the knowledge of environmentally relevant compounds (and associated metabolites) known to be present in urine and serum, we selected to investigate the presence of these constituents in untargeted data acquired via a Q-Exactive HF-X. We established a library that includes chemical ID, structure, formula, retention time, MS, and MS/MS spectra for over 100 environmentally relevant compounds (including parabens, phthalates, tobacco metabolites, pesticides, and volatile organic compounds), as well as 600 endogenous metabolites. This presentation will cover workflows associated with the construction and utilization of an in-house library for exposome research. An approach to scoring each library-matched assignment with a confidence level will be described. For example, signals assigned by library matching retention time and MS/MS spectra from authentic standards are more highly confident identifications. Peaks not associated with the in-house library of standards have less confident scores/grades using information from online libraries such as mass error tolerance, isotopic similarity, and theoretical fragment scores. This workflow provides rapid identification of a large number of analytes in biological samples with confidence scores for each assignment. [U2CES026544; 1U24DK097193]

15B 10:45 a.m. – 11:00 a.m.	Development of Integrated Computational and Empirical Tools for Higher-throughput and Confident Metabolite Identifications

PRESENTING AUTHOR: ***Lloyd W. Sumner, University of Missouri, United States***

CO-AUTHORS: ***Feng Qiu, Anil Bhatia, Zhentian Lei, Barbara W. Sumner, Mark Schroeder, Sven Meyer, and Aiko Barsch***

Our lab is addressing the metabolomics grand challenge of confident metabolite identifications using an integrated computational approach to first predict metabolite identities and then prove the identities using a suite of sophisticated instrumentation. Metabolite identifications are first attempted through retention time and spectral matching of experimental xC-MS or xC-MS/MS data with data from authentic compounds. We have generated and released several spectral libraries which have also been submitted to MassBank, HMDB and NIST. However, a large number of plant natural products/specialized metabolites are not commercially available. This fact encouraged us to build custom software entitled Plant Metabolite Annotation Toolbox (PlantMAT) that can predict large numbers of currently unknown metabolite structures using biochemistry principles and combinatorial enumeration. This process expands the defined metabolic space which is then searched using empirical UHPLC-MS and UHPLC-MS/MS data for predicting metabolite identities. We have also generated a MetExpert software to predict metabolite identity based upon GC-MS data, in silico derivatization and machine learning. Predicted metabolite identifications are then proved, refined or determined de novo through the coupling of UHPLC-QTofMS/MS to a solid phase extraction system (SPE) for automated purification and concentration of targeted analytes followed by offline NMR analyses. This cumulative UHPLC-MS-SPE-NMR system provides a powerful tool for higher-throughput, confident metabolite identifications. We are further developing a 4-Dimensional UHPLC-Trapped ion mobility (TIMS)-QTOFMS/MS platform for increased metabolome depth of coverage and increased metabolite identification confidence based upon measuring collisional cross sections as well as traditional retention time and mass spectral matching.

15C 11:00 a.m. – 11:15 a.m. **Quantitative mass spectrometry-based targeted metabolomics using fastly produced deuterated internal standards**

PRESENTING AUTHOR: ***Annelaure Damont, CEA, France***

CO-AUTHORS: ***Yu Min Kiw, Kathleen Rousseau, Sophie Feuillastre, Grégory Pieters, Christophe Junot, François Fenaille***

In recent years, large-scale quantification of metabolites in complex biological samples proved essential to more in-depth and accurately investigate the metabolism of organisms. In particular, targeted metabolomics quantification is well adapted to explore specific metabolic pathways or to evaluate the impact of environmental factors by studying relevant sets of metabolites. Mass spectrometry-based quantification requires stable isotopically labeled internal standards (SIL-IS) for high robustness and accuracy, and to overcome matrix effects on ionization efficiency. Considering the large panel of metabolites that need to be quantified, the limited availability of their labeled counterparts, and their often associated prohibitive cost, we implemented a fast and versatile strategy to generate deuterium-labeled metabolites from authentic standards via metal-catalyzed H/D-exchange reaction. Such single-step process allowed for the production, within 24 to 72 hours, of a set of some twenty deuterated compounds that can be used, in a raw and without further purification, for the quantification of the corresponding metabolites in plasma samples using an LC-Q-ToF. In summary, our strategy to rapidly and conveniently produce deuterium-labeled versions of numerous metabolites considerably, facilitates the implementation of targeted quantitative assays. It also demonstrated similar performance than common standard addition or isotope dilution (using 13C- and/or 15N-labeled metabolites) approaches for the quantification of amino and organic acids in human plasma. Our approach opens new possibilities for the quantification of almost any metabolite and, as such, will certainly facilitate the implementation of LC-MS assays for the absolute quantification of endogenous, xenobiotic or pollutant metabolites in complex matrices.

15D 11:15 a.m. – 11:30 a.m. **Accurate Identification of Known and Unknown Metabolites by Multidimensional NMR and Customized Metabolite Database**

PRESENTING AUTHOR: ***Cheng Wang, The Ohio State University, China***

CO-AUTHORS: ***Bo Zhang, Da-Wei Li, Lei Bruschweiler-Li, John Gunn, Rafael Brüschweiler***

Metabolite identification in metabolomics samples is a key step that critically impacts downstream analysis, including analysis of biochemical pathways and their perturbations resulting from mutations, aging, diet, exercise, or life style. To improve the identification accuracy of unknown and known metabolites beyond the scope of current spectroscopic databases, we designed an efficient, customizable method by combining 2D & 3D NMR with specifically curated metabolite database information, similar to the SUMMIT approach [1,2]. First, experimental chemical shifts of each compound were extracted from 2D & 3D NMR spectra by the maximum clique method [3]. In parallel, a unified metabolite database was curated that incorporates information from several public metabolite databases followed by the prediction of the metabolite 1H and 13C NMR chemical shifts using an empirical NMR predictor. The compound candidates were then rank-ordered by comparing predicted with experimental chemical shifts. The method is demonstrated for an untargeted analysis of mouse bile fluid containing both known and unknown hydrophilic metabolites. In this way, a substantial number of previously known metabolites can be accurately identified along with unknown metabolites, independent of any experimental NMR database information. [1] Bingol, K., Bruschweiler-Li, L., Yu, C., Somogyi, A., Zhang, F., Brüschweiler, R. Anal. Chem. 2015, 87, 3864-70. [2] Wang, C., He, L., Li, D. W., Bruschweiler-Li, L., Marshall, A. G., Brüschweiler, R. J. Proteome Res. 2017, 16, 3774-86. [3]Li, D. W., Wang, C., Brüschweiler, R. J. Biomol. NMR 2017, 68, 195-202.

15E 11:30 a.m. – 11:45 a.m. **A fast and reliable spectral quality assessment in metabolomics studies**

PRESENTING AUTHOR: ***Alberto Gil de la Fuente, CEU-San Pablo University, Spain***

CO-AUTHORS: ***Alberto Gil de la Fuente, Federico Traldi, Tomasz Kowalczyk, Michal Ciborowski, Abraham Otero, Coral Barbas, Joanna Godzien***

Untargeted metabolomic experiments where researchers aim to identify compounds differentiating the groups without a priori hypothesis have an increasing range of applications in biological studies. The final success of the study depends on the correctness of the identification process and this is one of the main bottlenecks due to the nature of the separation and mass spectrometry techniques. The limited amount of information obtained (m/z, retention time and intensity) makes tandem mass spectrometry (MSn) a crucial method for compound identification. Furthermore, the low availability of authentic standards additionally hinders identification. Consequently, a high-quality spectrum is paramount to improve the identification rate of the compounds, while a low-quality spectrum increases the risk of miss-identification. But, what exactly is a “high-quality” or a “low-quality” spectrum? A study of hundreds of MS/MS spectra from biological studies has been performed to establish criteria for the objective evaluation of spectral quality. A pentagonal-point evaluation system including as input variables: the overall intensity, the noise impact, the number of MS/MS scans obtained, the co-elution ions, and the cross-talk phenomena has been designed. From these input variables, a score is calculated to rank the spectra as “excellent”, “acceptable” or “inadequate”. We have carried out a validation of this quality evaluation system that shows that among the “inadequate” spectra the rate of misidentified compounds was 2x higher than among “acceptable” or “excellent” spectra . The evaluation system designed is available on-line at Ceu Mass Mediator (http://ceumass.eps.uspceu.es/).

SESSION 16: CANCER	Wednesday June 27	1:30 p.m. – 3:00 p.m.

16A KEYNOTE 1:30 p.m. – 2:00 p.m.	Global metabolic reprogramming of cancer

PRESENTING AUTHOR: ***Masaru Tomita, Institute for Advanced Biosciences, Keio University, Japan***

Institute for Advanced Biosciences (IAB), established in 2001, is a pioneer of “data-driven systems biology” located in Tsuruoka City, which is a beautiful historical town nestled between the mountains and the seaside with the purest Japanese cuisine and culture. This talk consists of three parts: Metabolic reprogramming of cancer We performed multi-omics analyses of colorectal cancer tissue samples, and revealed that the protooncogene protein MYC regulated global metabolic reprogramming of colorectal cancer by modulating 215 metabolic reactions. Importantly, this metabolic reprogramming occurred in a manner not associated with specific gene mutations in colorectal carcinogenesis. [Satoh 2017, Proc Natl Acad Sci U S A.] Plasma biomarker of major depressive disorder (MDD) Human Metabolome Technologies Inc., a spinoff company of IAB, has found that phosphorylethanolamine (PEA) can distinguish patients with MDD from patients suffering from other mood disorders such as bipolar and adjustment disorder. HMT established the manufacturing and sales of a PEA reagent kit, and will provide these kits to a wide range of clinical research institutes in 2018. [Kawamura 2018, Psychiatry and Clinical Neurosci] Saliva biomarker of cancer We conducted a comprehensive metabolite analysis of saliva samples obtained from oral, pancreatic and breast cancer patients, and identified dozens of principal metabolites that can be used to predict the probability of being affected by each individual disease. Saliva Tech Co LTD, another spinoff company of IAB, is conducting a practical service of saliva check at six (6) medical clinics in Japan at present. [Sugimoto 2009, Metabolomics]

16B 2:00 p.m. – 2:15 p.m.	Acquisition of Warburg-Like Phenotype in IDH1mut Lower Grade Gliomas as a Mechanism of Malignant Progression

PRESENTING AUTHOR: ***Mioara Larion, National Institutes of Health, United States***

CO-AUTHORS: ***Victor Ruiz-Rodado, Tomohiro Seki, Adrian Lita, Alejandra Cavazos Saldana, Tyrone Dowdy, Wei Zhang, Hua Song, Dionne Davis, Tathiane M. Malta, Thais S. Sabedot, Houtan Noushmehr, Sunmin Lee, Jane B. Neckers, Orieta Celiku, Aiguo Li, Christel Herold-Mende, Mark R. Gilbert, Murali Krishna Cherukuri***

Warburg effect is one of the hallmarks of cancer metabolism and its prevalence also spans over the brain tumors. The formation of lactate and upregulation of glycolysis has been the basis of many studies that either use 18F-FDG PET or hyperpolarized 13C pyruvate MRI as a readout of cancer metabolic activity in vivo. IDH1mut gliomas which are mostly encountered as lower grades, have been reported to display low glycolytic rates and low lactate production, primarily due to the hypermethylation of the promoter region of lactate dehydrogenase A (LDHA). Interestingly, IDH1mut glioma that have progress towards glioblastoma multiforme, appear as Warburg-like phenotype. Herein, using hyperpolarized MRI, we report two examples of IDH1mut glioma cell lines that were isolated from grade III gliomas (astrocytoma and oligodendroglioma) but they appear as transformed gliomas, with highly glycolytic and aggressive, Warburg-like phenotypes. In order to understand the mechanisms of malignant transformation, we undertook a detailed epigenetic, transcriptomic and metabolomics analysis of these cell lines and compared them with a non-aggressive IDH1mut cell line. The comparison between the aggressive and non-aggressive cell lines suggests that loss of promoter methylation in the glycolytic enzymes leads to increased mRNA and metabolite levels. The results of this study represent an example of malignant transformation that is linked with loss of promoter methylation in the glycolytic enzymes as a primary step of a mechanism by which IDH1mut low-grade gliomas progress towards glioblastomas.

16C 2:15 p.m. – 2:30 p.m.	Multiplatform metabolic and lipid fingerprinting of breast cancer: A pilot control-case study in Colombian Hispanic women.

PRESENTING AUTHOR: ***Julian Aldana, Los Andes University, Colombia***

CO-AUTHORS: ***Monica Cala, Jessica Medina, Julian Sanchez, Jose Guio, Julien Wist, Roland Meesters***

Breast cancer (BC) is a highly heterogeneous disease associated with metabolic reprogramming. The shifts in the metabolome caused by BC still lack data from Latin populations of Hispanic origin. In this pilot study, multiplatform metabolomic and lipidomic approaches (GC-MS, LC-MS 1H-NMR) were performed to establish a plasma and urine metabolic fingerprint of Colombian Hispanic women with BC. Fifty-eight women were selected for the study, Control patient (CP): 29 healthy women (Age 51 ± 8 years, BMI 27 ± 3 kg/m2), Breast cancer patient (BCP): 29 women diagnosed with breast cancer, mostly invasive ductal carcinoma between stage I and III (Age 50 ± 7 years, BMI 26 ± 3 kg/m2). The differentiating metabolites in plasma samples were involved in glycerolipid, glycerophospholipid, amino acid, fatty acid metabolism and the up-regulation of long chain fatty acyl carnitines and the down-regulation of cyclic phosphatidic acid (cPA). In urine samples an overall decrease of intermediates of the tricarboxylic acid cycle and metabolites belonging to amino acids and nucleotides were observed, along with an increment of lipid-related compounds. ROC analysis evaluated the combination of dimethyl heptanoylcarnitine and succinic acid as potential urinary markers, achieving a sensitivity of 93% and a specificity of 86%. Our findings propose relevant plasma and urine metabolites that could contribute to a better understanding of underlying metabolic shifts driven by BC in women of Colombian Hispanic origin. The mapped metabolic signatures in breast cancer were similar but not identical to those reported for non-Hispanic women, despite racial differences.

SESSION 16: CANCER	Wednesday June 27	1:30 p.m. – 3:00 p.m.

16D 2:30 p.m. – 2:45 p.m.	Plasma-derived Metabolite and Protein Biomarker Panels Have Additive Performance for Early Stage Pancreatic Cancer

PRESENTING AUTHOR: ***Johannes Fahrmann, The University of Texas MD Anderson Cancer Center, United States***

CO-AUTHORS: ***Leonidas E. Bantis, Michela Capello, Ghislaine Scelo, Jennifer B. Dennison, Nikul Patel, Eunice Murage, Jody Vykoukal , Deepali L. Kundnani, Lenka Foretova, Eleonora Fabianova, Ivana Holcatova, Vladimir Janout, Ziding Feng , Michele Yip-Schneider, Jianjun Zhang, Randall Brand, Ayumu Taguchi, Anirban Maitra, Paul Brennan, C. Max Schmidt, Samir Hanash***

Purpose: We applied a training and testing approach to develop and validate a plasma metabolite panel for the detection of early stage pancreatic ductal adenocarcinoma (PDAC) alone and in combination with a previously validated protein panel for early stage PDAC. Methods: A comprehensive metabolomics platform was initially applied to plasmas collected from 20 PDAC cases and 80 controls. Candidate markers were filtered based on a second independent cohort that included 9 invasive intraductal papillary mucinous neoplasm cases and 51 benign pancreatic cysts. Blinded validation of the resulting metabolite panel was performed in an independent test cohort consisting of 39 resectable PDAC cases and 82 matched healthy controls. The additive value of combining the metabolite panel with a previously validated protein panel was evaluated. Results: Five metabolites were selected as a panel based on filtering criteria. A combination rule was developed for distinguishing between PDAC and healthy controls using the training set. In the blinded validation study with early stage PDAC samples and controls, the five metabolites yielded AUCs ranging from 0.726 to 0.842 and the combined model metabolite model yielded an AUC of 0.892. Performance was further significantly improved by combining the metabolite panel with a previously validated protein marker panel consisting of CA 19-9, LRG1 and TIMP1. Conclusion: A metabolite panel in combination with CA19-9, TIMP1 and LRG1 exhibited substantially improved performance in the detection of early stage PDAC compared with a protein panel alone.

16E 2:45 p.m. – 3:00 p.m.	Metabolic profile and the risk of developing prostate cancer - A nested case-control study

PRESENTING AUTHOR: ***Ali A Moazzami, Department of Molecular Sciences, Swedish University of Agricultural Sciences, Sweden***

CO-AUTHORS: ***Hanna E Röhnisch, Cecilie Kyrø, Anja Olsen, Elin Thysell, Göran Hallmans***

Prostate cancer is the most frequently diagnosed cancer and the second cause of cancer-related death in men. Identifying modifiable risk factors and markers for disease risk are therefore important. Our aim was to identify metabolites associated with risk of prostate cancer using a nested case-control study design i.e. 777 pairs of prostate cancer cases and their matched controls (n = 1554) recruited from Northern Sweden Health and Disease Study Cohort (NSHDC). Metabolites were quantified with targeted MS and NMR-based metabolomics in fasting plasma samples. Association to disease risk was examined using conditional logistic regression conditioned on matching factors (BMI, age and sample storage time), followed with correcting for multiple testing. Statistical analyses were also done after restriction to non-aggressive and aggressive cases and stratification by baseline age. After correction for multiple testing, we identified a positive association between overall disease risk and plasma levels of two lysophosphatidylcholines (i.e., LPC C17:0 and LPC C18:0). The associations were more pronounced in older subjects (60 years), where the association for LPC C17:0 was significant even after Bonferroni correction. For younger subjects (40 and 50 years), glycine levels positively, and pyruvate levels negatively, associated with risk of overall disease. We also identified positive association between aggressive disease risk and plasma levels of six glycerophospholipids, whereas levels of acylcarnitine C18:2 displayed a negative association. A strong association was found for LPC 17:0 and aggressive disease in older individuals, where individuals in the top quartile had a 3.9 fold higher odds of developing aggressive disease.

SESSION 17: INTERNAL MEDICINE	Wednesday June 27	1:30 p.m. – 3:00 p.m.

17A SESSION KEYNOTE 1:30 p.m. – 2:00 p.m.	Molecular sub-phenotyping of obstructive lung disease

PRESENTING AUTHOR: ***Craig Wheelock, Karolinska Institute, Sweden***

CO-AUTHORS: ***C. Magnus Sköld, Åsa M. Wheelock***

Obstructive lung disease is an increasing global health problem of pandemic proportions. The definition of obstructive lung disease is currently based upon clinical parameters. However, this definition represents an umbrella diagnosis that can be derived from a multitude of etiologies including environmental exposures, genetic predispositions and developmental factors. It is necessary to perform molecular sub-phenotyping of individuals with obstructive lung disease to develop relevant diagnostic and treatment options for this heterogeneous patient group. The aim of our investigations is to develop an understanding of the molecular sub-phenotypes of disease towards the goal of identifying prognostic markers for discovering early signs and/or increased risk of developing disease prior to debilitating destruction of lung capacity. The identification of a panel of non-invasive biomarkers (e.g., blood and urine markers) that correlate with molecular lung profiles will enable clinicians to apply personalized interventions at earlier stages and improve patient quality of life. We have employed LC-HRMS-based metabolomics to identify that: 1) mild asthma is metabolically distinct from both moderate and severe asthma, and steroid treatment affects metabolism; 2) oxidative stress and the autotaxin–lysophosphatidic acid axis evidence sex-associated metabotypes in COPD patients; 3) severe asthmatics evidence an elevated urinary tryptophan metabotype profile; and 4) individuals with bronchopulmonary dysplasia have elevated thromboxane metabolites. In addition, we have shown that multi-omics integration drastically improves unsupervised molecular prediction of COPD sub-phenotypes, enabling molecular phenotyping to be performed in small cohorts. These findings demonstrate the power and utility of molecular phenotyping for identifying sub-groups of obstructive lung disease.

17B 2:00 p.m. – 2:15 p.m. | **Metabolite quantitative trait loci provide functional link between FADS2 and lung obstruction in asthmatics**

PRESENTING AUTHOR: ***Rachel Kelly, Brigham and Women's Hospital Harvard Medical School, United States***

CO-AUTHORS: ***Rachel S. Kelly, Bruce Levy, Benjamin A. Raby, Scott T. Weiss, Jessica Lasky-Su***

Rationale: FADS2 (fatty acid desaturase 2)encodes a crucial rate limiting enzyme within the omega-3/omega-6 fatty acid pathways, and has been linked to asthma. Metabolomics is ideally suited to explore the downstream implications of FADS2 variants on the asthmatic phenotype. Methods: Blood collected at recruitment in the Childhood Asthma Management Program was submitted for metabolomic and genome-wide profiling. Metabolite Quantitative Trait Loci (mQTL) analysis was used to identify metabolites associated with 72 SNPs in FADS2. Mediation analysis was conducted to determine if the genetic burden of airway obstruction was mediated through alterations in these metabolites. Results: 495 asthmatic children, including 59 with airway obstruction (%predicted FEV1/FVC<70%) were included. The mQTL (rs7112655) most strongly associated with obstruction (OR: 1.15, p=0.002) also associated with abundance of docosahexaenoate, alpha linolenate and arachidonate. Up to 14% of the effect of this SNP was mediated through these metabolites. Conclusions: In this study, we leverage population-wide integrative-omic data to demonstrate that variants near FADS2 may act as mQTLs driving differential abundance of key inflammatory mediating metabolites that influence asthma severity. The balance of omega-3 versus omega-6 fatty acid conversion regulated by FADS2 in the alpha-linoleic acid pathway is crucial for the resolution of inflammation and dampening of airway hyper-responsiveness. However, mediation analysis suggests that FADS2 may not be exerting its genetic influence on asthma through this pathway alone, but also through its role in the regulation of other metabolic pathways.

17C 2:15 p.m. – 2:30 p.m. | **The Blood Plasma Lipidome Distinguishes Fatalities from Survivors during Human Ebola Virus Disease**

PRESENTING AUTHOR: ***Jennifer Kyle, Pacific Northwest National Laboratory, United States***

CO-AUTHORS: ***J. E. Kyle, K. E. Burnum-Johnson, J. P. Wendler, A. J. Eisfeld, K. G. Stratton, K. K. Weitz, R. D. Smith, Y. Kawaoka, K. M.Waters, T.O. Metz***

The West African Ebola virus (EBOV) outbreak of 2013 to 2016 was the most devastating human EBOV epidemic to date. Much of what is known about EBOV pathogenicity has been elucidated in in vitro and in vivo models. Here we show that the plasma lipidome of human EBOV disease (EVD) reveals striking trends in patients that survived compared with fatalities. Samples were obtained from 11 EVD survivors, 9 EVD fatalities, and 10 healthy control Sierra Leonean volunteers. Total lipid extracts and proteins were isolated and subjected to liquid chromatography-tandem mass spectrometry. Transcriptomics were conducted on plasma PBMCs. Over 420 lipids were identified and quantified across 19 subclasses. Most of the lipids were altered at a subclass level and correlated with patient outcomes (e.g., decreased diacylglycerophophocholine (PC), lyso-PC (LPC) and diacylglycerophophoinositol (PI) among fatalities, and increased diacylglycerophophoserine (PS), and diacylglycerophophoglycerol (PG) in fatalities). Intra-subclass trends also correlated with outcome, both at the intact chain and fatty acid level. All ceramides (Cer) containing 16:0 or 18:0 fatty acids decreased in EVD survivors, whereas all but one PI containing 18:3 or 20:3 fatty acids increased. Ratios of PS lipids also correlated with outcome. Multi-omic data integration supported drastically altered lipid metabolism with EVD. The lipid profiles highlight the probable impact of extracellular vesicles and liver dysfunction on the lipidome, as well as the influence and importance of nutrition during states of critical illness. This research was funded by an administrative supplement to grant U19AI106772, National Institute of Allergy and Infectious Diseases.

17D 2:30 p.m. – 2:45 p.m. | **Serum metabolic signatures differentiate women with repeated embryo implantation failure from recurrent implantation success**

PRESENTING AUTHOR: ***Koel Chaudhury, Indian Institute of Technology Kharagpur, India***

CO-AUTHORS: ***Sourav Roy Choudhury, Mamata V. Joshi, B.N. Chakravarty***

Clinicians duringin vitrofertilization–embryo transfer (IVF–ET) in infertility clinics often encounter a group of women in whom implantation fails repeatedly despite several successful transfers of good quality embryos. Reduced endometrial receptivity or poor embryo quality or inappropriate transfer techniques are attributed for recurrent implantation failure (RIF) in such women. Conversely, a group of women exist who undergo recurrent implantation success (RIS) in each cycle, yet have a poor obstetric history. We were motivated to investigate whether any difference exists at the serum metabolic level between these two groups of women undergoing IVF? Blood samples were collected during implantation window from 28 women with RIF and 24 women with RIS. These women were reporting with primary infertility at the Institute of Reproductive Medicine, Kolkata, India. 700 MHz proton NMR metabolomics study indicated that metabolites largely related to energy metabolism, lipid metabolism and arginine metabolism pathway were significantly up-regulatedin women with RIF. Elevated levels of both ornithine and urea were further supported by the low serum level of endothelial nitric oxide synthase (eNOS), indicating possible impairment in nitric oxide production. However, the interplay between these molecules in RIF is complex and holds merit for further exploration.In-depth studies of the arginine metabolic pathway in endometrial tissues seem necessary to validate our findings. The identification of serum metabolic marker(s) in women with RIF could help with strategies of early therapeutic intervention, which may improve the chances of implantation considerably in women otherwise susceptible to IVF failure.

SESSION 17: INTERNAL MEDICINE	Wednesday June 27	1:30 p.m. – 3:00 p.m.

17E 2:45 p.m. – 3:00 p.m.	A biomarker for idiopathic pulmonary fibrosis identified through genetic epidemiology and a metabolomics-based case-control study

PRESENTING AUTHOR: ***Agustin Cerani, McGill University, Canada***

CO-AUTHORS: ***Agustin Cerani, Stephanie Ross, David A. Schwartz, Paul Wolters, Brent Richards***

Idiopathic pulmonary fibrosis (IPF) is a lethal disease without current effective treatments. There is, therefore, an urgent need to find and validate biomarkers for diagnosis, prognosis, as well as potential drug targets. We propose to combine genomics and metabolomics to meet these objectives. Methods/Results: To elucidate potential metabolites associated with IPF, we used an IPF genome-wide association study (GWAS) that identified novel single nucleotide polymorphisms (SNP) associated with isovaleryl dehydrogenase (IVD), whose inhibition is known to increase the metabolite isovalerylcarnitine (IVC). Concurrently, we collaborated in a metabolite GWAS in healthy subjects that found the same IPF-associated SNP to be associated with IVC blood levels. Through 2-sample Mendelian randomization, we observed that genetically decreased IVC levels were strongly associated with increased risk of IPF ($p< 2.9x10-10$). The non-coding allele that decreased IVC levels increased IVD expression ($p< 3.4x10-12$). Thus, we hypothesized that low IVC levels are associated with higher risk of IPF. To test our hypothesis, we ran a case-control pilot study and found that IVC levels (targeted UPLC-MRM/MS) are decreased in IPF cases relative to controls ($p < 2x10-3$). Therefore, these data strongly suggest IVC's metabolic pathway could play a role in IPF etiology. We are currently confirming our results in a larger case-control study. Conclusion: IPF presents seriously unmet clinical needs. Building on strong preliminary data, we propose that IVC levels are associated with higher risk of IPF. IVC represents a clinically relevant biomarker and its enzyme, IVD, could represent an entirely novel IPF drug target.

SESSION 18: AGRICULTURE	Wednesday June 27	1:30 p.m. – 3:00 p.m.

18A SESSION KEYNOTE 1:30 p.m. – 2:00 p.m.	Identifying compounds of quality in rice grown in Northern Australia

PRESENTING AUTHOR: ***Melissa Fitzgerald, University of Queensland, Australia***

CO-AUTHORS: ***Venea Dara Daygon, Mariafe Calingacion***

The Australian rice industry is exploring different options for expansion,and is currentlytesting whether a rice industrycould flourishin northern Australia. This regionprovides an environment more similar to tropical Asia, than the southern temperate region of Australia where rice has been grown for many years. An industry in northern Australia offers potential for diversevarietiesand potential new markets. In establishing rice in the north, the industry tested the yield and quality of current varieties against some of the new varieties targeted to the north.The same varieties weregrown in both northern and southern Australia over several seasons. The harvested rice samples were provided and we milled the rice, tested its quality,conducted sensory profiling of the samples by cooking them and conducting a preference test with 80 students, then measuring metabollites on several platforms. Sensory profilingrevealed that the rice from northern Australia was not liked due to unpleasant aroma and flavour.Metabolomic analysis of the volatile compounds demonstratedthe presence of apanel of compounds that have unpleasant aroma and low odour thresholds. These compounds includedindole and thiols,and their presencecorrelated with the sensory data. However, some of the varieties did not release indole, indicatinggenetic control for this unpleasant volatile compound.Wethen created mapping populations to determine the genetic basis of indole in order to provide selection tools to rice breeding programs, which led to the discovery that the pathways to indole differ by genetic background.

18B 2:00 p.m. – 2:15 p.m.	Understanding the genetics and chemistry of multifaceted rice grain quality traits using metabolomics and molecular markers

PRESENTING AUTHOR: ***Jeanaflor Crystal T. Concepcion, The University of Queensland, Australia***

CO-AUTHORS: ***Makara Ouk, Mariafe Calingacion, Mary J. Garson, Melissa A. Fitzgerald***

Rice grain quality is a determinant of rice breeding success. Although several studies have independently looked at the different traits of quality to infer the phenotype of the grain, not many studies have identified the various metabolites that are produced in different rice quality types. In recent years, genetic markers have proven to be effective in increasing the efficiency of breeding line selection and therefore shortening the breeding cycle. In complex traits such as rice grain quality, the availability of more specific phenotypes will increase the value of these genetic markers. Many traits of quality in rice are associated with starch; however, lipids interact with starch and have been shown to influence many of the traits of eating quality and aroma. Using a population developed specifically to segregate for many traits of rice quality, we genotyped and phenotyped the progenies for every trait of physical, textural and eating quality, as well as phenotyped for macronutrients, volatile compounds (GCGCTOFMS), lipids (UPLCMSMS) and fatty acids (GCMS). A bioinformatics analysis was conducted using all the phenotype data, in order to understand how each component contributes to the major traits of quality used in rice breeding. A QTL analysis was also conducted to identify candidate genes for each trait of quality, and for each important phenotype. The information will be delivered to rice improvement programs to enable them to select more accurately for particular traits of quality, and for novel quality traits identified here.

18C 2:15 p.m. – 2:30 p.m. **Untargeted Analysis of Volatiles from Brassica oleracea (cabbage) Cultivars linked to Sensory Profiling and Breath Gas Analysis**

PRESENTING AUTHOR: ***Gesine Schmidt, Nofima AS, Norway***

CO-AUTHORS: ***Erika Zardin, Kristine Myhrer, Ingunn M. Vågen, Gerd Guren, John-Erik Haugen, Paula Varela, Grethe Iren Borge***

Cruciferous vegetables (Brassicaceae, cabbages) are a good source for essential nutrients, fiber and health-promoting plant compounds. While consumption of vegetables in Norway has generally increased over the last years, sales of Brassicaceae stagnated. Sensory quality, assortment, availability and knowledge about culinary uses are among the primary drivers of consumers' food choice. Thus, to increase Brassica intake, consumers should have access to a larger spectrum of local produce, clearer product differentiation as well as information about health-related quality. In order to understand variations in flavor (taste and aroma) and their implications for health-related quality, we have grown 50 different cultivars of Brassica oleracea (white cabbage, cauliflower and leafy cabbage) in experimental field trials in Norway. Untargeted analysis of volatile organic compounds (VOC) by dynamic headspace extraction followed by GC-MS on frozen-thawed cabbage revealed that among the ca. 80 VOC detected, five chemical groups were predominant: isothiocyanates, nitriles, sulfides, aldehydes and alcohols, giving each cultivar a distinct volatile profile. To correlate the volatile profile with the sensory impression when eating cabbage, descriptive sensory analysis was conducted by a trained sensory panel assessing appearance, flavor and texture attributes on all raw cabbage cultivars. Further, in vivo mastication with real-time breath gas analysis by proton transfer reaction – time of flight – mass spectrometry (PTR-TOF-MS) were performed on selected raw cabbages, detecting the volatile metabolites dynamically released in the human oronasal cavity during mastication. Results from multivariate correlations between VOC, sensory data and targeted analysis of health-relevant phytochemicals will be presented.

18D 2:30 p.m. – 2:45 p.m. **Juice Index: An integrated Sauvignon blanc grape and wine metabolomics database**

PRESENTING AUTHOR: ***Farhana PInu, New Zealand Institute for Plant and Food Research Ltd, New Zealand***

CO-AUTHORS: ***Farhana R Pinu, Claire Grose, Sergey Tumanov, Victoria Raw, Abby Albright, Lily Stuart, Silas G Villas-Boas, Damian Martin, Roger Harker, Marc Greven***

Although Sauvignon Blanc (SB) grapes are cultivated widely throughout New Zealand, only wines from Marlborough region are famous for their typical varietal combination of herbal and tropical aromas. However, wine quality differs from season to season and within the region, which makesthe continual production of good quality wines is always challenging. Here, we developed a unique database of NZ SB grape juices and wines to develop tools to help winemakers to make blending decisions and assist in the development of new wine styles. Over 400 juices were collected from different regions in NZ over three harvesting seasons (2011-2013), which were then fermented under controlled conditions using a commercial yeast strain Saccharomyces cerevisiae EC1118. Comprehensive metabolite profiling of these juices and wines were performed by gas chromatography-mass spectrometry, and was combined with their detailed oenological and meteorological parameters. Our data clearly show that seasonal variation is more prominent than regional difference in both grape juices and wines. Moreover, we observed a clear variation between the juices and wines from different wineries, thereby highlighting the effect of vineyard management practices on juice and wine composition. In addition, we identified a group of juice metabolites that play central roles behind these variations, which could be used as potential chemical signatures for juice and wine quality assessment. This database is the first of its kind in the world and can be used to develop a predictive tool for wine quality and innovation when combined with mathematical modelling.

18E 2:45 p.m. – 3:00 p.m. **Profiling of fermented brewer's spent grain using GC-MS to discover novel applications for waste biomaterial**

PRESENTING AUTHOR: ***Sachindra T. Cooray, Nanyang Technological University, Singapore***

CO-AUTHORS: ***Wei Ning Chen***

Brewer's spent grain (BSG) is an underutilized by-product of beer manufacturing, generated in vast quantities throughout the world. Even though the majority of the starch present in BSG is removed during the brewing process, this waste material is extremely rich in nitrogen and fibre. However, currently, BSG is disposed in landfills or given away as animal feed without taking any significant use. The objective of this study is to employ fermentation techniques using fungi to enhance BSG's economic value. Fermentation is a preferred pretreatment and valorization technique used for biomass processing. BSG was subjected to solid-state fermentation using food grade fungi and incubated at 300C for 3 days. Afterwards, metabolites from the fermented BSG and unfermented BSG were extracted using solvents and freeze-dried. By employing GC-MS-based targeted and untargeted metabolomics, we were able to observe the metabolite profile changes during the fungi fermentation and distinguish significant changes in metabolites using multivariate statistics. The metabolites attributing to these substantial changes between unfermented BSG and fermented BSG were searched against NIST mass spectral library for identification. We observed enhanced levels in some important metabolites contributing to the total nutritional value of the fermented BSG. Taken together, our study provides evidence of the importance of a metabolomics study to provide information to shed light on fermentation processes. Furthermore, this metabolomics analysis has been useful in identifying novel applications for food waste material.

SESSION 19: FOOD & NUTRITION II	Wednesday June 27	3:30 p.m. – 5:15 p.m.

19A 3:30 p.m. – 3:45 p.m.	Biomarkers of red meat intake - a new tool to study colorectal cancer

PRESENTING AUTHOR: ***Catalina Cuparencu, Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark***

CO-AUTHORS: ***Åsmund Rinnan, Anne Raben, Lars Ove Dragsted***

Red meat intake contrasts white meat through its association to colorectal cancer (CRC) in observational studies. The nature of this association is often debated. No established objective methods exist to assess red meat intake, although such methods are needed to investigate how specific meats affect health outcomes. We aim to discover biomarkers of white, pink and red meats intake, assess their robustness andlink them to putative risk markers for CRC. We therefore conducted a cross-over meal study in healthy volunteers. Each subject was randomized to a sequence of four test meals: chicken, pork, meat and control. Urine samples were collectedup to 48h following intervention, and were analyzed by untargeted LC-ESI-qTOF-MS metabolomics. Univariate and multivariate analyses were used to explore the exposures and discriminate candidate biomarkers. Additionally, the heme iron content (HIC) of the 4 meals was calculated and used to predict any ions that increase proportionally to HIC. Fifteen and seven metabolites were biomarkers for general meat and chicken intake, respectively. Eight metabolites increased with increasing HIC, some of which are collagen related. One additional metabolite is a novel candidate marker for pork and beef intake and already shows robustness by remaining unaffected by chicken, egg white, pea-protein or cheese consumption. The biomarkers are undergoing validation in a cohort of the PREVIEW study (EU-FP7-nr.312057), a 3yintervention with high/low protein diets. PREVIEW also provides measurements of putative CRC risk that will be correlated to the intake of red meat assessed through biomarkers. Preliminary results will be presented.

19B 3:45 p.m. – 4:00 p.m.	You are what you eat: Metabolomic insights into the human diet

PRESENTING AUTHOR: ***Julia M Gauglitz, University of California San Diego, United States***

CO-AUTHORS: ***Morgan Panitchpakdi, Francesca Di Ottavio, Christine M. Aceves, Elizabeth Brown, Nicole Sikora, Katharina Spengler, Lindsay DeRight Goldasich, Greg Humphrey, Tara Schwartz, MacKenzie Bryant, Alan K. Jarmusch, Rachel J. Dutton, Rob Knight, Pieter C. Dorrestein***

The human diet is incredibly diverse, sourced from all major ecosystems, and its chemical and microbial composition is largely unknown. Food metabolites, food-derived microbial metabolites, and potentially food microbes, contribute to our health, mood, and perception of how foods taste in unknown ways. We have begun a community-based multi-omics approach (the Global FoodOmics project) to characterize foods, using untargeted LC-MS/MS and 16S rRNA sequencing. 2273 different samples have been analyzed, representing fungal (e.g. mushrooms), plant-based, animal-based foods, fermented foods, beverages, and complex foods which have been submitted by dozens of contributors, including donations from more than a dozen local companies, 3 local farms, and several local gardens. One distinguishing aspect is extensive metadata, containing more than 140 descriptors including processing method, ingredients, taxonomy, and a curated ontology for prepared foods. To investigate molecular identity, all mass spectra were deposited (MassIVE) and annotated using GNPS. Molecules from the following major categories were identified: lipids, small peptides, flavonoids, mycotoxins, pesticides, food additives and others. 16S rRNA amplicon sequencing revealed distinctions in microbial community profiles consistent with the metabolomics. Molecular networking of American Gut samples, ~2400 human fecal samples, and the 2237 food samples was performed in GNPS. 521 identifications were shared while 1528 and 2327 were unique to the American Gut and FoodOmics samples, respectively. We are poised to integrate our metabolome and microbiome analyses of food with those from projects such as the American Gut project in order to gain insight into how diet-derived metabolites impact gut health.

19C 4:00 p.m. – 4:15 p.m.	Development of solid phase analytical derivatization method for gas chromatography/mass spectrometry -based metabolomics of foods containing high amounts of sugars

PRESENTING AUTHOR: ***Eiichiro Fukusaki, Osaka University, Japan***

CO-AUTHORS: ***Moyu Taniguchi, Hiroshi Kawasaki, Ryoichi Sasano, Masahiro Furuno, Taichi Koizumi, Takeharu Nakahara, Shuichi Shimma***

Food metabolomics is a useful technology to understand the correlation between metabolites and their food function. Gas chromatography/mass spectrometry (GC/MS) is one of the most commonly used among the metabolomics tactics. However, GC/MS-based method sometimes faces serious difficulties in analyzing high amounts of sugars containing food samples. The most significant problem is serious drop of sensitivity due to ion source pollution caused by excess existence of sugars. It might cause overlooking slight amount of metabolites including amino acids, organic acids, etc. Especially, it is almost impossible to detect such slight amount of metabolites if they are co-eluted with sugars. In this study, solid phase analytical derivatization (SPAD) method was developed using a combination of cation exchange solid matrix and anion exchange solid matrix. The method removes excess sugars and facilitates derivatization (methoximation followed by trimethylsilylation) of amino acids and organic acids. The method was applied to Japanese sweet rice wine (Mirin) which contains high amounts of sugars. Consequently, GC/MS-based metabolomics of mirin was successfully performed. When SPAD method and the conventional method (unextraction, only dilution with water and derivatization) were compared, nine kinds of sugars were not detected by SPAD method among sixteen kinds of sugars detected by the conventional method. Moreover, although only two amino acids were detected by the conventional method, fifteen amino acids and seven organic acids were newly detected by SPAD method. These results demonstrated that the practical utility of SPAD method is high, and the applicability of GC/MS-based metabolomics would be expanded to various foods.

19D 4:15 p.m. – 4:30 p.m.	A comprehensive strategy for identification of food origin

PRESENTING AUTHOR: *Zora Jandric, International Atomic Energy Agency, Austria*

CO-AUTHORS: *Valentina Centonze, Marivil Islam, Andrew Cannavan, Russell Frew*

New applications of metabolomics have emerged demonstrating that the technique is an important tool for food authenticity testing. Mislabelling and adulteration is a problem in many areas of the food industry. In addition to economic and food safety implications, counterfeit foods may not have the same nutritional value as authentic products. Food commodities that are expensive and are part of complex supply chains are particularly vulnerable. Examples include oranges and honey, which are of interest because of their high nutritional value and high content of phenolics. Food authentication depends on measuring features that can discriminate foods of different origins. Using untargeted profiling, it is possible to acquire comprehensive information on the composition of a metabolite pool which can be used to identify food origin. In this research, we investigated the use of untargeted spectroscopic (FTIR-ATR) and metabolomic (UPLC-QToF-MS) measurements with subsequent data processing by chemometrics, for classification of the geographical origin of oranges and honeys, as well as the floral origins of honey. The two testing strategies were applied to 152 orange (Italy, Spain, South Africa, and India) and 232 honey (Greece, New Zealand, Australia and USA) samples obtained directly from producers and from a variety of retail sources. Linear discriminant analysis (LDA) and data-driven soft independent modelling of class analogy (DD-SIMCA) were applied to predict the origins of orange and honey samples. The correct classification rates achieved ranged from 85% to 100%. This innovative strategy could potentially be used for food origin authentication purposes.

19E 4:30 p.m. – 4:45 p.m.	Comprehensive, plasma-metabolome analysis of Japanese Kampo medicine “maoto” in healthy human subjects

PRESENTING AUTHOR: *Katsuya Ohbuchi, Tsumura & Co., Japan*

CO-AUTHORS: *Takashi Matsumoto, Chika Shimobori, Nozomu Sakurai,Hiroyuki Kitagawa, Masaya Munekage, Kazune Fujisawa, Yasuhiro Kawanishi, Tsutomu Namikawa, Hirotaka Kushida, Akinori Nishi, Masanori Arita, Kazuhiro Hanazaki, Masahiro Yamamoto*

Traditional herbal medicine (THM) is expected to overcome limitations of a single chemical agent through combined effects produced by various crude materials. To understand the complex nature of THM, comprehensive investigation of its components and the phenotypes was conducted. In this study, a Kampo medicine for febrile symptoms, maoto, was administered in four healthy subjects. Blood samples were collected before and after the maoto treatment, 7-time points in total. The plasma samples were subjected to wide-targeted and non-targeted metabolome analyses. Wide-targeted analysis on endogenous metabolites revealed that the administration decreased essential amino acids and increased various lipid mediators including EPA and DHA. Major maoto ingredients, such as ephedrine, prunasin, cinnamic acid and glycyrrhetinic acid, were detected in the plasma and showed diverse pharmacokinetics. Most of the ingredients were detected at early phase. On the contrary, glycyrrhetinic acid was absorbed into the systemic blood 4 hours after administration, Plasma concentration of ephedrine is strongly correlated with those of essential amino acids, as the key component in maoto pharmacology. Further analysis was performed using LC-Orbitrap MS. Many of compounds detected in maoto were undetected, but their derivatives, such as aglycons, sulfate- or glucuronate conjugates were detected in different concentrations among the subjects. These results suggest that comprehensive profiling is essential for clarifying the complex nature of THM and for developing the responder biomarkers.

19F 4:45 p.m. – 5:00 p.m.	A combination of lipidomics, MS imaging and PET scan imaging reveals differences in cerebral activity in rat pups according to the lipid quality of infant formulas

PRESENTING AUTHOR: *Jean-Charles Martin, INRA, France*

CO-AUTHORS: *Nacima Aidoud, Charlotte Baudry, Bernadette Delplanque, Benjamin Guillet, Karl Fraser, Pascale Leruyet*

We evaluated the relevance of adding docosahexaenoic :arachidonic acids (3:2) (DHA+ARA) to 2 representative commercial infant formulas, on brain activity and brain and eye lipids in an artificially reared rat pups model. The formula lipid background was either a pure vegetal blend, or a dairy-fat with the vegetal blend (1:1). Results at weaning were compared to Sham-dam milk fed pups. Brain functional activity was determined by PET-scan imaging, the brain and eye fatty acid and lipid composition by targeted and untargeted lipidomics, and DHA brain regional location by mass-spectrometry imaging. The brain functional activity was normalized to sham-controls when adding DHA+ARA in formulas. More than 400 and 700 lipid species were measured in both brain and eye, respectively. The DHA lipid species represented 17% in whole brain and 22% in the eye of the total lipid species. More than 2/3 of tissue DHA-glycerolipids remained insensitive to the dietary challenge. However, the nutritionally sensitive DHA lipidome was better correlated to brain functional activity than the sole DHA content ($r = 0.70$ vs $r = 0.48$, $P < 0.05$, respectively). In that respect, DHA phosphatidylserine species appeared especially influenced by the diets and related to brain activity. Brain DHA regional distribution revealed by MS imaging was more affected by the formula lipid background than the provision of PUFA. In conclusion, providing DHA+ARA in formulas alters the nervous tissues DHA content and lipidome in the neonate to be closer to dam milk fed controls, and normalizes brain functional activity.

SESSION 20: METABOLOMICS & MODEL ORGANISMS	Wednesday June 27	3:30 p.m. – 5:15 p.m.

20A 3:30 p.m. – 3:45 p.m.	Cheminformatics used with high resolution mass spectrometry identifies novel epimetabolites in mouse gene knockouts

PRESENTING AUTHOR: ***Oliver Fiehn, UC Davis, United States***

CO-AUTHORS: ***Elizabeth Axton, Megan Showalter, Dinesh Barupal, Sajjan Mehta, Diego Pedrosa, Matthew Mueller, Gert Wohlgemuth***

In untargeted metabolomics by LC-MS or GC-TOF MS, less than 30% of the compounds are identified. Without structural identifications, these molecules cannot reveal biological mechanisms or biochemical pathways. Epimetabolites transform primary, canonical metabolites into modified versions to cellular and physiological roles such as regulation of histone demethylation in epigenetics, inflammation in tissue injury, insulin sensitivity, cancer cell invasion, stem cell pluripotency status, inhibition of nitric oxide signaling and others. The West Coast Metabolomics Center has developed software for identifying epimetabolites in untargeted metabolomics, starting from the BinBase metabolome database covering more than 2,000 studies, mass spectra for more than 100,000 lipids in LipidBlast that are now integrated into the MassBank of North America, over releasing MS-DIAL for untargeted data processing and finally MS-FINDER for computationally identifying novel compounds by in-silico fragmentation prediction. We investigated 30 mouse knockout genotypes generated by the 'International Mouse Phenotyping Consortium' using five untargeted and targeted mass spectrometry platforms: primary metabolism, lipidomics, biogenic amines, bile acids/steroids and oxylipins. Combined, we identified over 880 unique metabolites in mouse plasma, including methylated, hydroxylated, acetylated and epoxidated epimetabolites. Surprisingly, all 30 mouse gene knockouts had clear metabolic phenotypes with more than 10-fold differences in plasma metabolites, including for genes with unknown function and genes with known cellular but unknown metabolic functions. We used ChemRICH and MetaMapp for pathway and enrichment statistics, validated expected metabolic functions for several knockouts and showcase the use of our tools for finding novel and unexpected metabolic phenotypes.

20B 3:45 p.m. – 4:00 p.m.	Modeling meets Metabolomics – The WormJam Consensus Metabolic Reconstruction as basis for metabolic investigations in Caenorhabditis elegans

PRESENTING AUTHOR: ***Michael Witting, Research Unit Analytical BioGeoChemistry, Helmholtz Zentrum München, Germany***

CO-AUTHORS: ***Janna Hastings, Nathan Lewis, Nicolas Le Novere, Frank Schroeder, Olivia Casanueva, Christoph Kaleta***

Metabolism is one of the attributes of life and supplies energy and building blocks to organisms. Therefore, understanding metabolism is crucial for the understanding of complex biological phenomena. Despite having been in the focus of research for centuries, our picture of metabolism is still incomplete. Metabolomics, the systematic analysis of all small molecules in a biological system, aims to close this gap. In order to facilitate such investigations a blue print of the metabolic network is required. Recently, two metabolic network reconstructions for the model organism Caenorhabditis elegans have been published, each having unique features. We have established the WormJam Community to merge and reconcile these (and other unpublished models) into a single consensus metabolic reconstruction. In a series of workshops and annotation seminars this model was refined with manual correction of incorrect assignments, metabolite structure and identifier curation as well as addition of new pathways. The WormJam consensus metabolic reconstruction represents a rich data source not only for in silico network-based approaches like flux balance analysis, but also for metabolomics, as it includes a database of metabolites present in C. elegans, which can be used for annotation. Here we present the process of model merging, correction and curation and give a detailed overview of the model. In the future it is intended to expand the model towards different tissues and put special emphasizes on lipid metabolism and secondary metabolism including ascaroside metabolism in accordance to their central role in C. elegans physiology.

20C 4:00 p.m. – 4:15 p.m.	NMR-based metabolomic analysis of the marine vent hyperthermophile Thermococcus onnurineus NA1

PRESENTING AUTHOR: ***Kehau A. Hagiwara, Institute of Marine and Environmental Technology; NIST, United States***

CO-AUTHORS: ***Frank T. Robb, Daniel W. Bearden***

Thermococcus onnurineus NA1 is a marine hyperthermophilic, carbon monoxide utilizing archaeon that grows optimally at 80°C. This microorganism can grow using heterotrophic or autotrophic (using CO) metabolism under anaerobic conditions. Recently, T. onnurineus has garnered interest as an industrial source of hydrogen gas. As with other members of the genus Thermococcus, it is known to biosynthesize osmoprotective and thermoprotective compounds with potential benefits in pharmaceutical and cosmetic industries. Consequently, previous studies have documented environmental stress responses and altered transcriptome profiles during heterotrophy and carboxydotrophy in this microorganism. However, the metabolic pathways utilized by T. onnurineus have yet to be elucidated. This study was undertaken to fill those gaps and is the first metabolomic exploration of T. onnurineus NA1. We utilized nuclear magnetic resonance (NMR) spectroscopy to characterize the metabolome profile in a robust, repeatable, and quantitative approach to identify small molecules produced by T. onnurineus. Changes in global metabolic profiles were recorded during growth with different carbon sources and studying the global metabolomes of these organisms. Initial statistical analyses of 1H NMR spectra revealed distinct metabolic differences corresponding to the mode of metabolism. Analysis of HSQC spectra allowed the identification of significant metabolites or chemical entities and, in future, may provide insight to the metabolic plasticity of T. onnurineus NA1.

20D 4:15 p.m. – 4:30 p.m. **Metabolomics analysis of the toxic effects of the production of lycopene and its precursors**

PRESENTING AUTHOR: ***April Miguez, Georgia Institute of Technology, United States***

CO-AUTHORS: ***April Miguez, Monica McNerney, Mark Styczynski***

In the field of metabolic engineering (the effort to manipulate cells to produce valuable chemicals), efforts for fine temporal and product specificity control have recently received great attention. Unfortunately, manipulating any given metabolic pathway to create a desired product can indirectly affect other parts of metabolism, making fine-tuning cells a challenge. In recent work, we have designed lycopene-producing E. coli for use as a low-cost diagnostic biosensor. To increase the rate of lycopene production, we heterologously expressed the mevalonate pathway to increase precursor availability. We found that simultaneous induction of these pathways increases lycopene production, but surprisingly, induction of the mevalonate pathway before induction of the lycopene pathway decreases both lycopene production and growth rate. Here, we sought to characterize the metabolic changes the cells may be undergoing during expression of these heterologous pathways using two-dimensional gas chromatography coupled to mass spectrometry. We found that the metabolic impacts of producing non-toxic levels of lycopene are of much smaller magnitude than the metabolic changes inherent to batch growth, and that cells could recover from mevalonate-associated toxicity if lycopene production was not also induced. The metabolites homocysteine and homoserine exhibited profiles that potentially link them to the growth inhibition caused by induction of mevalonate production. Based on this analysis, we predicted that extracellular methionine supplementation would limit mevalonate-associated growth inhibition, and we validated this prediction. This suggests potential future avenues toward engineering increased lycopene biosynthesis, as well as the general utility of metabolomics to inform metabolic engineering.

20E 4:30 p.m. – 4:45 p.m. **Metabolomics Analysis of Hypoxic Preconditioning in C. elegans**

PRESENTING AUTHOR: ***Haiwei Gu, Arizona State University, United States***

CO-AUTHORS: ***Dongfang Wang, Chun-Ling Sun, Qiang Fei, C. Michael Crowder, Sunny Lihua Cheng, Julia Yue Cui, Daniel Raftery***

Hypoxic preconditioning (HP) consists of low to mild oxygen reduction that is below the damage threshold, which increases resistance to subsequent hypoxia or even different noxious stimuli. Currently, a better understanding of the HP mechanism is of increasing interest because of its therapeutic potential and insights into metabolic adaptation and cell death. Most previous studies have focused on changes in proteins and gene expression, while metabolic alterations due to HP are largely unknown. Here we report the first metabolomics analysis of HP in C. elegans to provide a complementary and promising approach to further understand HP. Two C. elegans strains were used, N2 (wild type) and cep-1 (the sole C. elegans orthologue of mammalian tumor suppressor p53). The metabolites extracted from the worms were measured using a targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) platform. Both univariate and multivariate statistical analyses showed that HP caused significant and wide metabolic alterations to cep-1 and N2 worms. Generally, metabolism is attenuated under HP, which helps to consume less nutrients and promote cell survival. In particular, amino acid, nucleoside/nucleotide, glucose, and lipid metabolisms, along with the kynurenine pathway were affected by the HP process, which fits well with previous findings that HP and hypoxia induce broad genetic reprogramming in metabolism. This study indicates that metabolomics is a promising approach to understand HP. The significantly altered metabolites and metabolic pathways observed in this study could become targets for future approaches to enhance cellular survival, which ultimately will have significant potential in clinical settings.

20F 4:45 p.m. – 5:00 p.m. **Random Forest and Gaussian Graphical Model analysis of untargeted metabolomics data reveals strong dietary fat effects but little association with obesity phenotypes in the metabolome of genetically diverse Drosophila melanogaster larvae**

PRESENTING AUTHOR: ***Laura K Reed, University of Alabama, United States***

CO-AUTHORS: ***Vishal Oza, Joseph Aicher***

Obesity is a complex disease, shaped by both genetic and environmental factors such as diet. In this study, we use untargeted metabolomics and Drosophila melanogaster (the fruit fly), to model how diet and genotype shape the metabolome of obese phenotypes. Drosophila larvae from 16 distinct outbred genotypes were raised both on normal (ND) and high fat (HFD) diets, producing two phenotypic classes; half the genotypes stored more triglycerides on a ND relative to the HFD, while the rest stored more triglycerides on a HFD. We characterized 270 metabolites with definitive chemical IDs and 80 that were chemically unidentified. Using the Random Forest algorithm, we determined the “important” metabolites (many being unidentified) that differed between the HFD and ND larvae, as well as between the triglyceride phenotypic classes. Surprisingly, we found that on a HFD, the omega fatty acid oxidation pathway was up-regulated. In addition, the triglyceride storage phenotype and free fatty acid levels were uncorrelated, indicating that HFD fed Drosophila larvae have distinct fatty acid profiles, but those profiles does not propagate into triglyceride storage differences. However, dipeptides did show moderate differences between the phenotypic classes. Using Gaussian Graphical Models for the HFD and ND diet flies, we characterized the changes in metabolic network structure between the two diets. The edge symmetric difference was 0.786, indicating very different topologies. Taken together, these results show that, in the context of obesity, metabolomic profiles under distinct dietary conditions may not be reliable predictors of phenotypic outcomes in a genetically diverse population.

SESSION 21: NEW METHODS & TECHNOLOGIES II	Wednesday June 27	3:30 p.m. – 5:15 p.m.

21A 3:30 p.m. – 3:45 p.m.	A rapid quantitative method for large cohort metabolic fluxomics with unambiguous identification of all 13C-labeled metabolites and labeling cites

PRESENTING AUTHOR: ***Huiru Tang, Fudan University, China***

CO-AUTHORS: ***Qianfen Wan, Yulan Wang***

Quantifying and identifying 13C-labeled metabolites are essential for fluxomics with 13C-NMR as a natural choice. However, long relaxation and low sensitivity make 13C-NMR unsuitable for large cohorts and low abundance metabolites. Inversion-detection methods such as 1H-13C HMQC/HSQC improve sensitivity for quantifying the 13C-labeled metabolites with time consumption remaining a serious burden when hundreds of samples need measuring. Here, we report an HMQC-based 1H-NMR method for absolute quantification of 13C-labeled metabolites by taking polarization-transfer efficiencies and relaxation into consideration for different 13C-labeled groups; the naturally abundant TSP is conveniently used as chemical-shift reference and internal-standard for quantification. We validated this method with both the 13C-labeled and natural metabolites with known concentrations including 13C-U-alanine, 13CH3-methionine, 13C-U-glucose, 13C-formic acid together with natural alanine, lactate, glycine, uridine, cytosine and hypoxanthine. We further applied this method to analyze 13C-U-glucose flux in HepG2 cells infected with hepatitis B virus. We showed that HBV infection increased the cellular uptake of glucose, stimulated the glycolysis, and hijacked the pentose phosphate and hexosamine pathways to support the synthesis of RNA and DNA and nucleotides to facilitate HBV viral replication. This absolute quantitative and rapid method for large cohort metabolomics flux analysis (5-6 samples/hr) also offers unambiguous identification of all 13C-labeled metabolites with confirmed labeling cites. References: Q.F. Wan, et al, Anal Chem, 2017, 89, 3293–3299. I. Marin-Valencia, et al., Cell Metab. 2012, 15, 827-837. S. Heikkinen, et al. JACS. 2003, 125, 4362-4367. H. Xie, et al, Cell Metab. 2014, 19, 795-809.

21B 3:45 p.m. – 4:00 p.m.	NMR Guided Mass Spectrometry for Absolute Quantitation of Human Blood Metabolites

PRESENTING AUTHOR: ***G. A. Nagana Gowda, University of Washington, United States***

CO-AUTHORS: ***Danijel Djukovic, Lisa Bettcher, Haiwei Gu and Daniel Raftery***

While absolute quantitation of metabolites using NMR is relatively straightforward, this is not the case for mass spectrometry (MS), owing to numerous factors that include: the need for isotope labeled internal standards, their prohibitively high cost, the need to maintain their concentrations close to the target metabolites and/or the requirement of calibration curve for each target metabolite. Focused on alleviating this bottleneck, a new approach is described, in which metabolites from a single serum specimen quantitated based on our recently developed NMR method were used for absolute metabolite quantitation using MS. Concentrations thus derived for human serum samples using two different mass spectrometers exhibited excellent correlation with the NMR data (R2 > 0.99) with a median CV of 3.2%. Intriguingly, however, a few metabolites including glutamine, pyroglutamic acid, glucose and sarcosine correlated poorly with NMR; the results for glutamine and pyroglutamic acid agree with the phenomenon of glutamine cyclization discovered recently. Such poor correlations challenge the implicit assumption that generally almost all metabolites are stable during MS analysis and open a new avenue for identification of metabolites that are potentially fragile to ionization sources during MS analysis. The NMR guided MS quantitation approach is simple, easy to implement and, by obviating the need for an internal standard for each metabolite, offers a new avenue for quantitation of blood metabolites using MS, routinely. The demonstration that NMR and MS data can be compared and correlated opens new avenues for exploiting their combined strength for biomarker discovery and unknown metabolite identification.

21C 4:00 p.m. – 4:15 p.m.	Developing novel tools for in vivo functional analysis of gut microbiotas

PRESENTING AUTHOR: ***Fariba Assadi-Porter, University of Wisconsin-Madison, United States***

CO-AUTHORS: ***Hannah V. Carey, Marco Tonelli***

Host diet influences the structure and function of the gut microbiota in species that regularly consume food. We developed a metabolome-microbiome platform (MMP) that uses novel isotopic labeling to specifically trace microbiotas' metabolism in breath, cecal contents, and host tissues. We combined cavity ringdown spectroscopy (CRDS) and 1H-[13C]-NMR-based metabolomics to assess real-time breath and metabolome profiles. We applied MMP to hibernating ground squirrels as a test-bed to assess the role of certain bacteria contributing to seasonal changes in diet. Previously, we showed that the long-term fast of hibernation increases relative abundance of bacterial taxa that are adept at degrading abundant plant glycans in summer versus their reduction in winter. Here, we determined the functional significance of seasonally changing microbiotas by gavaging Spring and Summer squirrels and aroused hibernators in Winterwith 13C-labeled plant– and host–derived glycans, and comparing against their respective intraperitoneal applications. Measurement of 13CO2/12CO2 (δ13C) in breath was used as an index of bacterial degradation of 13C-substrates and linked to observed changes in 13C-edited metabolome profiles. Compared with robust responses in Summer, peak changes in δ13C after 13C-inulin gavage in Late Winter hibernators were nearly abolished, whereas squirrels in Early Winter and Spring showed variable responses consistent with their transitional microbiotas. Metabolome profiles mirrored seasonal changes in 13C-labeled short chain fatty acids. These suggest that during hibernation, the gut microbiota gradually loses the capacity to degrade a plant-derived glycan, possibly due to a combination of reduced bacterial abundance and a shift away from taxa that prefer dietary substrates.

21D 4:15 p.m. – 4:30 p.m.	A High Throughput Method for Free Fatty Acid Profiling for Epidemiological Studies by Multisegment Injection-Non-Aqueous Capillary Electrophoresis-Mass Spectrometry

PRESENTING AUTHOR: ***Sandi Azab, Department of Chemistry and Chemical Biology, McMaster, Canada***

CO-AUTHORS: ***Ritchie Ly, Philip Britz-Mckibbin***

Fatty acids (FA) are metabolites of high interest in clinical medicine due to their myriad roles relevant to human health and nutrition while also representing biomarkers of habitual diet. Gas chromatography (GC) remains the gold standard method for FA analysis as it offers excellent resolution, but it suffers from low sample throughput due to complicated sample workup and chemical derivatization procedures, as well as long elution times. Herein, we introduce multisegment injection-non-aqueous capillary electrophoresis-mass spectrometry (MSI-NACE-MS) as a high throughput platform for reliable determination of free FA in human serum with quality assurance. Following a simple methyl-t-butyl ether extraction procedure using 50 µl of human serum,seven or more samples are analyzed simultaneously within a single run (< 4 min/sample), including a quality control that monitors for long-term system drift and allows for batch correction. Optimization of both background electrolyte composition and coaxial sheath liquid conditions wasperformed to enhance sensitivity with sub-micromolar detection limits (0.1-2.5 µM) for over 20 FA with good linearity over a 50-fold concentration range (R2> 0.990) and acceptable intermediate precision with a median coefficient of variance of 11%. Additionally, a cross-platform comparison of MSI-CE-MS relative to GC/MS was performed on 50 serum samples demonstrating good mutual agreement for free FA determination with a minimal average bias. MSI-NACE-MS offers a high throughput yet low cost platform for routine profiling of free FAin serum extracts on a single instrument(> 200 samples/day) with high data fidelity as required for large-scale epidemiological studies or clinical investigations.

21E 4:30 p.m. – 4:45 p.m.	Separating metabolites based on their structural properties: Building a path to molecular structure prediction

PRESENTING AUTHOR: ***Gang Xing, Pfizer Inc., United States***

Cellular metabolites consist of vastly diverse chemical structures, many of which are highly hydrophilic and difficult to retain using traditional reverse-phase chromatography. We present a mixed mode LC strategy, ERLIC Salt Protonation Double Ion Exchange (ESPDI) chromatography, developed to retain and systematically elute these traditionally challenging analytes. ESPDI exploits a combination of pKa, hydrophilicity, and hydrogen-bonding ability to achieve baseline separation of multiple structural isomers including hexoses and hexose phosphates. Coupled to the Thermo QExactive Plus mass spectrometer with negative mode ESI, the method is capable of detecting ~400 metabolites spanning a wide chemical diversity. These 400 metabolites with retention times served as a training set to create a molecular structure predictor model, with good correlation (R2=0.82) between predicted and actual retention times. Interfacing with comprehensive databases, this model may be applied to computationally reduce the number of potential structures a mass spectral feature represents, enabling streamlined “unknown” identification. Beyond untargeted capabilities, the method’s application to investigate cellular fluxes using isotopically labeled nutrients will be shown.

21F 4:45 p.m. – 5:00 p.m.	Exploring ion-chromatography mass spectrometry (IC-MS/MS) for untargeted metabolic profiling of central metabolism and beyond

PRESENTING AUTHOR: ***James Stephen Oswin McCullagh, University of Oxford, United Kingdom***

CO-AUTHORS: ***John Walsby-Tickle, Joan Gannon, Elisabete Pires, Thomas Cadoux-Hudson, Joseph Harvey, David Hauton, Christopher J. Schofield***

Central carbon metabolism drives the production of cellular energy, maintains cellular redox balance and converts sugars to metabolic precursors. These processes, which include glycolysis, pentose phosphate pathway (PPP), TCA cycle, purine, pyrimidine and extended sugar phosphate metabolism, are highly conserved from an evolutionary perspective yet are often associated with perturbations in a wide range of diseases including cancer. HILIC, ion-pairing chromatography and derivatised gas chromatography have traditionally been used with mass spectrometry for analysis of polar and ionic compounds but coverage of central metabolism is not always comprehensive or robust and has remained problematic. The development of ion-chromatography coupled directly to mass spectrometry (IC-MS) provides an approach well suited to coverage of central metabolism and beyond. We evaluated IC-MS in a multi-chromatographic, untargeted workflow and addressed the comprehensive characterisation metabolism in a range of cell, tissue and blood plasma extracts. Here we demonstrate it can comprehensively and robustly capture metabolites of central metabolism and additional physiologically ionic compounds. We applied this workflow to investigate metabolic changes associated with R132H mutations in isocitrate dehydrogenase (IDH1), a mutant form of the TCA cycle enzyme found in over 70% of grade 2 & 3 glioma, 30% of myeloid leukaemia and around 10% of cholangiocarcinoma. We characterised established metabolic perturbations but also discovered changes in metabolic pathways not previously reported. We use this discovery to demonstrate the efficacy and exciting potential of IC-MS as a complementary chromatographic approach for untargeted metabolomics.

SESSION 22: DIABETES, DIET AND GLUCOSE INTOLERANCE	Thursday June 28	10:30 a.m. – 12:00 p.m.

22A 10:30 a.m. – 10:45 a.m.	Glucagon-dependent substrate selection in hepatic gluconeogenesis revealed by stable isotope tracers and metabolomics

PRESENTING AUTHOR: ***Wenyun Lu, Princeton University, United States***

CO-AUTHORS: ***Russell A. Miller, Yuji Shi, Junyoung O. Park, Morris J. Birnbaum, Joshua D. Rabinowitz***

Under fasting conditions, the level of pancreatic hormone glucagon increases, promoting the release of glucose from the liver by accelerating the breakdown of glycogen, enhancing gluconeogenic flux, and increasing the hepatic consumption of amino acids. In type 2 diabetes, dysregulated glucagon signaling contributes to elevated hepatic glucose output and fasting hyperglycemia that occurs in this condition. Yet the mechanism by which glucagon stimulates gluconeogenesis remains incompletely understood. Contrary to the prevailing belief that glucagon acts primarily on cytoplasmic and nuclear targets, we find a glucagon-dependent stimulation of mitochondrial anaplerotic flux from glutamine that increases the contribution of this amino acid to the carbons of glucose generated during gluconeogenesis, using stable isotope tracers and metabolomics. This effect is PKA-dependent and is associated with glucagon-stimulated calcium release, activation of mitochondrial alpha-ketoglutarate dehydrogenase, and increased glutaminolysis. Mice with reduced levels of hepatic glutaminase 2 (GLS2), the enzyme that catalyzes the first step in glutamine metabolism, show lower glucagon-stimulated glutamine-to-glucose flux in vivo, and GLS2 knockout results in higher fasting plasma glucagon and glutamine with lower fasting blood glucose levels in insulin-resistant conditions. These data emphasize the importance of gluconeogenesis from glutamine, particularly in pathological states of increased glucagon signaling, while suggesting a possible new therapeutic avenue to treat hyperglycemia.

22B 10:45 a.m. – 11:00 a.m.	The impact of obesity on metabotype of type 1 and type 2 diabetes in youth

PRESENTING AUTHOR: ***Yuanyuan Li, University of North Carolina at Chapel Hill, United States***

CO-AUTHORS: ***Yuanyuan Li, Delisha Stewart, Wimal Pathmasiri, Susan McRitchie, Elaine Urbina, Elizabeth Mayer-Davis, Dana Dabelea, Susan Sumner***

The incidence of type 1 and type 2 pediatric diabetes (T1D and T2D) increased substantially in the past 20 years, but most studies have focused on adults. This research aims to understand the metabotypes in pediatric diabetes. Plasma samples from T1D, T2D, and controls were selected from existing adolescent diabetes research bio-repositories. These samples were divided into lean and obese sub-groups by BMI percentage. Targeted LC-MS and NMR metabolomics were used to assess perturbation of endogenous metabolites that differentiate control, T1D and T2D. The monitored metabolites included amino acids, acylcarnitines, phospholipids, biogenic amines, and sugars. Although T1D and T2D showed similar hyperglycemia and chronic inflammation, T1D displayed much greater metabolic perturbation Vs. control, than T2D Vs. control. Pediatric diabetes presented a decrease of plasma amino acids, a slight decrease of long-chain acylcarnitines; and an increase of short-chain acylcarnitines, possibly due to better adaptive metabolic plasticity in children. Trimethylamine N-oxide (TMAO) decreased in pediatric T1D and T2D. In contrast to lean controls, obese controls demonstrate insulin resistance, chronic inflammation, and perturbation of amino acid metabolism, albeit without hyperglycemia. Obesity impacted pediatric T1D and T2D differently. Obesity influenced dyslipidemia in T1D, causing significant increases of LDL and total cholesterol, accompanied by profile changes of phosphatidylcholines and lysophosphatidylcholines. For T2D, obesity rather aggravated gluconeogenesis and further impaired glucose metabolism, perturbing metabolites such as creatinine, kynurenine, taurine, and hexose. Our findings provide insights into the biochemical basis of pediatric diabetes, which will be helpful for creating personalized therapies.

22C 11:00 a.m. – 11:15 a.m.	High protein feeding in humans increases short chain triglycerides associated with hepatic de novo lipogenesis

PRESENTING AUTHOR: ***Evelina Charidemou, University of Cambridge, United Kingdom***

CO-AUTHORS: ***Xuefei Li, Elise Orford, Ben McNally, James A. West, Tom Ashmore, Julian L. Griffin***

Rationale: Energy-dense, low-satietyWestern dietshaveled toincreased prevalence of obesity andType 2 diabetes mellitus (T2DM). Over half of diabeticssuffer fromdyslipidaemiaand30-50% experience delayed rates at which chyme exits the stomach (gastric emptying; GE).Weaimedto define, in healthy humans,howsolid/liquid ratiosof mealsandmacronutrient compositionaffectGEandlipid metabolism. Methods: After a 12-hour fast, subjects (n=9) consumedone offiverandomisedmealscontaining 13C-octanoic acid.A standardised2 MJmeal (S) (low liquid carbohydrate; CHO), was modified to contain purely liquidCHO(MS). MSwas furtheraltered to provideisoenergeticmealshigh in fat (HF), protein (HP) andCHO(HC). Isotope ratio-mass spectrometrydetermined13C-enrichment inbreathexhalates for GE rates,andblood lipid profiles were determined byliquid chromatography-mass spectrometry. Results: Macronutrient composition did not affect GE. HF (lowest liquid CHO) had the fastest GE rate, whilst of HC, the reverse was true.We also demonstrated a correlation between stomach-released fat and plasma lipids. However, HP had more abundant plasma lipids than anticipated. We illustrated that HP, high in glutamate, increased plasma triglycerides(TAGs)associated withde novolipogenesis (DNL) and in liver-secreted lipoproteins. Furthermore, palmitate-13C-enrichment was increased from 13C-glutamate in cultured AML-12 hepatocytes. Increased levels of glutamate increased DNL TAGs and expression of DNL genes dose-dependently. Conclusions: IncreasedTAGs indicative of DNL were detected after HP. Moreover, thisfolloweda 12-hour fast, which typically supresses DNL, and maythereforehavegeneralimplications for high-protein diets and T2DM.

22D 11:15 a.m. – 11:30 a.m. **Chronic kidney disease is associated with altered metabolic response to insulin**

PRESENTING AUTHOR: ***Baback Roshanravan, University of Washington, United States***

CO-AUTHORS: ***Leila R. Zelnick, Daniel Djucovic, Haiwei Gu PhD, Kristina Utzschneider, Thomas R. Ziegler, Jessica Alvarez A, Jorge Gamboa, Bryan Kestenbaum, Jonathan Himmelfarb, Steven E. Kahn, Daniel Raftery, Ian H. de Boer***

The inflammatory milieu of chronic kidney disease (CKD) leads to decreased sensitivity to the actions of insulin. In order to evaluate potential mechanisms and manifestations of insulin resistance in CKD, we examined changes in the plasma metabolome before and after infusion of intravenous insulin among participants with and without CKD. A total 95 non-diabetic underwent a hyperinsulinemic euglycemic clamp procedure, among whom 59 had CKD defined by an estimated glomerular filtration rate <60 mL/min per1.73m2.We used a targeted LC/MS metabolomics platform to quantify 74 plasma metabolites before the clamp (fasting) and during steady-state insulin infusion. We used linear mixed models regressinglog-transformed metabolitesconcentrations on sample time (clamp versus fasting), CKD status (CKD versus non-CKD), and the interaction of the two, adjusting for age, sex, race/ethnicity, and weight.Insulin infusionlead to marked changesin metabolites involved in lipid metabolism, fatty acid metabolism, and nicotinamide metabolism.The pathways most impacted by insulin weretryptophan metabolism, the TCA cycle, and nitrogen metabolism. In particular, insulin administration resulted in marked changes in the plasma concentrations of niacinamide, taurine, prostaglandin E, threonine, and valine. CKD was associated with an attenuated response to insulin in plasma concentrations of 17 of 74 (23%) metabolites, of which 10 were amino acids. CKD was associated with altered insulin response inniacinamide, arachidonic acid, glutamine/glutamate, and taurine/hypotaurine metabolic pathways. These findings suggest disruption in mitochondrial metabolism as a potential mechanism or manifestation of insulin resistance in CKD.

22E 11:30 a.m. – 11:45 a.m. **Data Pipeline for Clinical Diabetes Metabolomics - From Targeted Mass-Spectra to Patient Stratification**

PRESENTING AUTHOR: ***Tommi Suvitaival, Steno Diabetes Center Copenhagen, Denmark***

CO-AUTHORS: ***Linda Ahonen, Ashfaq Ali, Cristina Legido Quigley, Lars Ove Dragsted, Peter Rossing***

Introduction Targeted metabolomics is entering diabetes clinics to provide a blood-molecular snapshot reflecting patient's diet, lifestyle, disease status and treatment. High-throughput quantitative analysis of relevant metabolites can also provide new insights into disease subtypes and comorbidities that are otherwise hard to characterize. Methods We quantify metabolites using a selected reaction monitoring method built on ultra-high performance liquid chromatography triple-quadrupole mass spectrometry. We have developed a flexible and methods-rich computational pipeline for pre-processing, normalization, calibration, quality control and statistical analysis of the mass spectral data--all coupled with dynamic reproducible reporting. The pipeline is built on Skyline, which is a cutting-edge targeted mass spectrometry tool broadly used in proteomics, and R, which as a flexible and reproducible statistical computing platform. We demonstrate the approach with results from a small cross-sectional study of type 1 diabetes (T1D) patients with or without a kidney complication (macro- or normo-albuminuria; N=25+25, respectively, all with 3 replicate analyses). We test for metabolite-wise differences with a random-effects model, taking into account sample replication and adjusting for 14 relevant clinical variables. Results We produced an automated quality control report with visualizations of the measured metabolites individually and as whole. The report covers peak picking, technical variation, normalization and calibration. In the study, 7 amino acids were elevated in macro-albuminuric patients (FDR<0.05), and also two bile acids and kynurenine were deregulated. Conclusion We have developed a high-throughput pre-processing and quality control pipeline for targeted metabolomics. These solutions are contributing to the implementation of precision medicine at a diabetes clinic.

22F 11:45 a.m. – 12:00 p.m. **GC-MS-based metabolomics to study cardiac substrate metabolism in a dish**

PRESENTING AUTHOR: ***Julia Ritterhoff, University of Washington, United States***

CO-AUTHORS: ***Lauren Abell, Rong Tian***

The adult heart uses mostly fatty acids (FAs) for its ATP generation and glucose to a lesser extent. However, for in vitro studies, adult cardiomyocytes (CM) are cultured in FA-free medium, which poorly reflects conditions in vivo. Using GC-MS-based targeted metabolomics, this study compared the intermediary metabolism of isolated CMs cultured in standard medium containing 5.5mM glucose (G-medium) to CMs cultured with 5.5mM glucose and a physiological level of long-chain fatty acids (0.4mM, F+G-medium). There were no differences in morphology, viability or ATP content in CMs cultured in G- or F+G-medium up to 48hr. FA supplementation resulted in a 3-fold reduction in glucose uptake from the medium and a high uptake of FA. Targeted GC-MS analysis showed higher levels of TCA intermediates and glutamic acid in CM cultured in F+G-medium. Stable isotopic tracing in CM cultured in F+G-medium showed that U-13C FA labeled 50-60% and U-13C glucose labelled 5-10% of TCA intermediates, suggesting FA oxidation (FAO) is predominant. In CM cultured in G-medium, U-13C glucose labelled 10-20% of TCA intermediates, suggesting a significant contribution of unlabeled amino acids (aa) from the medium. Analysis of the spent medium showed that FA supplementation markedly reduced the consumption of glutamine (42%), leucine (53%) and isoleucine (75%), suggesting that FAO reduced aa utilization. Together, these data show that adult CMs cultured in medium containing glucose primarily consume aa. FA supplementation overcomes the dependence on aa and moderately reduces glucose utilization, rendering the contribution of FAO to a level comparable to in vivo cardiac metabolism.

SESSION 23: BIOSPECIMENS, SAMPLE PREPARATION, AND QUALITY	Thursday June 28	10:30 a.m. – 12:00 p.m.

23A 10:30 a.m. – 10:45 a.m.	mQACC: A community-led effort to strengthen quality assurance (QA) and quality control (QC) practices in metabolomics research and reporting

PRESENTING AUTHOR: ***Jonathan Mosley, U.S. Environmental Protection Agency, United States***

CO-AUTHORS: ***Abbas Bandukwala, Dan Bearden, Richard Beger, Bianca Bethan, Clary Clish, Surendara Dasari, Leslie Derr, Warwick Dunn, Annie Evans, Steve Fischer, Thomas Flynn, Thomas Hartung, David Herrington, Rick Higashi, Ping-Ching Hsu, Christina Jones, Maureen Kachman, Helen Karuso, Gary Kruppa, Katrice Lippa, Padma Maruvada, Ioanna Ntai, Claire O'Donovan, Mary Playdon, Dan Raftery, Daniel Shaughnessy, Amanda Souza, Tim Spaeder, Barbara Spalholz, Fariba Tayyari, Baljit Ubhi, Mukesh Verma, Tilmann Walk, Ian Wilson, Keren Witkin, Krista Zanetti***

The Metabolomics Quality Assurance and quality Control Consortium (mQACC) is a community-led effort to strengthen quality assurance (QA) and quality control (QC) practices for untargeted metabolomics research and reporting. mQACC was established through an inaugural meeting, funded by the National Institutes of Health, held in October 2017. Data from a survey published by Dunn, et al. (Metabolomics, 2017) was used to establish meeting objectives: 1.) Identify the most useful metrics for assessing study and data quality for untargeted metabolomic studies; 2.) Identify and prioritize processes to ensure appropriate reporting of QA/QC data; and 3.) Identify and prioritize the types of test materials that are needed in the field of metabolomics for QA/QC in untargeted studies. Key priorities for each meeting objective were identified and scored for importance, resulting in several primary themes. Although priorities were identified for long-term efforts, three immediate priorities were moved forward: 1.) Prepare a meeting report for publication; 2.) Prepare a manuscript documenting the complete experimental procedure for untargeted metabolomics, including the QC practices; and 3.) Identify 2-3 reference materials that need to be developed quickly. mQACC currently includes over 40 representatives from the United States, Europe, and Asia, including instrument manufacturers, commercial laboratories, and government and academic stakeholders. The consortium has continued to be active since the inaugural meeting, holding monthly teleconferences and establishing working groups to address these initial priorities. In addition to describing the mQACC priorities, we will highlight how the metabolomics community can get involved in this important effort.

23B 10:45 a.m. – 11:00 a.m.	Exploring the impact of sample preparation to profile intracellular metabolites of 3D organoids and 2D cell cultures

PRESENTING AUTHOR: ***Caroline Mathon, PMI R&D, Philip Morris Products S.A., Switzerland***

CO-AUTHORS: ***Caroline Mathon, David Bovard, Quentin Dutertre, Sandra Sendyk, Mark Bentley, Julia Hoeng, Arno Knorr***

Cell culture metabolomics has expanded incredibly in recent years, as it has many potential applications and advantages, especially in the field of toxicology. The first critical step in exploring the cellular metabolome is the sample preparation. For a metabolomics analysis, an ideal sample preparation combines a maximal number of extracted metabolites for a large number of samples/replicates in a highly reproducible and accurate manner and merges consistency across several studies over an extended time frame. Sample preparation is dependent upon the cell culture type (adherent or suspended cells, 2D cell cultures or 3D organoids) as well as the different cell lines. The aim of this study is to evaluate several extraction processes for intracellular metabolite profiling of both 2D and 3D bronchial and liver cell cultures prior to their analysis by liquid chromatography high-resolution accurate-mass spectrometry (LC-HRAM-MS) using isotope-labeled internal standards. The exogenous metabolite extraction was evaluated by exposing different cell cultures to a mixture of chemicals, like nicotine and nicotine-derived nitrosamine ketone. On the other hand, the endogenous metabolite extraction was performed using a comprehensive metabolite standard library. Results concerning process efficiency, matrix factor, and extraction yields were compared among the different extraction processes. The step of extract evaporation (N2 stream and speed vacuum) was identified as the main step for our sample preparation protocol that can influence the response of some metabolites drastically and increase the variability of metabolites measured by LC-HRAM-MS. Additionally, matrix factor and extraction yields were different based on the analyzed cell cultures.

23C 11:00 a.m. – 11:15 a.m.	The use of methanol for extraction creates dozens of artifacts in untargeted metabolomics studies

PRESENTING AUTHOR: ***Rainer Schuhmacher, BOKU University Vienna, IFA-Tulln, Austria***

CO-AUTHORS: ***Maria Doppler, Claudia Sauerschnig, Christoph Bueschl***

Many metabolomics studies use extraction mixtures containing methanol. Reaction of metabolites with methanol can result in artifacts (compounds formed during sample handling, storage or measurement). The aim of this work was to evaluate if and to which extent methanol derived solvent artifacts are formed when a routine untargeted metabolomics workflow is used. For tracking of artifacts a stable isotope assisted approach was applied. Wheat leaf samples were extracted with a mixture of native and deuterium labeled methanol and extracts were measured with LC-HRMS i) immediately after extraction, ii) once per day for one week after storage of extracts at 10°C in the autosampler and iii) after storage at -20°C or -80°C for 9 days. Automated data evaluation resulted in 73 solvent artifacts deriving from various substance classes which represent about 10% of all detected wheat leaf metabolites. Remarkably, about half of the detected artifacts were formed de novo. The abundance was within the same range as for all plant metabolites, demonstrating that not only low abundant signals were affected. Furthermore, additional artifacts were formed during storage and the abundance of those, immediately formed during extraction, increased over time. In contrast, extracts stored frozen did not significantly change over time. Our study clearly demonstrates that care needs to be taken when methanol is used for extraction to not misinterpret artifacts as biological compounds. Moreover, to prevent further artifact formation in the extracts, we recommend to either measure extracts immediately or to store them in a freezer until measurement.

23D 11:15 a.m. – 11:30 a.m.	Optimizing LC-QToF based untargeted fecal metabolomics

PRESENTING AUTHOR: ***Ken Cheng, Chalmers University of Technology, Sweden***

CO-AUTHORS: ***Carl Brunius, Rikard Landberg***

Feces is a complex matrix offering the possibility to study interactions between diet, host and gut microbiota by analyzing the metabolites from all these origins. NMR and GC-MS are commonly used approaches for untargeted fecal metabolomics, whereas there are currently fewer LC-MS based applications. Among those LC-MS applications, different procedures for sample preparation, including different solvents for extraction and fecal materials have been used. Currently, there is a lack of standard practice for fecal sample preparation and analysis. Our aim was thus to evaluate how different parameters during sample preparation, including extraction solvents (MeOH, acetonitrile and water), feces-to-solvent ratio (1:1 to 1:40) and fecal material handling (fresh and freeze-dried), affected metabolite recovery. The aim was further to obtain a high-throughput, robust method with broad metabolite coverage. Several outcome aspects were assessed using an xcms-based pipeline: numbers of extracted features, feature intensities and distribution of missing features. Furthermore, reproducibility and intensity of selected components were evaluated. Results showed that MeOH as extraction solvent gave the best analytical performance and the feces-to-solvent ratio 1:5 provided optimum trade-off between metabolite coverage and saturation in the QTOF MS. Furthermore, the distribution of missing features was similar in fresh and freeze-dried fecal samples, with missing features depending predominantly on whether features were present in individual samples. In addition, freeze-drying greatly facilitated normalizing sample wet weight to dry matter content. This analytical approach will be applied to study host × microbiota × diet interactions in several large-scale observational as well as dietary intervention studies.

23E 11:30 a.m. – 11:45 a.m.	The influence of culture media upon observed cell secretome metabolite profiles: the balance between cell viability and data interpretability

PRESENTING AUTHOR: ***Evangelia Daskalakis, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Sweden***

CO-AUTHORS: ***Nicolas J. Pillon, Anna Krook, Antonio Checa, Craig E. Wheelock***

The application of metabolomics to investigating the cell secretome has garnered popularity due to the method's large-scale data output,biochemical insights, and prospects for novel compound discovery. However, there are no standardized protocols for the use of cell growth media, a factor that can exert profound effects upon the detected metabolites, and thus in the interpretability of the resulting data. Herein, we applied a liquid chromatography-high resolution mass spectrometry-based metabolomics approach to examine the influence of 5 different media combinations upon the obtained secretome of two phenotypically different cell lines: human embryonic kidney cellsand L6 rat muscle cells. These media combinations were, M1: Medium-199, M2: Medium-199 + 2% fetal bovine serum (FBS), M3: Dulbecco's Modified Eagle's Medium (DMEM), M4: DMEM + 2% FBS and M5: Krebs-Henseleit Modified Buffer. The effect of incubation (37°C) vs. refrigeration (4°C) on DMEM medium over a 24 hr period was also investigated. A total of 53 polar metabolites exhibiting differential patterns on cell type- and medium-specific basis were identified. We observed that choice of media was the primary contributor to the secreted metabolite profile detected. The addition of FBS resulted in unique detected metabolites, compared to media-only controls (M199 and DMEM alone). Glutamine and pyroglutamate, were more abundant in incubated relative to refrigerated DMEM medium.These data indicate that the experimental design of cell secretome studies is a primary driver of the observed metabolite profile. Based upon these findings, we highlight a series of considerations in designing a cell secretome-based metabolite profiling experiment.

23F 11:45 a.m. – 12:00 p.m.	MS multiplatform untargeted metabolomics for the analysis of dried blood spots (DBS) from female soccer players before and after competition

PRESENTING AUTHOR: ***Vinicius Veri Hernandes, Unicamp/CEU San Pablo, Brazil***

CO-AUTHORS: ***Francisco Javier Rupérez, Coral Barbas***

Dried blood spots (DBS) are an interesting sample collection method which can be alternative to venipuncture, due to the many advantages presented such as minimum blood withdraw and easier in situ collection and storage. We will present results from DBS collected from female soccer players from Brazil Under-20 National Team during the 2015 South American Championship, in the quest for cardiovascular damage evidence caused by the intense and short-term effort. In total, 7 matches were played and 4 points of collection were established: before and after both first and last matches. Samples were collected on ordinary filter paper, due to easier access and time limitations. Prior to GC-MS, CE-MS and LC-MS analysis following CEMBIO protocols, a single extraction method was optimized considering extraction technique (vortex or bead-assisted), total extraction time and solvent composition. Final conditions were selected according to the number of features and their coefficient of variation. After instrumental analysis, results from the players were normalized with different methods to achieve better QC samples clustering for the following multi and univariate statistical analysis. Among the compounds that showed significant variations, amino acids were the class of metabolites that changed the most. Remarkably, branched chain amino acids, which have been previously related to insulin resistance and cardiac complications, were significantly correlated with the total distance traveled by each player during the game.

SESSION 24: PLANT METABOLOMICS II	Thursday June 28	10:30 a.m. – 12:00 p.m.

24A 10:30 a.m. – 10:45 a.m.	Annotation of specialized metabolite innovation by plants in the form of acylsugar metabolomes: strategies and challenges

PRESENTING AUTHOR: ***A. Daniel Jones, Michigan State University, United States***

CO-AUTHORS: ***Steven A. Hurney, Thilani M. Anthony, Xiaoxiao Liu, Banibrata Ghosh, Pengxiang Fan, Abigail M. Miller, Gaurav Moghe, Bryan Leong, Yann-Ru Lou, Daniel J. Murphy, Zoe Tsang, Satya Swathi Nadakuduti, Cornelius S. Barry, Robert L. Last***

Gene duplication and mutation in plants drive the evolution of metabolic novelty, particularly in plants and fungi, and advances in genomic technologies have driven exploration of the evolution of plant chemistry in non-model plants. Our profiling of acylsugar metabolites has shown that numerous members of the plant family Solanaceae accumulate varied groups of acylsugar specialized metabolites, leading to discoveries of BAHD acyltransferases responsible for metabolic acylations involved in acylsugar biosynthesis in tomato, Petunia, and Salpiglossis sinuata. Untargeted LC/MS profiling of acylsugars in plant species across the family Solanaceae has revealed evidence of vast acylsugar diversity, encompassing at least 23 different acylgroups. Acylsugars across the family exhibit diversity in the numbers of acyl groups, their positions of attachment, and heterogeneity of carbohydrate cores. Chromatographic, LC/MS, and NMR techniques are challenged to distinguish members of this family of compounds where there are billions of potential permutations that might be assembled from known building blocks. Many isomers are not distinguished by MS/MS spectra, and purified authentic standards are scarce. We present new evidence of acylsugars based on inositol and glycoinositol cores from a distant relative of tomato (Solanum quitoense) and propose recommendations for distinguishing isomeric acylsugar metabolites.

24B 10:45 a.m. – 11:00 a.m.	Semi-targeted discovery of new natural products using diagnostic fragmentation filtering

PRESENTING AUTHOR: ***Mark W Sumarah, Agriculture and Agri-Food Canada, Canada***

CO-AUTHORS: ***Justin B. Renaud***

Natural products are often biosynthesized as classes of related compounds that share structural similarities. When analyzed by LC-MS/MS, these compounds often produce diagnostic product ions or neutral losses. Parent ion and neutral loss scanning have traditionally been performed using a triple-quadrupole mass spectrometer, allowing for detection of precursor ions with a common product ion or neutral loss. High resolution platforms including time-of-flight and Orbitrap mass analyzers can't perform these specific acquisitions; however, they can achieve the same outcome through post-acquisition data processing. We have developed a software module for MZmine capable of mining high resolution, data-dependent acquisition (DDA) datasets for MS/MS spectra with target product ion(s) and/or neutral losses, in what we define as diagnostic fragmentation filtering (DFF). This DFF software has led to the discovery a new class of structurally related cyclooctadepsipeptides in cultures of Fusarium avenaceum, as well as multiple microcystin algal toxins in fresh water that would evade traditional targeted screening. Similarly, we used this DFF approach in the screening for new analogues of the mycotoxin fumonisins, where we discovered novel non-aminated fumonisins produced by species of Aspergillus. These compounds provided new insight into fumonisin biosynthesis and their mechanism of toxicity. DFF is a powerful yet underutilized semi-targeted data analysis approach that could lead to new discoveries in multiple analytical fields. The development of this MZmine module will allow for more widespread use of this important technique.

24C 11:00 a.m. – 11:15 a.m.	Primary metabolite responses of Brachystegia boehmii and Colophospermum mopane to fire stress in Miombo and Mopane African woodlands

PRESENTING AUTHOR: ***Carla António, Instituto de Tecnologia Quimica e Biologica Antonio Xavier, Portugal***

CO-AUTHORS: ***Jossias A. Duvane, Tiago F. Jorge, Ivete Maquia, Natasha Ribeiro, Ana Ribeiro-Barros***

Miombo and Mopane are ecological and economic important woodlands from Africa, highly affected by a combination of climate change factors, and anthropogenic fires. Although most species of these ecosystems are fire tolerant, the mechanisms that lead to adaptive responses (metabolic reconfiguration) are unknown. In this study, we have characterized the primary metabolite composition of typical legume trees from these ecosystems, namely, Brachystegia boehmii (Miombo) and Colophospermum mopane (Mopane) subjected to different fire regimes. Fresh leaves from each species were collected in management units and landscapes across varied fire frequencies in the Niassa National Reserve and Limpopo National Parkin Mozambique. Primary metabolites were extracted and analyzed with a well-established GC-TOF-MS metabolomics platform. In B. boehmii, 39 primary metabolites were identified from which seven amino acids, two organic acids and two sugars increased significantly, whereas in C. mopane, 41 primary metabolites were identified from which eight amino acids, one sugar and two organic acids significantly increased with increasing fire frequency. The observed changes in the pool of primary metabolites of C. mopane might be related to high glycolytic and TCA-cyle rates, which provided increased levels of amino acids and energy yield. In B. boehmii, the high levels of amino acids might be due to inhibition of protein biosynthesis. The osmoprotectant and ROS scavenging properties of accumulated metabolites, in parallel with a high-energy yield, might support plant survival under fire stress.

24D 11:15 a.m. – 11:30 a.m.	Rapid Screening of Ellagitannins in Natural Products via Targeted Reporter Ion Triggered Tandem Mass Spectrometry

PRESENTING AUTHOR: ***Jeremiah J Bowers, Clemson University, United States***

CO-AUTHORS: ***Harsha P. Gunawardena, Anaëlle Cornu, Ashwini S. Narvekar, Antoine Richieu, Denis Deffieux, Stéphane Quideau, Nishanth Tharayil***

Complex biomolecules present in their natural sources have been difficult to analyze using traditional analytical approaches. Ultrahigh-performance liquid chromatography (UHPLC-MS/MS) methods have the potential to enhance the discovery of a less well characterized and challenging class of biomolecules in plants, the ellagitannins. We present an approach that allows for the screening of ellagitannins by employing higher energy collision dissociation (HCD) to generate reporter ions for classification and collision-induced dissociation (CID) to generate unique fragmentation spectra for isomeric variants of previously unreported species. Ellagitannin anions efficiently form three characteristic reporter ions after HCD fragmentation that allows for the classification of unknown precursors that we call targeted reporter ion triggering (TRT). We demonstrate how a tandem HCD-CID experiment might be used to screen natural sources using UHPLC-MS/MS by application of 22 method conditions from which an optimized data-dependent acquisition (DDA) emerged. The method was verified not to yield false-positive results in complex plant matrices. We were able to identify 154 non-isomeric ellagitannins from strawberry leaves, which is 17 times higher than previously reported in the same matrix. The systematic inclusion of CID spectra for isomers of each species classified as an ellagitannin has never been possible before the development of this approach.

24E 11:30 a.m. – 11:45 a.m.	Compositional diversity of Traditional Chinese Medicine Ge-Gen-Tang

PRESENTING AUTHOR: ***Kaoru Yoshida, Sony Computer Science Laboratories, Inc., Japan***

The quantity and variety of compositions of herbal medicines are influenced by various factors related with their materials, formulations and manufacturing processes. One of the oldest formulations of Traditional Chinese Medicine,Ge-Gen-Tang, which consistsof seven different crude drugs (Ge-Gen, Ma-Huang, Gui-Zhi, Bai-Shao, Sheng-Jiang and Da-Cao), is now widely used in Asia mainly for treating the early stage of cold. We collected commercial powdered products of Ge-Gen-Tang from six different manufactures, S1-4 from Japan, S5 from Taiwan and S6 from Hongkong, and analyzed their compositions. Through LC-LTQ-Orbitrap-MS with the positive ion mode, total 3467 peaks were detected for mass <1500. Using our newly developed computational analysis system, Categorical Mapper, 2516 different formula were assigned to 3509 peaks, of which 1400 formula were provided with chemical information. After identifying each formula as one composition, individual compositions were classified into 61 categories based on their chemical, biological and medical properties. In hierarchical cluster analysis, S2-4 labeled with the same formulation of the component crude drugs were separated from each other. S1, S4 and S6 were relatively close to each other and the others were individually separated. S5 was highest for Ma-Huang, Gui-Zhi and Bai-Shao specific compositions, reflecting its formulation. For terpenoids, S5 was highest, while S2 was lowest, reflecting their aromas. For flavonoids, S3 was highest, while S5 was lowest, reflecting their colors. For pesticides and herbicides, S6 were highest, followed by S5. Thus, the compositional diversity of theseGe-Gen-Tang products has been elucidated in this study.

24F 11:45 a.m. – 12:00 p.m.	Combination of global and tracer based stable isotope labeling allows enhanced characterization of submetabolomes and unknowns: Investigation of the phenylalanine derived submetabolome of Fusarium Head Blight in wheat

PRESENTING AUTHOR: ***Maria Doppler, BOKU University Vienna, IFA-Tulln, Austria***

CO-AUTHORS: ***Christoph Bueschl, Bernhard Kluger, Barbara Steiner, Justyna Rechthaler, Hermann Buerstmayr, Marc Lemmens, Gerhard Adam, Rainer Schuhmacher***

Untargeted metabolomics approaches are often limited by identification of metabolites which complicates biological interpretation. We adapted our routinely used stable isotope labeling LC-HRMS based workflow and combined global metabolome and tracer labeling. This approach does not only allow separation of biological data from background and noise signals, but also helps to classify the detected metabolites into structure classes according to their metabolic origin of the tracer. Additionally, the tracer related information helped to narrow down often numerous database hits for each metabolite of the submetabolome. In this study this approach was used for the comprehensive description of the phenylalanine derived submetabolome of wheat under different conditions. 13CO2 was used for global metabolome labeling and 13C9 phenylalanine was taken as a tracer in order to create a reference list of all detectable wheat derived metabolites. Those metabolites were analyzed in more detail in a study investigating resistance mechanisms of wheat against the plant pathogen Fusarium graminearum. At five different time points after treatment with the pathogen or water as a control, samples were harvested, milled, extracted and measured with LC-HRMS. Automated data processing allowed the detection of about 1000 plant metabolites including 175 metabolites belonging to the Phe-submetabolome. The presentation will illustrate the new isotope-assisted metabolomics approach and focus on the very diverse Phe-derived submetabolome.and its implication in resistance against Fusarium head blight.

SESSION 25: WELLNESS AND AGING	Thursday June 28	1:30 p.m. – 3:00 p.m.

25A 1:30 p.m. – 1:45 p.m.	Metabolomics Analysis Reveals Altered BCAA Metabolism in Insomnia Patients

PRESENTING AUTHOR: ***Arjun Sengupta, University of Pennsylvania, United States***

CO-AUTHORS: ***Elizabeth Harders, Anup Sharma, Ubeydullah ER, Allan I Pack, Matthew S Kayser, Philip Gehrman, Aalim M Weljie***

Approximately 1/3 of a general population experiences symptoms of insomnia. Clinically, insomnia is associated with physiological hyperarousal, but relationships with metabolism are unclear. The bidirectional relationship of insomnia with adverse medical outcomes is well known, however, the underlying mechanisms remain underexplored. Here we use comprehensive metabolomics to gauge the metabolic effect of insomnia in otherwise healthy individuals. NMR metabolic profiling of serum at high temporal resolution showed distinct changes in the metabolic profile in insomnia patients compared to age- and sex-matched good sleepers over two days. Insomnia subjects manifested clear metabolic desynchrony in terms of altered circadian oscillation of circulatory metabolites along with significantly different global metabolic profile from healthy controls. Nighttime metabolism of insomnia patients were dominated by altered branched chain amino acid catabolism and increased accumulation of glucose. These observations suggest that insomnia is associated with quantitative metabolic dysregulation and supports the hyperarousal hypothesis. These results were compared to a study of HIV patients with co-morbid depression under treatment for insomnia with comprehensive serum metabolomics analysis using NMR spectroscopy, polar LC-MS/MS and untargeted lipidomics. Improvement of the insomnia severity index (ISI) post treatment was found to strongly correlate with changes in BCAA and branched chain keto acid (BCKA) metabolites along with long chain acylcarnitines and several lipid features. These results suggest that BCAA catabolism is mechanistically linked with occurrences of insomnia irrespective of the cause. This result also connects epidemiological evidence that insomnia may lead to other metabolic disorders such as diabetes and metabolic syndrome.

25B 1:45 p.m. – 2:00 p.m.	Plasma Metabolite Biomarker Score Segregates Individuals between Adequate versus Insufficient Sleep

PRESENTING AUTHOR: ***Christopher Depner, University of Colorado Boulder, United States***

CO-AUTHORS: ***Paul J Bisesi, Rachel R Markwald, Charmion Cruickshank-Quinn, Kevin Quinn, Nichole Reisdorph, Kenneth P. Wright Jr.***

Introduction: An objective and easily assessed biomarker of insufficient sleep has great potential to enhance clinical assesmentsof overall sleep health, advance clinical care targeting sleep health, and contribute to precision medicine. Here, we analyzed the plasma metabolome in humans undergoing experimental sleep restriction to identify small molecule biomarkers associated with insufficient sleep. Methods: We conducted a cross-over randomized 15 day in-laboratory study where 16 (8M/8F) healthy participants aged 22.4±4.8y (mean±SD) completed three baseline days (9h scheduled sleep opportunity/night) followed by five day insufficient (5h/night), and adequate (9h/night) sleep conditions. Blood was collected every 4h across 24h on the final day of baseline, insufficient, and adequate sleep conditions, and plasma was analyzed by untargeted liquid chromatography/mass-spectrometry. Results: Partial least squares discriminant analysis VIP scores identified 34 potential metabolite biomarkers of insufficient sleep. Out of these 34 metabolites, 6 significant metabolites were selected based on logistic regression analysis and used to calculate a weighted biomarker score as: $\sum \beta k^*$metabolite-k abundance, where βk is the regression coefficient for metabolite-k. The area under a receiver operator curve for this biomarker score to discriminate insufficient sleep versus baseline was 0.929 (95% CI 0.848-0.988), indicating “excellent” performance. Conclusion: We identified a biomarker score of insufficient sleep based on 6 plasma metabolites with the potential to improve quantitative assessments of overall sleep health. These findings set the stage for larger trials to confirm and improve biomarker performance and to develop and analyze the efficacy of precision medicine based countermeasures targeting the negative health consequences of sleep loss.

25C 2:00 p.m. – 2:15 p.m.	Determinants of the urinary and serum metabolome in children from six European populations

PRESENTING AUTHOR: ***Hector C. Keun, Imperial College London, United Kingdom***

CO-AUTHORS: ***Chung-Ho E. Lau, Alexandros P. Siskos, Lea Maitre, Oliver Robinson, Toby Athersuch, Elizabeth J. Want, Leda Chatzi, Martine Vrijheid, Muireann Coen***

Background. Metabolic phenotyping of urine and serum can help deconvolve the link between early life exposure and disease risk predisposition, yet to date we lack large-scale metabolome studies in children that combine analyses of these biological fluids. We sought to address this and to understand the major determinants of the metabolome in children by exploiting a unique biobank established as part of the HELIX early-life exposome project (http://www.projecthelix.eu) Methods. Metabolic phenotypes of matched urine and serum samples from 1,192 children recruited from birth cohorts in six European countries (aged 6 – 11) were measured by high-througput 1H nuclear magnetic resonance (NMR) spectroscopy and a targeted LC-MS/MS metabolomic assay (Biocrates AbsoluteIDQ p180 kit). Results. Metabolic associations to BMI z-score included a novel association with urinary 4-deoxyerythreonic acid (from threonine catabolism), valine, p-cresol sulphate, pantothenate, serum carnitine and serum branched-chain amino acids. Dietary-metabolite assoications were identified including urinary creatine with meat intake, urinary hippurate with vegetables and fruits, and urinary proline betaine and scyllo-inositol with fruit intake. Metabolic pathway correlations were also identified including a link between serum threonine and production of urinary 4-deoxyerythronic acid. Conclusions. Using a multi-platform metabolic phenotyping approach, we have established a pan-European reference metabolome for urine and serum from healthy children, identifying a novel metabolic association between threonine catabolism and BMI. The six European populations studied share common metabolic characteristics and metabolic associations with age, gender, BMI z-score and diet. This study provides a novel resource for investigating the links between the exposome and child health.

25D 2:15 p.m. – 2:30 p.m. **The effect of simulated shift work on circulating metabolite rhythms**

PRESENTING AUTHOR: ***Debra J Skene, University of Surrey, United Kingdom***

CO-AUTHORS: ***Elena Skornyakov, Namrata R. Chowdhury, Rajendra P. Gajulad, Benita Middleton, Brieann C. Satterfield, Kenneth Porter, Hans P.A. Van Dongen, Shobhan Gaddameedhi***

Metabolic profiling has revealed clear time-of-day rhythms in human plasma. During night shifts behavioural schedules (e.g. sleep and meal times) are shifted and become misaligned to the circadian timing system. In light of the increased risk of metabolic disorders in shift workers, we investigated the effect of this misalignment on metabolite rhythms. Healthy participants (n=14, 25.8±3.2 y, 4 females) underwent a 7-day in-laboratory study. Participants were randomized to a 3-day simulated day or night shift schedule followed by a 24-h constant routine (CR) protocol (designed to expose endogenous rhythms free of confounds from food, sleep, light, activity, and posture). Plasma obtained every 3 h during the CR was analysed for markers of the central circadian clock: melatonin, cortisol. Metabolites were assayed using targeted LC/MS metabolomics. Of the 132 circulating metabolites analysed, 65 showed significant 24-h rhythmicity following either or both simulated shift schedules. However, only 3 metabolites maintained the same phase alignment relative to clock time, similar to that observed for melatonin and cortisol. By contrast, 24 metabolites showed a ~12-h phase shift following night shift compared to day shift; 19 metabolites lost rhythmicity following night shift; and 19 metabolites showed significant rhythmicity following night shift only. Thus for >90% of metabolites, 24-h rhythmicity was not locked to the central circadian clock. Rather, their rhythms aligned with the behavioral timing of the prior 3-day simulated shift schedule. These findings provide a window onto metabolic pathways potentially involved in the elevated risk of metabolic disorders in shift work.

25E 2:30 p.m. – 2:45 p.m. **A systems biology approach to identify a fuel source of cold induced thermogenesis in brown fat in young and old mice.**

PRESENTING AUTHOR: ***James E. Cox, University of Utah, United States***

CO-AUTHORS: ***Claudio J. Villanueva, Judith Simcox, Gisela Geoghegan, John Alan Maschek, Claire L. Bensard, Marzia Pasquali, Ren Miao, Sanghoon Lee, Lei Jiang, Ian Huck, Erin E. Kershaw, Anthony J. Donato, Udayan Apte, Nicola Longo, Jared Rutter, Renate Schreiber, Rudolf Zechner***

Cold-induced thermogenesis is an energy-demanding process that protects endotherms against a reduction in ambient temperature. Glucose and free fatty acids liberated from white adipose tissue are known fuel sources for heat production but do not completely account for all the energy expended to drive thermogenesis. To identify additional fuel sources, an untargeted UPLC-QToF-MS lipidomics analysis was performed on plasma from 3- and 24-month old mice exposed to 4°C versus 24°C for 5 hours. Cold exposed mice showed increased levels of long chain acyl-carnitines (Fold change >2, p-value<0.01). These results were confirmed by a validated LC-QQQ-MS quantitative assay. To identify the source of these acyl-carnitines, siRNA knockdown studies targeting hepatic Cpt1a/b show a requirement for hepatic acyl-carnitine synthesis for thermogenesis. A tracer approach was employed to determine the fate of circulating acyl-carnitines. Injection of 14C-palmitoylcarnitine to mice demonstrated that brown adipose tissue (BAT) took up 8-fold more acyl-carnitines during cold exposure. To verify that acyl-carnitines are metabolized by BAT, 13C-palmitolycarnitine was incubated with brown adipocytes. Using GC-MS, label was observed to be incorporated in TCA cycle metabolites. In addition, with aging, mice show reduced plasma acyl-carnitine levels in response to the cold and display a cold-sensitive phenotype. This can be reversed with carnitine or palmitoylcarnitine supplementation. The holistic approach used in this study demonstrates a mechanism of thermogenesis whereby white adipose tissue provides long-chain fatty acids for hepatic carnitilation to generate plasma acyl-carnitines as a fuel source for peripheral tissues in mice.

25F 2:45 p.m. – 3:00 p.m. **Multiomics profiling of insulin resistant and insulin sensitive individuals during exercise delineates personalized molecular signatures**

PRESENTING AUTHOR: ***Daniel Hornburg, Stanford, School of Medicine, United States***

CO-AUTHORS: ***Daniel Hornburg, Kevin Contrepois, Sara Ahadi, Jeniffer Quijada, Michael Snyder, Francois Haddad, Kegan Moneghetti, Eric Wei, Hassan Chaib, Ming-Shian Tsai***

Exercising has a variety of beneficial effects on health and disease. Aerobic exercise, for instance, is a recommended intervention in Type 2 Diabetes having positive effects on insulin resistance. Scattered evidence suggests a complex response of the organism to exercise on multiple physiological layers. However, the underlying molecular mechanisms are poorly understood and have not been characterized at a personalized level. Metabolites and lipids play essential roles in a variety of biological functions including energy homeostasis, cell signaling, and inflammation. To identify the molecular effects of aerobic exercise we characterized longitudinal alterations in the plasma metabolome and lipidome of insulin resistant (IR) and insulin sensitive (IS) individuals. We profiled the plasma metabolome and lipidome of 34 IR and IS volunteers during and after runningfor a total of 9 time-points per individual. For untargeted metabolomics, we employed liquid chromatography coupled with mass spectrometry (LC-MS). In addition, we used the targeted Lipidyzer™ platform to profile more than 1000 lipids in high-throughput. The Lipidyzer facilitates absolute quantification of lipids and can unambiguously distinguish isobaric lipid species by the integrated differential ion mobility cell. Employing an untargeted LC-MS pipeline we identified significant changes in the metabolome as early as 15 min post-exercise. Among others, aerobic exercise increased levels of lactic acid, maleic acid, pyruvic acid, and reduced the abundance of certain amino acids in the blood. Moreover, we observed a strong effect on various lipids.

SESSION 26: MICROBIOME & METABOLOMICS II	Thursday June 28	1:30 p.m. – 3:00 p.m.

26A 1:30 p.m. – 1:45 p.m.	Lactobacillus acidophilus disrupts collaborative multispecies bile acid metabolism

PRESENTING AUTHOR: *Thomas Metz, Pacific Northwest National Laboratory, United States*

CO-AUTHORS: *Sydney E. Dautel, Nymul Khan, Kristopher R. Brandvold, Colin Brislawn, Janine Hutchison, Karl K. Weitz, Heino M. Heyman, Hyun-Seob Song, Zehra Esra Ilhan, Eric A. Hill, Joshua R. Hansen, Xueyun Zheng, Erin S. Baker, John R. Cort, Young-Mo Kim, Nancy G. Isern, John K. DiBaise, Rosa Krajmalnik-Brown, Janet K. Jansson, Aaron T. Wright, Hans C. Bernstein*

Bile acids are metabolic links between hosts and their gut microbiomes. Here we present a study designed to investigate the effect that a common probiotic, Lactobacillus acidophilus, has on microbial interactions that lead to formation of secondary bile acids. A model microbial consortium was built from three human gut microbes, Clostridium scindens, Collinsella aerofaciens, and Blautia obeum, and cultured in the presence and absence of cholateand deoxycholate, and with and without L. acidophilus (n = 3, each). Multi-omics analyses, includingGC-MS-based metabolomics, LC-MS/MSanalysis of bile acids,and activity-based proteomics, were performed. An unidentified molecule detected during bile acid analysis was isolated, partially purified, and identified by NMR and IMS-MS analyses as the uncommon secondary bile acid ursocholate.We report two primary findings.The first is that ursocholate was produced by a multi-species chemical synthesis pathway. This result highlights a new microbe-to-microbe interaction mediated by bile acids. The second finding was that the probiotic strainL. acidophilusquenched the observed multi-species interactions and effectively halted consortial synthesis of ursocholate.This result was then contextualized by performing targeted bile acid and microbiome measurements in fecal samples from a human clinical studythat investigated secondary bile acid abundances as outcomes of gastric bypass surgery (n = 10 controls and 14 patients with surgery). Levels of ursocholate corresponded with the efficacy of surgery, highlighting that it may be important to investigate the implications of both patient- and microbe-derived metabolites to help gain a predictive understanding of a patient's response to bariatric surgery.

26B 1:45 p.m. – 2:00 p.m.	Metabolomic insights into microbial metabolism of flavan-3-ols and generation of brain-targeting bioactive metabolites in a humanized gnotobiotic mouse model

PRESENTING AUTHOR: *Danyue Zhao, Rutgers University, United States*

CO-AUTHORS: *Danyue Zhao, Lap Ho, Ilaria Mongo, Elieen Carry, Justin Brathwaite, Steven Sims, Tal Frolinger, James E. Simon, Jeremiah Faith, Qingli Wu, Giulio M., Pasinetti*

Flavan-3-ols are a polyphenol subgroup purported with multifarious health benefits. Although abundantly present in dietary botanicals, their limited bioavailability, especially in the brain, arouses controversies over application of flavanol-rich botanicals as prophylactic therapy to neurodegeneration. Nonetheless, as exemplified by our previous studies, certain microbial-generated phenolic acid metabolites (MPAMs) accumulatedin the brain and effectively attenuatedneurodegeneration in mouse models of depression and Alzheimer's disease. In this work, we examined the production and bioavailability of bioactive MPAMs in humanized gnotobiotic mice. A highly efficient ultra-high performance liquid chromatography with triple quadrupole mass spectrometry (UPLC-QqQ/MS) method was developed and validated for examining 22 characteristic MPAMsin biosamples. Gnotobiotic mice were gavaged with gut microbiomefrom different human donorsand treated with a flavanol-rich preparation (FRP) for 10 days before a final dose. Accumulation of (epi)catechin and MPAMs were then measured in plasma, cecum and brain specimens. Results from method validation assays indicate excellent sensitivity of our analytical method, with limits of detection below 1.0 ng/mL for most analytes, and achieved promising selectivity, accuracy, precision and recovery. Preliminary inspections over the catabolism of pure (epi)catechin and generation of MPAMs in vitro using compositionally-different human gut microbiota helped select microbiome banks that lead to higher production of certain MPAMs. Bioanalytical data revealed distinct metabolite profiles in tissues from animals carrying compositionally-different microbiomes. Treatment dose also significantly influenced the generation and bioavailability of MPAMs.Our findings further corroborate the importance of gut microbiota and interindividual heterogeneity in polyphenol metabolism and generation of brain-targeting bioactive MPAMs.

26C 2:00 p.m. – 2:15 p.m.	Discovery of novel metabolites in co-culture of basidiomycetes Trametes versicolor and Ganoderma applanatum

PRESENTING AUTHOR: *Song Yang, Qingdao Agricultural University, China*

CO-AUTHORS: *Lu Yao, XiaoYan Xu, Sadilek Martin, XiaoTing Shen, LiPing Zhu*

Transcriptomic analysis of cultured fungi suggests that many genes for secondary metabolite synthesis are presumably silent under standard laboratory condition. In order to discover novel metabolites, 136 fungi-fungi symbiotic systems were built up by co-culturing seventeen basidiomycetes, among which the co-culture of Trametes versicolor and Ganoderma applanatum demonstrated the strongest coloration of confrontation zones. Metabolomics study of this co-culture revealed that sixty-two features were either newly synthesized or highly produced in the co-culture compared with individual cultures. 13C-labeled metabolome further indicated that 20 induced features were derived from T. versicolor, 6 from G. applanatum, and 5 features were synthesized by both fungi. Molecular network analysis highlighted a subnetwork including two novel xylosides (compounds 1 and 2). Compound 1 was identified as N-(4-methoxyphenyl)formamide 2-O-β-D-xyloside. Compound 2 was determined as xylobioside. 13C-labeling further suggested that a series of novel xylosides were likely induced through the direct physical interaction of mycelia. Moreover, compound 3 was increased 15.4-fold in the co-culture and observed 13C incorporation, was identified as a phenyl polyketide, 2,5,6-trihydroxy-4, 6-diphenylcyclohex-4-ene-1,3-dione. Additionally, 3-phenyllactic acid and orsellinic acid were detected for the first time in G. applanatum, which may be ascribed to response against T. versicolor stress. The biological activity study indicated that novel metabolites had the potential either to enhance the cell viability of human immortalized bronchial epithelial cell line of Beas-2B, or to inhibit cell viability of leukemic cell line U937 and. The current work sets an important basis for further investigations including novel metabolites discovery and biosynthetic capacity improvement.

26D 2:15 p.m. – 2:30 p.m. **Urinary and Stool Metabolome of Inflammatory Bowel Diseases: Elucidating the Impact of Exclusive Enteral Nutrition Therapy as an Alternative to Corticosteroid Treatment**

PRESENTING AUTHOR: ***Mai Yamamoto, McMaster University, Canada***

CO-AUTHORS: ***Lara Hart, Nikhil Pai, Philip Britz-McKibbin***

Inflammatory bowel disease (IBD) is a chronic remitting and relapsing digestive disorder that is increasingly diagnosed in pediatric patients in North America. Pharmacological interventions are sub-optimal for young patients due to the adverse effects of corticosteroids (CS), which are often used for symptomatic treatment of IBD. Alternatively, exclusive enteral nutrition (EEN) is a dietary intervention with fewer side effects for children, however the mechanism for its efficacy remains poorly understood. In this work, 31 pediatric patients with IBD [12 Ulcerative Colitis (UC), 19 Crohn's disease (CD)] were recruited and allocated to two treatment arms. Urine and stool samples were collected over 8 weeks for patients receiving CS or EEN therapy. Capillary electrophoresis–mass spectrometry was used for nontargeted metabolite profiling of matching urine and stool samples, whereas data was rigorously filtered following missing value imputation, batch correction and normalization. Baseline metabolic profiles showed a series of microbial metabolites that were elevated in the stool and urine of CD patients as compared to UC cases. Importantly, specific microbial metabolites were also found to undergo dynamic changes following CS or EEN therapy, indicating a distinct involvement of microflora in nutrient digestion and colonic transit time. Additionally, there was a consistent reduction in amino acids measured in stool following therapy suggesting improved absorption of nutrients in all treated patients. Our study aims to fill the knowledge gap that exists between the clinical effectiveness and the biochemical mechanisms of CS and EEN as required for improved treatment of IBD in pediatric patients.

26E 2:30 p.m. – 2:45 p.m. **Global metabolomics to reveal multiple pathways associated with polymyxin B activity and resistance in Pseudomonas aeruginosa**

PRESENTING AUTHOR: ***Mei-Ling Han, Monash University, Australia***

CO-AUTHORS: ***Yan Zhu, Darren J. Creek, Yu-Wei Lin, Hsin-Hui Shen, Alina D. Gutu, Samuel M. Moskowitz, Tony Velkov, Jian Li***

Background: Polymyxins are the last-line antibiotics against multi-drug resistant Pseudomonas aeruginosa; however, polymyxin resistance has been increasingly reported. Understanding the detailed mechanisms of polymyxin activity and resistance is crucial for preserving their clinical usefulness. Methods: We performed untargeted metabolomics to investigate the responses of both polymyxin-susceptible PAK [polymyxin B (PMB) MIC 1 mg/L] and -resistant PAKpmrB6 (MIC 16 mg/L) strains to PMB (4, 8, and 128 mg/L) at 1, 4, and 24 h. Both hydrophilic interaction liquid chromatography (HILIC) and reversed-phase (RP) LC coupled with high-resolution mass spectrometry (HRMS) were employed for metabolite detections. Metabolomics data were analyzed using univariate and multivariate statistics, those showing >2-fold changes were subjected to metabolic pathway analysis. Results: Apart from the dramatic increase in the 4-amino-4-deoxy-L-arabinose synthesis pathway, PMB significantly decreased phospholipid and fatty acid levels as well as elevated the abundance of lipoamino acids in both strains at 1 and 4 h. Interestingly, the syntheses of lipopolysaccharide and peptidoglycan were significantly decreased in PAK, but increased in PAKpmrB6 due to PMB treatment at 1 h, suggesting that PMB causes different metabolic responses between polymyxin-susceptible and -resistant P. aeruginosa. Moreover, the increased trehalose-6-phosphate and decreased glutathione disulfide levels indicated that polymyxin B potentially induces osmotic imbalance and oxidative stress in P. aeruginosa. Conclusions: This metabolomics study is the first to elucidate the complex and dynamic interactions of multiple cellular pathways associated with polymyxin mode of action in P. aeruginosa. Our results provide mechanistic insights for the discovery of much-needed novel polymyxins against Gram-negative "superbugs".

26F 2:45 p.m. – 3:00 p.m. **Effects of whole-grain and refined-grain dietary patterns on the plasma metabolome and bile acid profiles, and impact of gut microbial metabolism in a controlled feeding study in humans**

PRESENTING AUTHOR: ***Sandi L. Navarro, Fred Hutchinson Cancer Research Center, United States***

CO-AUTHORS: ***Fayth L Miles, Bigina NR Ginos, Meredith AJ Hullar, Timothy W Randolph, Ali Shojaie, Haiwei Gu, Danjiel Djukovic, Dongfang Wang, Mario Kratz, Marian L Neuhouser, Paul D Lampe, Daniel Raftery, Johanna W Lampe***

Diets higher in whole grains, legumes, and fresh fruits and vegetables (WG) compared to diets high in refined grains and added sugars (RG) have been associated with reduced risk of several chronic diseases. In a randomized, controlled, crossover intervention, we evaluated the effects of a 28 day WG diet compared to a RG diet on LC-MS-based targeted aqueous metabolite and bile acid profiles in fasting plasma. Further, we determined the association of these profiles with urinary excretion of enterolactone (ENL), an anti-inflammatory bioactive produced through gut microbial metabolism of plant lignans. Eighty healthy participants (40 men, 40 women; 18-45 years) completed both diet periods. Linear mixed models were used to evaluate differences in response between diets for 121 metabolites and 29 bile acids and their association with ENL. Pathway enrichment analyses were carried out for metabolites using KEGG pathways. Eighteen metabolites, mainly involved in inositol and tryptophan metabolism, and three bile acids, taurolithocholic acid, taurocholic acid, and glycocholic acid, ligands for receptors involved in modulation of inflammation and glucose homeostasis, were statistically significantly different between diets at day 28 (FDR<0.05). One metabolite, hippuric acid, and five secondary bile acids, lithocholic acid, glycodeoxycholic acid, ursodeoxycholic acid, isolithocholic acid and taurodeoxycholic acid, were positively associated with ENL excretion (FDR<0.05). These differences in metabolites and bile acids, and increased ENL exposure, suggest beneficial effects of a WG dietary pattern compared to RG on inflammation pathways and glucose homeostasis—effects that may be associated with a gut microbial community capable of producing ENL.

SESSION 27: NEW METHODS & TECHNOLOGIES III	Thursday June 28	1:30 p.m. – 3:00 p.m.

27A 1:30 p.m. – 1:45 p.m.	Highly Sensitive Metabolomic Approach to Measure Redox States of Respiratory Chain Sensor: Coenzyme Q

PRESENTING AUTHOR: ***Renu Pandey, The University of Texas at Austin, United States***

CO-AUTHORS: ***Meghan Collins, Christopher L. Riley, Edward M. Mills, Stefano Tiziani***

Targeting oxidative phosphorylation through inhibition of mitochondrial respiratory chain (RC) complexes has been proposed as a novel approach for cancer chemotherapy. As an essential electron carrier of RC, coenzyme Q (CoQ) has a central role in oxidative phosphorylation and reactive oxygen species (ROS) generation. Redox status of CoQ has been suggested as a metabolic sensor of RC dysfunction and oxidative stress. Hence, accurate measurement of redox states of CoQ is crucial to understand alteration of cellular redox homeostasis in cancer and neurodegenerative diseases. Nevertheless, the instability of reduced form and artifactual oxidation during extraction and analysis hamper their accurate measurement. To address this issue, we developed a rapid, highly sensitive and specific ultra-performance liquid chromatography-high-resolution mass spectrometry (UHPLC-HR-MS) method combined with efficient extraction protocol for simultaneous determination of redox states of CoQ9 and CoQ10 in vitro and in vivo. Chromatographic separation of the analytes was achieved on a Kinetex C18 column with the isocratic elution of 5 mM ammonium formate in 2-propanol/methanol (60:40) within 4 min. A full MS/all ion fragmentation (AIF) acquisition mode was used for detection and determination. The developed method validated as per FDA guidelines showed good linearity (r2 ≥ 0.9991), intraday, inter-day precision (CVs ≤ 11.9%) and accuracy (≤ ±15.2%). In contrast to existing methods, the current method offers enhanced sensitivity (up to 52 fold) with LOD ranging from 0.01-0.49 ng mL-1. The developed method can be used in future studies investigating the pathophysiological role of CoQ in diseases associated with RC dysfunction and oxidative stress.

27B 1:45 p.m. – 2:00 p.m.	Profiling and annotation of flavonoids using a product ion-dependent MSn data acquisition method on a Tribrid Orbitrap mass spectrometer

PRESENTING AUTHOR: ***Reiko Kiyonami, Thermo Fisher Scientific, United States***

CO-AUTHORS: ***Iwao Sakane, Seema Sharma, Graeme Mcalister, Caroline Ding, Andreas Huhmer***

The untargeted profiling of flavonoids provides insights into their biological functions and potential health benefits for humans. However, comprehensive identification of flavonoids from real samples remains challenging because of the limited availability of authentic standards and the structural diversity of this class of compounds. Previous studies relied upon extensive expert knowledge about fragmentation rules, a priori knowledge of the structures of flavonoids, and simple MS2 based analyses that are often not sufficient for complete structural characterization. Here we present a new flavonoid profiling workflow that uses comprehensive fragment ion information from HCD MS-MS and higher order FTMSn for rapid flavonoid identification and quantitation on a Tribrid Orbitrap mass spectrometer. As the proof of concept of the workflow, flavonoid extracts from different types of natural products were tested. A C18 column was used for flavonoid separation, and a modified Orbitrap Fusion™ Tribrid™ mass spectrometer was used for collecting HRAM MS and MSn (up to MS5) data. The collected data were processed using Compound Discoverer™ 3.0 software. A novel structure ranking algorithm included in the Compound Discoverer 3.0 software was applied to the MS and MSn data for confident structure elucidation of the unknown flavonoids based on ChemSpider database and custom flavonoids database. The MSn data were critical, especially for the identification of flavonoid glycocnjugates.

27C 2:00 p.m. – 2:15 p.m.	Evaluation of Novel Software Tools for Automating Metabolomic Flux, Kinetics and Pathway Mapping

PRESENTING AUTHOR: ***Loren Olson, SCIEX, United States***

CO-AUTHORS: ***Baljit Ubhi, Raghav Sehgal, Abhishek Jha***

In recent years,LC-MS/MS techniques have vastly increased the capabilities and breadth of metabolomics analysis. Traditional measurement of relative or absolute concentrations of metabolite levels has evolved to measuring the kinetic flux of labeled species through the metabolome. These techniques allow one to quantitate the amount and change in metabolite levels as well as separate/elucidate anabolic and catabolic pathway velocities. We evaluate the merits of high resolution data independent (DIA), data dependant (DDA) and targeted (MRM) acquisition approaches for positional isotopomer analysis and present PollyPhi, a novel software pipeline for data processing, analysis and visualization for characterizing the cellular metabolic flux. DIA approaches were employed to evaluate the utility for kinetic measurements and compared to data dependent acquisition approaches. Flux of heavy isotopes can be observed in the MS data layer but even with high resolution mass measurement, the positional isotopomer cannot be determined. In this experiment we subjected a kidney cell culture model to varying amounts of glucose to simulate differences in dietary sugar exposure during a 3-week cell culture to confluence. A 24 hr flux experiment was performed with labeled (13C6) and unlabeled glucose. High speed MS/MS acquisition modes specific to flux measurement is advantageous and in some cases, affords the ability to show differences in pathway contribution where ambiguity exists. Furthermore, the stochastic nature of data dependent approaches limits the use of MS/MS to identification only. Measurement of flux kinetics and structural identification of positional isotopomers greatly enhanced by using DIA and targeted MS/MS.

27D 2:15 p.m. – 2:30 p.m.	Untargeted Adductomics Method to Profile Human Serum Albumin Cys34 Modifications in Newborn Dried Blood Spots for Characterization of the Fetal Exposome

PRESENTING AUTHOR: ***Yukiko Yano, UC Berkeley, United States***

CO-AUTHORS: ***Hasmik Grigoryan, Courtney Schiffman, William Edmands, Lauren Petrick, Todd Whitehead, Catherine Metayer, Katie Hall, Stephen M. Rappaport***

Many carcinogens are reactive electrophiles generated through metabolism of chemicals from the diet, lifestyle habits, exposure to xenobiotics, and the microbiome. While these electrophilic metabolites are short-lived and cannot be measured directly in blood, they can be quantified by measuring their protein adducts. We previously developed an untargeted adductomics method to detect human serum albumin (HSA) adducts modified at Cys34 in plasma/serum. Here, we extended that assay to profile HSA-Cys34 adducts in non-uniform portions (equivalent to ~5-mm punches) from dried blood spots (DBS). The workflow includes extracting HSA in solution from DBS, precipitating hemoglobin and other interfering proteins, digesting with trypsin, and detecting HSA-Cys34 adducts via nano-flow liquid chromatography-high resolution mass spectrometry. We optimized the method to overcome the small sample volume and matrix effects associated with DBS analysis. For validation, the method was applied to 49 archived DBS collected from newborns whose mothers either actively smoked during pregnancy or were nonsmokers. Twenty-six HSA-Cys34 adducts were identified, including Cys34 oxidation products and mixed disulfides with low-mass thiols (e.g., cysteine, homocysteine, and glutathione), as well as other modifications. Using an ensemble of statistical approaches, the Cys34 adduct of cyanide was found to consistently discriminate between smoking and nonsmoking mothers with fold change (smoking/nonsmoking) of 1.31. Our DBS-based adductomics method is currently being applied to discover in utero exposures to reactive chemicals and metabolites that may influence disease risks later in life.

27E 2:30 p.m. – 2:45 p.m.	Open biphasic microfluidics for on chip metabolite extraction

PRESENTING AUTHOR: ***Ulri Nicole Lee, University of Washington, United States***

CO-AUTHORS: ***Ulri N. Lee, Jean Berthier, Jiaquan Yu, Erwin Berthier*, and Ashleigh B. Theberge* *co-senior authors***

Liquid-liquid extraction, a common sample preparation method, partitions small hydrophobic molecules from a complex aqueous matrix into an organic phase for downstream analysis by mass spectrometry. Here, we present an open microfluidic device that enables stable biphasic interfaces for extraction of molecules directly from microscale cell culture. The device consists of a lower aqueous channel for cell culture and an upper organic solvent channel; the aqueous and organic channels are connected through geometrically tuned microscale apertures. Broader applications of this technology include the ability to culture limited numbers of cells from patients while streamlining the sample preparation/analysis workflow for improved personalized medicine and cancer diagnostics. The metabolite profiles observed after analysis are, in part, dependent on the polarity of the extraction solvent. To extend the use of this device to a broader range of solvents, we derived equations to optimize the aperture size based on solvents with varying polarity, density, and interfacial tension. We tested our model with organic solvents (cyclohexane, ethyl acetate, and chloroform) interfaced with cell culture media in 125-800 μm square apertures and identified conditions for stability. Importantly, the open nature of this device enables the user to add and remove microliter quantities of solvent over the aqueous filled apertures with simple pipetting. This microscale extraction method is an advance from traditional liquid-liquid extraction methods because it enables direct coupling to microscale cultures, full recovery of microliter volumes of solvent, and can be arrayed for multiplexed, high throughput studies.

27F 2:45 p.m. – 3:00 p.m.	Metabolic Reaction Network-based Recursive Metabolite Identification for Untargeted Metabolomics

PRESENTING AUTHOR: ***Xiaotao Shen, Chinese Academy of Sciences, China***

CO-AUTHORS: ***Zheng-Jiang Zhu***

Metabolite identification is the long-standing challenge for LC-MS-based untargeted metabolomics and a major hurdle in functional metabolomics studies. The widely used strategy in metabolite identification is to match experimental MS2 spectra with the standard spectral library. This strategy, however, is subject to a substantial limitation as only less than 10% of known metabolites in HMBD and METLIN have standard MS2 spectra, and that expanding the spectral library is limited by the availability of chemical standards and high cost. In spite of much effort made to predict the MS2 spectra in silico, accuracy still requires substantial improvement. To address this challenge, we developed Metabolite identification and Dysregulated Network Analysis (MetDNA) software, which implemented a novel metabolic reaction network (MRN)-based recursive algorithm for metabolite identification. This algorithm allows the large-scale metabolite identifications with a small tandem spectral library. A small number of metabolites (~100) are first identified using the spectral library, and their experimental MS2 spectra are used as surrogate MS2 spectra for identifying their neighbor metabolites in MRN. This strategy facilitates metabolite identifications without expanding the tandem spectral library. Strikingly, using our newly developed recursive algorithm, the surrogate strategy allows significant expansion of identified metabolites with the MRN. As a result, MetDNA significantly increases the number of identified metabolites from ~100 to >2,000 metabolites in one experiment. We also showcased the versatility of our workflow using different instrument platforms, data acquisition methods, and biological sample types, and demonstrated that MetDNA is a platform-independent and versatile software tool.

Poster Sessions

POSTER SESSIONS 1 AND 2 – **Monday and Tuesday –** all odd number presenters will be at their posters.
POSTER SESSIONS 3 AND 4 – **Wednesday and Thursday –** all even number presenters will be at their posters.

FOOD AND NUTRITION

P-1 Understanding changes in the metabolome and bioactivity of black raspberries through thermal processing and storage

PRESENTING AUTHOR: ***Jessica Cooperstone, The Ohio State University, United States***

CO-AUTHORS: ***Matthew D. Teegarden, Thomas J. Knobloch, Christopher M. Weghorst, Steven J. Schwartz, Devin G. Peterson, Jessica L. Cooperstone***

Pre-clinical and clinical studies have implicated black raspberries (BRBs) and their associated phytochemicals in the modulation of several chronic diseases, including oral and aerodigestive cancers. Most research on the health benefits of BRBs is conducted using freeze-dried or minimally processed products, yet BRBs are typically consumed as thermally processed goods like jams and syrups. Here, we used UHPLC-QTOF-MS untargeted metabolomics to profile global chemical changes that result from 1) thermal processing of BRB powder into a nectar and 2) its subsequent storage. Stored samples were then applied to SCC-83-01-82 premalignant oral epithelial cells and anti-proliferative activity was assessed. A total of 547 chemical features were found to differ by at least two-fold ($P<0.05$) between rawBRB powder and nectar, including 170 features unique to the nectar. Some of these were identified as key degradation products of anthocyanins along with several other proposed phenolic degradants. Of high-abundant features, key BRB phytochemicals including quercetin derivatives, procyanidin monomers and dimers, and some phenolic acids were stable to thermal processing, while the ellagitannin profile was differentially modulated. Interestingly, minimal differences were noted in anti-proliferative activity when raw and stored nectar extracts were applied in vitro, despite large chemical changes. As proof of concept, cyanidin-3-O--rutinoside and its degradation product, protocatechuic acid, were administered in different ratios maintaining an equimolar dose, and anti-proliferative activity was maintained. This demonstrates that single, isolated phytochemicals do not explain the complete bioactivity of a complex food product, and the utility of metabolomics to profile global chemical changes in foods.

P-2 Discriminating sticky rice growing in different regions in Thailand using UPLC-HRMS-based metabolomics

PRESENTING AUTHOR: ***Umaporn Uawisetwathana, National Center for Genetic Engineering and Biotechnology, Thailand***

CO-AUTHORS: ***Supavadee Yatapan, Watchareewan Jamboonsri, Nitsara Karoonuthaisiri***

This study developed metabolomics profiles of Thai sticky rice from three different cultivating regions (i.e. Chiang Mai, Nong Khai and Sakon Nakhon) in order to evaluate metabolomics application for rice authenticity and traceability. Genotypic confirmation, phenotypic characterization and chemical profiling using liquid chromatography-high resolution mass spectrometry were investigated. Percentage of genotype identity greater than 96% was used for metabolomics study. Phenotypic characteristic of the rice grain samples such as color and shape were not significantly different while the quality such as stickiness of the rice starch collected from Northeast region was significantly higher than those in North region. Interestingly, metabolic profiles of these rice samples were also different which correlated to their stickiness. The metabolic profiles of the rice samples could discriminate samples at the level of region and province. Therefore, LC-HRMS-based metabolomics is a promising method to trace the sources of rice and to identify the authenticity. Furthermore, this method can be applied to validate adulterants detection in the sticky rice which will have a high impact for import-export trades.

P-3 Targeted Metabolomics approach decipher Influence of Rootstock on Fruit Juice Metabolome of Kinnow Mandarin

PRESENTING AUTHOR: ***Manpreet Kaur Saini, National Agri Food Biotechnology Institute, Mohali & Panjab University, India***

CO-AUTHORS: ***Neena Caplash, Sukhvinder Pal Singh, Charanjit Kaur***

Rootstock-scion interactions affect the accumulation of metabolites in citrus which determines the flavor, nutrition and processing attributes of juice. Our objective was to apply targeted metabolomics to decipher the effects of six rootstocks on the fruit juice metabolome of 'Kinnow' mandarin (C. nobilis × C. deliciosa). The multidimensional analysis was performed using targeted metabolomics approach employing LC-MS and GC-MS to gain a wider coverage of metabolites. Results suggest that 'Kinnow' scion grafted on Sour Orange rootstock showed a higher concentration of polyphenolics, limonin, and antioxidant activity, while those grafted on Shekwasha and Pectinifera rootstocks showed a higher concentration of sucrose, organic acids and aroma volatiles. Overall, Rough lemon and Cleopatra mandarin performed optimally for all metabolites, inducing fair levels of vitamins (B-complex and C), polyphenolics, aroma volatiles and lower levels of organic acids and limonin in 'Kinnow'. Metabolite profiling of 'Kinnow' mandarin may assist growers in obtaining quality fruits with desirable traits.

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POSTER SESSIONS 3 AND 4 – **Wednesday and Thursday –** all even number presenters will be at their posters.

FOOD AND NUTRITION

P-4 Metabolomics-based approach for the study of Garcinia mangostana (mangosteen) ripening stages

PRESENTING AUTHOR: ***Anjaritha Aulia Rizky Parijadi, Osaka University, Japan***

CO-AUTHORS: ***Sastia Prama Putri, Sobir Ridwani, Fenny Martha Dwivany, Eiichiro Fukusaki***

Metabolomics, the study of total profiling of metabolites, is an important tool to support postharvest fruit development and ripening studies. Monitoring the changes in the metabolome during fruit ripening is important to obtain insight into the mechanisms involved and improve postharvest management strategies. In this study, a metabolomics-based approach for analysis of small hydrophilic metabolites was conducted to study the ripening of mangosteen. Mangosteen is one of the most important tropical fruits, which is often called as the queen of fruits due to its excellent flavor. Mangosteen is a climacteric fruit with ethylene playing a major role in the regulation of the ripening process and affecting the ripening rate. GC/MS analysis in combination with multivariate analysis of mangosteen from seven ripening stages was performed. We first categorized mangosteen samples in different ripening stages based on color changes, an established indicator of ripening. Using gas chromatography/mass spectrometry, small hydrophilic metabolites were profiled from non-ripened to fully ripened (ripening stages 0 to 6). These metabolites were then correlated with color changes to verify their involvement mangosteen ripening. The results from this study suggests several metabolites might be indicated to play a role in mangosteen fruit ripening process.

P-6 Metabolomics assisted identification of antimalarial metabolite from Piper betle leaf extract.

PRESENTING AUTHOR: ***Mamita Debnath, University of Calcutta, India***

CO-AUTHORS: ***Swagata Karak, Susmita Das, Bratati De***

Drug resistance is a global challenge faced towards the efforts to control malaria. New and effective treatments to significantly reduce cases of malaria deaths are gaining interest now. Plant derived antimalarial alternatives can be an optimum approach in treatment of malaria. The rapidly emerging field of metabolomics can be used to identify a wide range of metabolites in plants associated with treatment of malaria. The present study is aimed to find out antimalarial actives in Piper betle leaves through GC-MS based metabolite profiling. The leaves of Piper betle L. (Family-Piperaceae), commonly known as betel leaves, are widely consumed as masticator and mouth freshener in Asia. Methanol extracts of eight varieties of betel leaves, collected in 2012 as well as in 2015 from West Bengal, India, exhibited antimalarial properties through in vitro heme biocrystallization inhibitory assay. The leaf extracts were categorized into three groups with high, medium and low activities on the basis of IC50 values. PLS-DA segregated the three groups on the basis of metabolite profiles. GC-MS assisted metabolite profiling (using Fiehn metabolomics library and matching retention time of many metabolites with that of authentic compounds) helped in identification of 125 metabolites belonging to different groups including phenols. A phenol, 1, 2, 4- benzenetriol was found to be significantly correlated to anti-malarial activity.

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FOOD AND NUTRITION

P-7 The Anaphylactoid Mechanism of Injections with Natural Compounds Based on Proteomics and Metabolomics

PRESENTING AUTHOR: ***Dou De-Qiang, Liaoning University of Traditional Chinese Medicine, China***

CO-AUTHORS: ***Chen Jing, Ran Xiao-Ku***

Abstract: Background Injection with natural compounds (INC) is an important method in the application of natural medicine, but its adverse drug reactions (ADRs) occur frequently, particularly the anaphylactoid reaction, which accounts for more than 77% of all reactions and has become a serious threat to public health. Objective To elucidate the anaphylactoid mechanism and establish the surveillance method for ADR of INC. Methods Proteomics (iTRAQ method) and metabolomics were applied for the mechanism of anaphylactoid study. Results 22 differential proteins related to anaphylactoid reactions were extracted to construct the pathways of anaphylactoid reaction. Further, 7 proteins were selected and assayed by ELISA method in the BN rat model. 47 differential metabolites were identified and 28 of them were confirmed by the authentic standards. Conclusions The processes of anaphylactoid reactions could be divided into generation and effect processes. The former could be classified as direct stimulation, complement (classical and alternative), coagulation, kallikrein-kinin, and integrated pathways and their candidate biomarkers were suggested. The effect process could be concluded mainly compose of histidine metabolism, arachidonic acid metabolism, energy metabolism, purine metabolism and so on. The anaphylactoid screening of constituents of INC disclosed that the residual proteins are the principal factor to cause anaphylactoid reactions Thus, the anaphylactoid surveillance method of INC was established for the first time on the basis of the in vivo experiments in vitro experiments and protein determination with gel permeation chromatography over TSK column.

P-8 Aroma profile of Japanese peaches in association with the ripening characteristics of cultivars

PRESENTING AUTHOR: ***Fukuyo Tanaka, National Agriculture Research Organization, Japan***

CO-AUTHORS: ***Yoko Iijima, Keiki Okazaki, Yukari Kazami, Fumiyo Hayakawa***

Peaches (Prunus persicaL.) are popular fruit species worldwide. Even though Japan possesses numerous peach cultivars, both classic and newly developed, their eating quality and especially their aroma remain to be characterized because commercially available peaches show large variations in ripening degree, and hence aroma. It has been challenging to resolve this issue using large-scale experiments. A recent integrated study of GC/MS profiling and sensory analysis attracted a great deal of attention by food researchers because of the effective discovery of key components of consumer preferences. We aimed to sort peach cultivars into several types by their flavor and texture characteristics for supplying appropriate peaches according to consumer preferences. In order to establish a sorting strategy, we extracted key components reflecting sensory attributes and texture using the following integrated analyses: 1) GC/MS profiling, 2) aroma extract dilution analysis (AEDA), and 3) quantitative descriptive analysis (QDA). We investigated six typical cultivars (early or late, hard or soft in texture, rich or weak in aroma); they were stored at three different conditions to achieve different ripening degrees. We will discuss these aroma-active components in association with the texture and ripening characteristics of cultivars.

P-9 Metabolomic, Genetic, and Phenotypic Analysis of Blueberry Populations

PRESENTING AUTHOR: ***Kevin Knagge, David H Murdock Research Institute, United States***

CO-AUTHORS: ***Jason Winnike, Kyle Blankenship, Mary Ann Lila, Massimo Iorizzo, Samantha Case, Kelsey Zielinski, Sirius Li***

Blueberries are a valuable commercial crop native to North America. Blueberries offer a variety of health benefits due to antioxidant capacity related to their polyphenol profile. However, consumer aesthetics can be a difficult variable when growers are attempting to develop ideal blueberry populations. Often researchers, breeders, and consumers must work together in analyzing phenotypic, genomic, and molecular data to determine ideal blueberry breeding strategies. In our laboratory, different genetic variants of blueberries were examined using plant metabolomics based approaches. Blueberry extracts were examined using NMR and GC/MS analytical methods. Data was cross-referenced to genetic data to find QTLs (quantitative trait loci) associated with certain small molecules. Data was also cross-referenced to phenotypic data, specifically sensory panel data, to correlate compounds to taste response relative to QTLs.

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FOOD AND NUTRITION

P-10 Role of EPA on the bioaccessibility, cell uptake, and metabolism of beta-carotene

PRESENTING AUTHOR: ***Bo Zhang, Department of Chemistry and Biochemistry, The Ohio State University, United States***

CO-AUTHORS: ***Rafael Brüschweiler, Rachel Kopec***

Long-chain omega-3 fatty acids (e.g. eicosapentaenoic acid (EPA)), may be directly obtained from dietary sources like cold-water fish, and are essential for human health. EPA plays a crucial role in brain function, normal growth and development, and has demonstrated benefits in reducing adverse cardiovascular events. Likewise, the carotenoid β-carotene (BC) is a lipophilic provitamin synthesized in orange and green fruits and vegetables. BC serves as provitamin A, and its consumption is also associated with a decreased risk of certain cancers. Due to the commensal benefits of omega-3 and carotenoids, it is sensible to supplement them together in the diet to promote human health. However, the acute effects of EPA on BC bioaccessibility and bioavailability are unknown. Therefore, we studied the direct interaction(s) between EPA and β-carotene, and the corresponding impact on their respective bioaccessibilities and bioavailabilities using an in-vitro Caco-2 cell model coupled with a targeted HPLC-PDA approach. Preliminary results indicate that EPA increases the micellarization of BC, although this increase does not translate into a subsequent increase in BC uptake by the Caco-2 cells. In contrast, it demonstrates decreasing BC uptake with increasing EPA levels, supporting the hypothesis of a shared mechanism of uptake in this model. To further elucidate the underlying cellular changes that occur during EPA supplementation, a combined HPLC-MS and NMR-based metabolomics and lipidomics approach is being applied to provide identification and cross-system confirmation of metabolites, including metabolites of interest involved in the regulation of cell osmolality and lipid uptake.

P-11 Metabolite changes with different fermentation temperature of the Korean alcoholic beverage brewed with nuruk

PRESENTING AUTHOR: ***HyeRyun Kim, Traditional Alcoholic Beverage Research Team, Korea, South***

The traditional alcoholic beverages such as makgeolli and yakju are cultural products that retain Korean history and tradition. Globalization of these Korean alcoholic beverages is being promoted via emphasis on their tradition and modernization of their quality. The taste and flavor of Korean alcoholic beverage are mainly determined by the metabolic products such as free sugars, amino acids, organic acids, and aromatic compounds which are produced during fermentation process of raw materials by the microorganisms present in nuruk. In this study, we brewed Korean alcoholic beverage using different fermentation temperature and then investigated aromatic components as changes in volatile metabolites.The volatile metabolites of Korean alcoholic beverage were simultaneously analyzed by headspace solid phase microextraction (HS-SPME) and gas chromatography mass spectrometry (GC-MS). Metabolites profiling of Korean alcoholic beverage were affected by fermentation temperature. As a result, the samples taken at different fermentation temperature were clearly distinguishable in the score plot generated by combining PC1 (38.12% of the total variance) with PC2 (12.41% of the total variance). To investigate the metabolites related to the fermentation temperature of Korean alcoholic beverage, the aromatic components related to fermentation temperature were explored through the metabolite profiling, correlation analysis, and heatmap analysis. We found the aromatic components which are influenced by the fermentation temperature in Korean alcoholic beverages and set the condition of fermentation temperature which can produce the Korean alcoholic beverages havinggood flavorcomponents.

P-13 Metabolomic characterization of hop cultivars by LC-MS-based metabolic profiling

PRESENTING AUTHOR: ***Risa Takagi, Suntory Global Innovation Center Limited, Japan***

CO-AUTHORS: ***Hiroo Matsui, Yoshihide Matsuo, Koichi Nakahara, Daisuke Miura***

Hop (Humulus Lupulus) is one of the most important materials in brewing industry, which provides refreshing bitter taste and floral aroma to beer as well as foam stability. Recently, brewers have been seeking to produce beers with desired flavors. Several metabolites including resins and polyphenols in hop are known to provide impacts for such beer features. It is known that the compositions of these compounds are influenced by the difference of hop cultivar and breeding area. Thus, metabolomic characterization of hop cultivars will contribute to predict hop properties. For this purpose, LC-MS-based metabolite profiling of hop-derived metabolites were performed in the present study. Metabolites of hop (13 cultivars, harvested 5 different farms in 2017) were extracted using 80% methanol (20 mL/mg FW). For non-targeted metabolomic analysis, metabolites were analyzed using LC-MS equipped with a reversed-phase C-18 column LC and Q-Exactive (Thermo) system. Targeted metabolite profiling of several known key bitter compounds were also performed using LC-MS system equipped with QTRAP 6500 (SCIEX). All acquired data were normalized with quality control sample that were prepared by mixing same volume of all hop extracts, and these data were used for multivariate statistical analysis. By principal component analysis, clear clusters of each cultivar and cultivate region were observed. We found several compounds that were strongly contribute to the separation of these clusters. These compounds may affect to the characteristics of bitter taste and floral aroma of each hop cultivars.

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FOOD AND NUTRITION

P-14 Vegetable signatures derived from human urinary metabolomic data in controlled feeding studies

PRESENTING AUTHOR: ***Wen-Harn Pan, Institute of Biomedical Sciences, Academia Sinica, Taiwan***

CO-AUTHORS: ***Ke-Shiuan Lynn, Mei-Ling Cheng, Hsin-Chou Yang, Yu-Jen Liang, Mei-Jyh Kang, Fong-Ling Chen, Ming-Shi Shiao, Wen-Lian Hsu***

Backgrounds: Examination of changes in urinary metabolomic profiles after vegetable ingestion may lead to new methods of assessing plant food intake. We conducted a proof-of-principle methodological study with three series of feeding experiments with three vegetables (spinach, celery, and onion). In the first exploratory study, 10 healthy subjects were provided with one of the vegetables (200 g) each day at lunch after 1 week of washout and a low-phytonutrient diet on the previous day. Their urine samples were pooled and measured for metabolites at fasting, before lunch, and 0–2 h, 2–4 h, and 4–7 h after lunch. We attempted to identify a metabolite signature for each vegetable, and subsequently validated the results with a second feeding study, wherein a single vegetable was ingested each time and individual data was analyzed. Finally, we tested the signatures with a third feeding study in which all three vegetables were fed simultaneously. Results: A total of 1, 9, and 3 non-overlapping urinary metabolites were associated with the intake of spinach, celery, and onion, respectively. The PCA signature of these metabolites followed a similar “time cycle” pattern, which maximized at approximately 2–4 h after intake. In addition, the metabolite profiles for the same vegetable were consistent across samples, regardless consumed individually or in combination. Conclusion: We identified indicative urinary metabolites for spinach, celery, and onion, demonstrating the potential to use their intensity profiles as metabolomic signatures for assessing plant food intake.

P-16 Analysis of Chinese, Korean, and American ginseng roots by LC-MS2 and LC-MSn on a modified Orbitrap tribrid mass spectrometer

PRESENTING AUTHOR: ***Stephanie N. Samra, Thermo Fisher Scientific, United States***

CO-AUTHORS: ***Kate Comstock, Arpana Vaniya, Caroline Ding, Seema Sharma***

Traditional Chinese Medicine (TCM) has been in practice for thousands of years, incorporating various herbs or roots as form of medical treatment. Alternative to Western medicine, TCM incorporates a holistic approach that is not well understood or accepted by Western medicine. One such root incorporated into many TCM treatments is ginseng, originating from the genus Panax. This root can be cultivated in the wild or purchased from holistic store fronts or TCM pharmacopeias at a variable cost depending on the origin, variety, and quality of the root. Ginseng roots of varying quality and variety from Chinese, Korean, and American origin were purchased from a TCM pharmacopeia and were interrogated by LC-MS2 and LC-MSn. Samples were finely ground by mortar and pestle followed by an 80% methanol extraction. Samples were chromatographically separated by a 20 minute reverse phase gradient using a Thermo Scientific™ Hypersil Gold (2.1x150mm, 1.9 µm) column and then analyzed on a modified Thermo Scientific™ Orbitrap™ tribrid mass spectrometer. MS2 and MSn data was collected for added confidence in compound identification and structure elucidation. The overall efficiency of MS2 and MSn data acquisition was improved with real-time background subtraction and inter-run inclusion and exclusion lists based on real-time LC-MS feature detection. Data was processed with Thermo Scientific Mass Frontier™ 8.0 and Thermo Scientific™ Compound Discoverer™ 3.0 software using mzCloud and a local MS2 library of ~2,000 natural products for identification. Additional MSn data and mass spectral trees provided added confidence in identification.

P-17 Metabolomics-based profiling of taste components specially focusing on D-amino acids in Kijoshu (a Japanese sake)

PRESENTING AUTHOR: ***Moyu Taniguchi, Osaka University, Japan***

CO-AUTHORS: ***Moyu Taniguchi, Asako Shimotori, Yosuke Nakano, Shuichi Shimma, Eiichiro Fukusaki***

Kijoshu is a kind of Japanese sake (a brewage), and it has received high reputations in international competitions. To obtain valuable insights for efficiently developing the new sake products for exploiting overseas markets, characterizing the relationships between the components and taste differences among various sake products including Kijoshu using metabolomics would be effective. Japanese sake has been recently reported to contain trace D-amino acid that might contribute to modulation of flavors. Therefore, amino acid chirality should be considered for further profiling of Japanese sake although usual food metabolomics studies have not paid attention on metabolites enantiomers. Previously, we developed a novel high-throughput analytical method widely targeting amino acid enantiomers using liquid chromatography-time of flight mass spectrometry (LC-TOFMS). In this study, to perform metabolomics study of Kijoshu by means of a combination of enantioselective amino acid analysis and comprehensive analysis of other low molecular weight hydrophilic compounds by gas chromatography-mass spectrometry (GC-MS) were performed. By subsequent orthogonal projections to latent structures (OPLS) regressions subjected the component profiling data as explanatory variables and a sensory evaluation data obtained as the response variable, the prediction models of sensory were successfully constructed. The variable importance in the projection (VIP) of several D-amino acids are rather high. The results suggest those D-amino acids might contribute to modulation of tastesin Kijoshu.

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FOOD AND NUTRITION

P-18 A serum metabolomics study on the therapeutic effects of Fructus Ligustri Lucidi on calcium deficiency in aged female rats using GC-TOF/MS and UPLC-Orbitrap/MS

PRESENTING AUTHOR: ***Mengheng LI, The Hong Kong Polytechnic University, China***

CO-AUTHORS: ***Chi-On Chan, Sisi Cao, Man-Sau Wong, Daniel Kam-Wah Mok***

Fructus Ligustri Lucidi (FLL) is the dried ripe fruit of Ligustrum lucidum Ait. (Oleaceae), which has been widely used for treatment of rheumatic bone and osteoporotic bone pain in China for more than one thousand years. The active constituents of FLL have been identified with oleanic acid (OA) and ursolic acid (UA) as the two representative triterpenoids. Previous animal studies showed that FLL extract as well as these two active constituents OA/UA could inhibit bone resorption through suspending RANKL induced osteoclasts formation and subsequent TRAP activity. In this study, a serum metabolomics analysis using GC-TOF/MS and UPLC-Orbitrap/MS was conducted to acquire the metabolic profiles of rats received different treatment, including high calcium diet, medium calcium diet, FLL, OA and OA/UA. Principal component analysis and partial least squares-discriminant analysis were employed to identify differences among the metabolic profiles coming from different groups. Our study suggested that the metabolites responsible for the differences among groups including those involved in energy metabolism, amino acid metabolism and bile acid metabolism. In conclusion, metabolomics provides a powerful approach to identify the metabolic changes induced by calcium deficiency as well as the changes arise from interventions of FLL and OA/UA. The results provide important information to delineate the mechanisms of the effects of FLL and OA/UA in improving calcium balance and bond properties.

P-19 Comparison and profiling of beta-damascenone precursors among sweet potato (Ipomoea batatas) cultivars

PRESENTING AUTHOR: ***Tai Kaneshima, Suntory Global Innovation Center Limited, Japan***

CO-AUTHORS: ***Daisuke Miura, Koichi Nakahara, Yoshihide Matsuo***

beta-Damascenone is one of a key flavor of Shochu, a popular spirits in Japan that made from sweet potato (Ipomoea batatas), and it is already known to be derived from raw materials. beta-Damascenone is a potent flavor compound, possessing an extremely low odor threshold of 0.002 ug/L in water. Regulating the amount of beta-damascenone in the product leads to quality improvement. beta-Damascenone is known to be produced from its glycoside precursors by acid-catalysed transformations from their glycoconjugates, and are known to be contained in plants such as roses, apples, tea leaves and also sweet potatoes. Thus, comparison and profiling of beta-damascenone precursors among sweet potato cultivars will contribute to predict sweet potato products properties. For this purpose, LC-MS-based metabolite profiling of sweet potato-derived metabolites were performed in the present study. Metabolites of sweet potato (6 cultivars, harvested in 2017) were extracted with 80% methanol. Targeted metabolite profiling of several known precursors of beta-damascenone were also performed using LC-MS system equipped with QTRAP 6500 (SCIEX). All acquired data were normalized with quality control sample that were prepared by mixing same volume of all sweet potato extracts, and these data were used for multivariate statistical analysis. By principal component analysis, clear clusters of different color of tuberous root and their peel region were observed. We found several compounds that were strongly contribute to the separation of these clusters. These compounds may affect to the characteristics of amount of beta-damascenone of each sweet potato variety.

P-20 Prediction modeling of brewing characteristics of grains from its metabolite

PRESENTING AUTHOR: ***Takuji Kobayashi, National Research Institute of Brewing, Japan***

CO-AUTHORS: ***Shogo Hirata, Tsutomu Kumazaki, Kana Morikawa, Yuko Hata, Hiroshi Kamigakiuchi, Ryota Saito, Hisashi Yazawa, Ken Oda, Masaki Okuda, Kazuhiro Iwashita***

Sake, soy sauce, miso (Japanese bean paste), and beer are primarily prepared from grains, and the characteristics of raw materials are well known to significantly affect the product quality. While making sake, rice is not only an ingredient but also critical for controlling the fermentation and quality of sake. Despite several methods available to assess the properties of rice, most are sample- and time-consuming and do not specify the quality of end-products. Thus, we attempted to develop a novel method for predicting the brewing characteristics of rice using <1 g of sample. First, brown rice extracts were prepared from 33 samples with 50% methanol using 0.1 g of brown rice and analyzed by UPLC-QTof-MS. Consequently, 736markers were detected within a low coefficient of variation used as explanatory variables. Then, analytical data of physical and chemical properties of rice grains, the enzymatic activity of its rice-koji, fermentation properties of sake mash, and standard analytical value and flavor contents of sake were obtained as objective variables. Finally, prediction models were developed by orthogonal projections to latent structures (OPLS). Owing to the verification of prediction accuracy, 34 and 19 of 54 objective variables exhibited good ($R2 > 0.7$) and excellent ($R2 > 0.8$) models, respectively. Overall, we developed a method for predicting the properties of rice and brewing characteristics from 1 g of brown rice. This study highlights that the quality of products, including beer, and soy sauce etc., prepared from grains can be estimated from its metabolite using only few samples.

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FOOD AND NUTRITION

P-21 Biocontrol: Combating Anthracnose fungi by using actinomycetes

PRESENTING AUTHOR: ***Rinrada Suntivich, Dr., Thailand***

CO-AUTHORS: ***Suganya Yongkiettrakul, Vanicha Vichai, Sumalee Supothina, Surasak Jiemsup, Chanwit Suriyachadkun, Umaporn Uawisetwathana, Nitsara Karoonuthaisiri, Ubolsree Leartsakulpanich, Lin-Tang Goh, Chris Cheah Hun Teong, Jason Goh***

The anthracnose disease caused by Colletotrichum species is one of the major causes that affect production of chili worldwide. Several approaches have been used to manipulate the disease including biological control. The application of this approach could significantly reduce the usage of chemical fungicides that are harmful to health and the environment. Actinomycetes in our microbial collection have been explored for their potential use as biocontrol agent. BCC72154 has been selected as it showed potent anti-fungal activities, good antagonistic activities and low human cytotoxicity. The co-cultivation study of BCC72154 and C. Acutatum were performed by using LC-MS/MS-based metabolomics. In this work, we have established a protocol for metabolite extraction and profiling for microbial co-cultivation study. Metabolic changes in mono- and co-cultivation of an antagonistic actinomycetes strain with an anthracnose pathogen were observed and could provide better understanding on the interspecies interactions involved in antagonism.

P-22 Biomarkers of fish intake and their association with type 2 diabetes risk

PRESENTING AUTHOR: ***Lin Shi, Chalmers University of Technology, Sweden***

CO-AUTHORS: ***Carl Brunius, Ingvar A. Bergdahl, Ingegerd Johansson,Carolina Donat Vargas, Hannu Kiviranta, Kati Hanhineva, Agneta Åkesson, Rikard Landberg***

Conflicting evidence exists regarding the association between fish intake and type 2 diabetes (T2D) incidence, potentially due to error-prone self-reported dietary intake assessments and co-exposure to persistent organic pollutants (POPs) present in fish. We aimed to identify biomarkers of fish intake, assess their associations with T2D risk, and to investigate whether associations could be affected by POP exposures. In a Swedish population-based prospective cohort, plasma samples from 503 case-control pairs at baseline as well as 10-year follow-up samples from a subset of 187 pairs were analyzed using untargeted metabolomics and determination of 21 POPs (only187 pairs). Principal component analysis (PCA) was performed on 67 metabolites identified that were associated with fish intake measured by food frequency questionnaires. We found that PC1 showed strongest association with fish intake and had high reproducibility over 10 years, but was not related with T2D. However, among metabolites with high PC1 loadings, 11 were positively associated with risk of T2D while 7 were inversely associated. In total, 16 POPs were positively correlated with fish intake, and were also positively associated with T2D risk. Adjusting risk models for POPs exposures substantially lowered odds ratios for fish intake and fish-related metabolites. Overall, no association between fish intake and T2D risk was observed in the current population. Several fish-related metabolites were associated with T2D risk in different directions, when assessed individually, suggesting different roles in relation to T2D development. Co-exposure to POPs present in fish may cancel out any potential protective role of fish intake on T2D risk.

P-23 Investigation of relationship between combination of several sake-making parameters and sake metabolites

PRESENTING AUTHOR: ***Hisashi Yazawa, National Research Institute of Brewing, Japan***

CO-AUTHORS: ***Hajime Kozato, Yuko Hata, Ken Oda, Kazuhiro Iwashita***

Japanese sake, traditional alcoholic beverage made from rice, koji-rice, and water, has been known to contain over 280 metabolites that affect its quality. To investigate metabolome of various types of sake and other alcohol beverages in detail, we developed a novel “sake metabolome analysis method” using liquid chromatography with quadrupole–time-of-flight mass spectrometry. Using this method, we investigated the relationship between combination of several sake-making parameters (rice polishing ratio, rice cultivars, and yeast strains) and sake metabolites by a combined experiment of such parameters using small-scale fermentation. The results indicated all parameter affected significantly on sake metabolite and only few markers were significantly affected by sole parameters. This analysis also revealed that the effect of rice cultivar of koji-rice on sake metabolome was greater than that of rice cultivar of kake-rice. It has known by experientially, but this study clarified experimentally for the first time. This analysis can help evaluate the combined effect of several parameters. For example, we demonstrated different influence of each parameter on several amino acids. In this analysis, many unidentified markers were still remained. Investigating these unidentified markers will give us new insight for improvement of sake taste and quality.

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FOOD AND NUTRITION

P-24 Lipidomics of 38 different table cuts, co-products and fat deposits from New Zealand grass-fed Wagyu beef

PRESENTING AUTHOR: ***Arvind K. Subbaraj, AgResearch Ltd., New Zealand***

CO-AUTHORS: ***Arvind K. Subbaraj, Karl Fraser, David Cameron-Smith, Cameron Craigie, Emma N. Bermingham***

We conducted a non-targeted lipidomics study of 38 different parts from New Zealand grass-fed Wagyu beef. Twenty six table cuts, nine co-products and three sub-samples of fat deposits were analysed (n = 5) using LC-HRMS in positive and negative electrospray ionisation modes. Data processing and normalisation was done using XCMS and in-house scripts. Lipid identification based on MS2 spectra was performed using LipidSearch™ software. A total of 364 lipid species from 18 lipid classes were identified (Class-Number: SM-17; PS-45; PI-12; PG-15; PE-71; PC-49; PA-2; dMePE-3; LdMePE-3; LPS-1; LPE-9; LPI-1; LPG-2; LPC-5; FA-1; CL-29; Cer-2; and TG-97). To understand lipid diversity across the animal, lipid profiles both between cuts and within cuts were compared (α=0.05). For example, between cuts, liver had the highest concentrations of the lipid classes Cer, LPC, LPG, LPI, dMePE, LdMePE, PS, and the lowest concentration of TG. The lipids Cer(d18:1/24:0)-H, LPC(18:0)+HCOO, LPC(20:3)+HCOO, LPG(20:0)-H, LPI(18:0)-H, dMePE(18:0/18:1)-H, LdMePE(18:0)-H, LPE(17:0)-H, PC(20:0/18:2)+HCOO, PE(18:0/20:3)-H, PS(18:1/22:0)-H, PS(37:3)-H, PS(39:3)-H, PS(41:3)-H, PS(41:4)-H, and PS(43:5)-H i.e., species with predominantly saturated or unsaturated C18 FAs, were at higher concentrations in liver. Within the liver, the lipids Cer(d18:1/24:0)-H, CL(18:3/18:1/18:2/18:2)-H, dMePE(18:0/18:1)-H, LdMePE(18:0)-H, LPC(18:0)+HCOO, LPE(18:0)-H, LPG(20:0)-H, PA(18:0/18:1)-H, PC(16:0/18:1)+HCOO, PE(18:0/20:4)-H, PG(20:0/20:0)-H, PI(18:0/20:4)-H, PS(39:1)-H, SM(d41:1)+HCOO, and TG(18:0/16:0/18:1)+NH4 were the most concentrated species of their respective classes. Similar analyses were done for all cuts. An understanding of the Wagyu beef lipidome and how it differs across different parts, in conjunction with measures of meat quality, will provide a better understanding of the roles of lipid species/classes/networks in meat flavour and nutrition.

P-25 Germinated soy germ with increased soyasaponin Ab improves BMP-2-mediated osteogenesis and protects against in vivo bone loss in ovariectomy-induced osteoporosis

PRESENTING AUTHOR: ***Sik-Won Choi, National Institute of Crop Science, South Korea***

CO-AUTHORS: ***Shin-Hye Kim, Kwang-Sik Lee, Hyeon Jung Kang, Mi Ja Lee, Hyun Young Kim, Ki Chang Jang, Kie-In Park, Woo Duck Seo***

Osteoporosis is frequently induced following menopause, and bone fractures result in serious problems including skeletal deformity, pain, and increased mortality. Therefore, safe and effective therapeutic agents are needed for osteoporosis. This study aimed to clarify the bone protecting effects of germinated soy germ extracts (GSGE) and their mode of action. GSGE increased expression of alkaline phosphatase (ALP) and osteocalcin (OCL) by stimulating the expression of runt-related transcription factor 2 (Runx2) and osterix (Osx) through activation of Smad signaling molecules. Furthermore, germination of soy germ increased levels of nutritional components, especially soyasaponin Ab. The anabolic activity of soyasaponin Ab in GSGE was also evaluated. GSGE and soyasaponin Ab significantly protected against ovariectomy (OVX)-induced bone loss and improved bone-specific alkaline phosphatase (BALP) level in mouse serum. These in vitro and in vivo study results demonstrated that GSGE and soyasaponin Ab have potential as therapeutic candidate agents for bone protection in postmenopausal osteoporosis

P-26 A metabolomics study to identify specific markers of different processed meat product intake

PRESENTING AUTHOR: ***Roland Wedekind, International Agency for Research on Cancer, France***

CO-AUTHORS: ***Pekka Keski-Rahkonen, Inge Huybrechts, Erwan Engel, Pietro Ferrari, Marc Gunter, Augustin Scalbert***

Background: Processed meat (PM) is an established carcinogen to humans. However the estimation of PM intake remains a challenge, particularly for distinguishing different PM products which can be cured and non-cured, fermented and non-fermented. The goal of this work was to identify biomarkers of intake for various PMs that can be implemented in epidemiological studies. Methods: A variety of meat products were digested in vitro and metabolite profiles analysed by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). Compounds specific to different types of PM were first identified. A randomized cross-over dietary intervention was then carried out in which 12 healthy adults consumed one of the following foods: bacon, salami, hot dogs, fried pork or tofu for 3 days in succession. Blood and urine samples were collected and analysed by LC-HRMS and specific signals associated with intake of these foods were identified. Results: Several metabolites specific for different meat processing methods such as smoking and fermentation were identified in the in vitro digested meat products. Identification of markers of intake for the 5 food products tested in the intervention study is ongoing and results will be presented. Conclusion: In this study we were able to identify specific markers of different PM products. These markers will be validated in samples of a large European population-based study for which both biospecimens and self-recorded dietary intake data for PMs is available. Newly identified PM biomarkers will then be measured in a nested case control study to investigate their association with colorectal cancer development.

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POSTER SESSIONS 3 AND 4 – **Wednesday and Thursday –** all even number presenters will be at their posters.

FOOD AND NUTRITION

P-27 Effects of soy isoflavone enriched diet on metabolism: a serum lipidomics and urinary metabolomics analysis

PRESENTING AUTHOR: ***Xueheng Zhao, Cincinnati Children's Hospital Medical Center, United States***

CO-AUTHORS: ***Carlo Clerici, Elisabetta Nardi, Roberta Russo, Liang Niu, Lindsey Romick-Rosendale, Kenneth DR Setchell***

Diet has long been acknowledged for its role in contributing to disease risk and prevention. Soy isoflavones exhibit antioxidant, antimicrobial, and anti-inflammatory properties and diets rich in isoflavones has been implied in reducing risk in various diseases including cancer.However, the molecular foundations of their effects on human health remain poorly understood. The aim of this study is to elucidate the metabolic effects of soy isoflavones on the human serum and urinary metabolome. We conducted a pilot study with a double blinded, placebo controlled crossover design. Participants consumed a novel soy germ (isoflavone enriched) pasta containing 33 mg isoflavones per 80 gram serving daily for 3 consecutive weeks, while conventional pasta (lacking isoflavones) was used as placebo. A 2-week washout period was used to diminish the carryover effect. Thirteen healthy participants (28-67 years of age, 6 males and 7 females) completed the study. Fasting blood samples and 24 hour urine samples were collected from each participant. Serum lipidomics analysis was measured with liquid chromatography coupled to high resolution mass spectrometry (UPLC-QTOF-MS) and urine metabolome was analyzed by nuclear magnetic resonance (NMR). Over 290 lipid species were identified by lipidomics approach and 61 urinary metabolites were detected by NMR. Univariate and multivariate statistical analysis revealed that dietary intervention has a subtle metabolic effect in healthy adults. Among the significantly altered metabolites, plasmalogens have been shown to possess potential antioxidant and anti-inflammatory properties by previous studies. Based on these findings, modulation of the affected metabolic pathways by soy isoflavones warrants further study.

P-28 Non-targeted metabolic profiling according to regional characteristics of soybean.

PRESENTING AUTHOR: ***Eun Mi Lee, Kookmin University, Korea, South***

CO-AUTHORS: ***Byeong Gon Sin, Do Yup Lee***

Soybeans is one of the most important crops in the world that is enriched with important nutrients such as carbohydrates, proteins, essential fatty acids, and flavonoids. The nutritional quality and metabolic uniqueness of soybean is determined by a range of environmental factors (e.g. climate and soil) as well as genetic factors. Thus, the metabolic investigation may be essentially valuable In this study, we performed non-targeted metabolomic analysis of soybean seeds by using gas chromatography-time of flight mass spectrometry (GC-TOF MS) and liquid chromatography-orbitrap mass spectrometry (LC-Orbitrap MS). A total of 227 metabolites were acquired, and structurally identified, further employed for statistical analysis, which fairly covered a range of chemical entity, thus allowed comprehensive metabolic phenotyping. The resultant profiles integrative of primary and secondary metabolites were clearly discriminated by multivariate statistical model according to six representative cultivation regions in Korea (Gyeonggi-do, Gangwon-do, Chungcheong-do, Gyeongsangbuk-do, Gyeongsangnam-do, Jeollabuk-do, Jeollanam-do). The metabolic cluster re-composited with five metabolites (malonylgenistin, malonyldaidzin, N-acetylornithine, LysoPE, xylose) showed excellent discrimination power for the profiles of all six regions, which was determined by receiver operating characteristic (ROC) analysis. The subsequent interrogation on covarianced structures of the metabolome revealed region-specific metabolic traits that systematically isolated list of metabolites and linked it to different region of the soybean cultivation. Our result proposed metabolite analysis can be applied to authentic methodology that identifies origin of agricultural products, and also provide enriched information on nutritional values according to cultivation region.

P-29 THE USE OF METABOLOMICS TO PREDICT CHEESE FLAVOUR DEVELOPMENT

PRESENTING AUTHOR: ***Asal HajNajafi, PhD Student, Australia***

CO-AUTHORS: ***Oliver Jones, Harsharn Gill, Bogdan Zisu***

The flavour of all cheese develops during the ripening process, when complex biochemical changes take place. The ripening and associated flavour development of hard cheeses, such as cheddar, is well defined but the same processes are not well studied in soft cheeses. This makes it difficult for manufactures to produce such cheese with uniform flavour; this can hinder mass production of these products. Similarly, it is hard to isolate processes that cause 'off flavours' or predict when they will occur. Such problems often only become apparent when the cheese ripening process is finished; since this can take months (or even years) these issues can have a significant, negative economic impact. Metabolomics (the scientific study of the small bio-molecules present within biological systems) shows great promise for the study of cheese ripening as there are many small molecules contributing to the flavour profile of cheese and the highly interconnected nature of the taste also means that important information is most likely to be found in correlation patterns as opposed to individual signals. In this study we have used Solid Phase Micro-Extraction and Gas Chromatography Mass Spectroscopy based Metabolomics to analyse flavour development during the ripening of Australia Camembert cheese in association withLion Dairy & Drinks (National Foods). We present the optimised analytical methodology and show how it has been used to generate new knowledge about the volatile compounds that contribute to the flavour of Australian cheese.

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FOOD AND NUTRITION

P-30 Effects of microbial stain and cultivation conditions on the formation of volatile metabolites in Aspergillus oryzae fermentation

PRESENTING AUTHOR: ***Park, Min-Kyung, Ewha Womans University, Korea, South***

CO-AUTHORS: ***Kim Min-Joo, Seo Jeong-Ah, Kim Young-Suk***

Aspergillus oryzae is widely used as a microbial starter in some fermented foods, including soy sauce, fermented soybean pastes, and alcoholic beverages, especially, in Eastern Asia. The formation of volatile metabolites related to the quality of fermented products is mainly affected by microorganisms and cultivation conditions, including temperature, cultivation time, initial pH, and aerobic/anaerobic conditions. In this study, the profiles of volatile metabolites were compared between two types of A.oryzae isolated from some fermented products, nuruk (AON), which is a mixture of airborne microorganisms and sake koji (AOS), respectively. Additionally, we also investigated the impacts of cultivation conditions, such as temperatures, cultivation times, and initial pH on the formation of volatile metabolites Stir bar sorptive extraction (SBSE) coupled with gas chromatography-mass spectrometry (GC-MS) was performed to extract and analyze volatile metabolites produced by A.oryzae. Then, data sets were processed by partial least squares-discriminant analysis (PLS-DA) to evaluate statistical differences in the volatile metabolites of samples. In PLS-DA results, AON sample (negative PC1) was clearly discriminated from ones inoculated with AOS (positive PC1). 3-Methylbutan-1-ol, ethyl acetate, and 3-methylbutanoic acid mainly contributed to the positive PC1, whereas 1-phenylbutan-2-one, dodecanoic acid, and 2-ethylhexen-1-ol were responsible for the negative PC2. Additionally, the group of AOS was more scattered on the score plot in comparison with that of AON. These results demonstrated that volatile metabolites produced by A.oryzae could be varied depending on cultural conditions, such as temperature, cultivation time, and initial pH, as well as its strain.

P-31 Integrated metabolomic analysis of beef quality for taste properties in the longissimus dorsi muscle of beef cattle, Hanwoo

PRESENTING AUTHOR: ***Hyun-Jeong Lee, National Institute of Animal Sciences in Korea, Korea, South***

CO-AUTHORS: ***Jin Young Jeong, Minseok Kim, Kondreddy Eswar Reddy, Seul Lee, Soohyun Cho***

Tissue metabolites are direct and essential regulators of biological processes and reflect phenotypic traits such as breed, sex, and tissue types, and possibly meat quality and flavors. This study aimed to characterize beef quality based on metabolome profiles and the relationship between beef quality and taste properties in Hanwoo, Korean native cattle. Metabolomic analysis was performed using proton nuclear magnetic resonance imaging (1H NMR) and gas chromatography mass spectrometry (GC/MS) analysis of the longissimus dorsi muscle. A score and loading plots were determined via orthogonal partial least-squares discriminant analysis (OPLS-DA). Metabolites related to beef quality grade were screened using variable influences on projection (VIP) and analyzed using multivariate statistical analysis. Based on multivariate analysis, samples were classified on the basis of their beef quality grades. Several metabolites were found to be taste-related metabolites. Overall, related metabolites such as acetate, alanine, anserine, carnosine, creatine, glucose, glutamine, glutathione, glycerol, lactate, leucine, taurine, and myo-inositol had high sensitivity in beef quality grade analysis via NMR. Furthermore, pyridine, N-methyltrifluoroacetamide, trifluoroacetamide, methylamine, butanoic acid, oxalic acid, butyrolactone, 3-pyridinol, L-alanine, pyroglutamic acid, L-aspartic acid, and L-glutamic acid were identified on GC/MS analysis. Sweet and umami flavors tended to be higher in beef samples with relatively high quality grades. In conclusion, comprehensive analysis using a multiplatform approach suggests that the potential metabolites associated with beef quality can elucidate metabolic networks for taste properties and provide information regarding the formation of sensory traits in beef; however, further studies are required.

P-32 Comparison of muscle metabolome between two carnivorous fish species as affected by high-starch diets

PRESENTING AUTHOR: ***Ivan Viegas, University of Coimbra, Portugal***

CO-AUTHORS: ***Mariana Palma, Ludgero Tavares, Lauren Trenkner, João Rito, John Jones, Nick Wade, Ivan Viegas***

Aquaculture development and sustainability are highly dependent on the feed optimization, especially for carnivorous species. Barramundi (Lates calcarifer) and European seabass (Dicentrarchus labrax) are important farmed fish in almost opposite regions of the world, but both are is highly dependent on fishmeal. Plant-derived ingredients can contribute to reduce costs and nitrogenous effluents while sparing wild stocks. However, the metabolic effects of diets enriched with vegetable carbohydrates are still not completely understood. A Nuclear Magnetic Resonance-metabolomics approach was followed to evaluate the effects of two diets: a high-protein vs. high-starch content, in the fish muscle (fillet) metabolome of both barramundi and seabass juveniles. Proton spectra of aqueous muscle extracts were acquired on a Varian VNMR 600 MHz (Varian Inc., Palo Alto, CA, USA) spectrometer equipped with a 3 mm 1H(X)-PFG inverse configuration probe. Spectra were processed using ACD/Labs software and further multivariate analysis was performed using the web-based platform MetaboAnalyst. Multivariate analysis (PCA) showed differences between species but the diet overlap within species. Applying PLS-DA, we were able to successfully observe separation between both species and diets. Despite being carnivorous fish with some different nutritional requirements, high-starch diets promoted similar responses in both species. VIPs values are indicative of variations in the aromatic regions and the amino acid profile. This methodology revealed to be quick, robust, reproducible and easily applicable for aquaculture farmers and food industry.

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FOOD AND NUTRITION

P-33 Absolute Quantification of Five Phenolic Compounds by LC-MS/MS in the Nine Thai Herbal Medicines

PRESENTING AUTHOR: ***Manmas Vannbhum, Mahidol University, Thailand***

CO-AUTHORS: ***Pravit Akarasereenont, Jantanee Wattanarangsan, Siriphan Manocheewa, Kwanjeera Wanichthanarak, Suveerawan Limsuvanc, Ranida Boonrak, Manmas Vannabhum, Sakda Khoomrung***

Thai Herbal Medicines (THM) has been used for health promotion and treatment more than seven centuries. To integrate the use of THM, it is highly important to establish more of scientific evidence particularly efficacy, safety, and quality. In this study, we developed and validated an analytical method for absolute quantification of gallic acid, vanillic acid, caffeic acid, p-coumaric acid, and ferulic acid, in the nine widely used THM. The method was developed based on ultra-performance liquid chromatography coupled to electrospray tandem mass spectrometry (UPLC-MS/MS) in connection with reverse phase chromatographic separation. The problem regarding matrix effects leading to low precision and accuracy of the measurement was overcome by classical standard addition approach. The %recovery (spiking experiment) of all compound were from 87±7-103±7 (N=5). The method was shown to be highly reproducible in terms of retention time of the analytes (intraday; 0.0-0.3% RSD; N = 5, interday; 0.1-0.3% RSD; N = 5) and peak area (intraday; 1.3-5.0% RSD; N = 5, interday; 2-6.9% RSD; N = 3). The limit of detections (LODs) and limit of quantification (LOQs) were determined using the lowest concentration of each individual standards with a signal to noise ratio (S/N) of 3 and 10, respectively. The LOD of all metabolites were in the range of 0.7-30 ng/mL while the LOQ ranged between 2.5-0 ng/mL. At the end of this study, we applied our method for absolute quantification of these metabolites and hierarchical clustering analysis review classification of these nine THM products on the presence of these metabolites.

P-34 UPLC-QTOF-MS and GC-TOF-MS Based Serum Metabolomics of the Bone Protective Effect of Sambuci Williamsii Ramulus in Ovariectomized Rats

PRESENTING AUTHOR: ***Daniel Kam-Wah Mok, The Hong Kong Polytechnic University, Hong Kong***

CO-AUTHORS: ***Daniel Kam-Wah Mok, Tung-Ting Sham, Meng-Heng Li, Chi-On Chan, Hui-Hui Xiao, Man-Sau Wong, Xin-Sheng Yao***

Conventional estrogen eplacementtherapy is the first line treatment for osteoporosis in menopausal women but it increases the risk of reproductive cancers.An alternative approach to alleviate the risk concern comes to traditional Chinese medicine. In our previous study, Sambuci Williamsii Ramulus(SWR) could improve bone propertiesin vivo.However, the mechanism of itsprotectiveeffectagainst osteoporosis is not fully understood. A non-targeted serum metabolomics study was conducted to investigate the role ofSWR on osteoporosis usingUPLC-QTOF-MS and GC-TOF-MS. In the animal study, 40 female Sprague-Dawley rats were randomly assigned to: ovariectomized (OVX) model, sham group with sham surgery, premarin-treated (conjugated estrogen) andethanol fraction of SWR-supplemented OVX groups.After the serum metabolic profiles were collected, multivariate statistics was applied to identify significant differences in the profiles among different groups of animal. In the PLS-DA score plot,clear distinctionshave been observed between OVX and sham groups while treatment groups were between them, suggesting that premarin and SWR treatments were able to partially restore the metabolism towards the normal state. From the biomarker candidates extracted from OPLS-DA of sham group against OVX group, both SWR and premarin interventions showed the upregulation of tryptophan, branch-chained amino acids, the restoration of most polyunsaturated fatty acids and lysophospholipids to normal. However, only SWR group showed markedly reduced level of cresol sulfate. This is the first metabolomics study reported that combined LC- and GC-MS technologies to give a more comprehensive picture of SWR in the treatment of osteoporosis.

P-35 Lipidomics Study of the Effect of Polygoni Cuspidati Rhizoma et Radix Water Extract on Non-Alcoholic Fatty Liver Disease in Rats

PRESENTING AUTHOR: ***Chi-On Chan, The Hong Kong Polytechnic University, Hong Kong***

CO-AUTHORS: ***Chi-On Chan, Tung-Ting Sham, Huan Zhang, Shun-Wan Chan, Daniel Kam-Wah Mok***

Non-alcoholic fatty liver disease (NAFLD) is the most common form of liver dysfunction and it affects 20-30% of the adult population around the world. NAFLD has a strong association with obesity, insulin resistance and dyslipidaemia and these factors are also features of the metabolic syndrome. Treatments and preventions of NAFLD and metabolic syndrome represent a priority health challenge. PolygoniCuspidati Rhizoma etRadix(PCRR, Huzhang), is a Chinese herbal medicine that is extensively used as a hepatoprotective and cholagogic agent. UPLC-MS analysis revealed that the PCRR water extract contains rich polyphenols such as polydatin and resveratrol. Animal study showed that the PCRR water extract could lower the lipid content in liver and the total cholesterol level in serum significantly. Lipidomics study was conducted to understand the effects of PCRR treatment in the liver metabolisms in high fat diet (HFD)-induced hypercholesterolemic Sprague-Dawley rats. The lipidomics profiles of PCRR treatment group were significantly different from HFD groups. More than 20 metabolites were found as potential biomarkers and indicated that PCRR could effectively lower fatty acids content in liver. We believed that treatment of PCRR could modulate the fatty acid synthesis results in improvements in NAFLD.

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FOOD AND NUTRITION

P-36 Non-Targeted Metabolomics Approach for the Development of a Novel Taste Evaluation Method for Litopenaeus vannamei (white leg shrimp)

PRESENTING AUTHOR: ***Sastia Prama Putri, Osaka University, Japan***

CO-AUTHORS: ***Safira Latifa Erlangga Putri, Felicia Irene Saputra, Gede Suantika, Magdalena Lenny Situmorang, Eiichiro Fukusaki***

Shrimp is one of the highest economic value commodities in aquaculture industry and the worldwide need of shrimp crustaceans increases almost 10% annually. Despite its importance in global food industry, one important concern in shrimp industry is the lack of quality evaluation procedures. At present, shrimp producers primarily focus on the compliance with food safety requirement and overall quality of the product which relate primarily to appearance, freshness, and uniformity of the size. Shrimp market price is determined by count size, which is the number of shrimp pieces contained per unit weight. As such, the present quality evaluation standards for shrimp lacks taste evaluation method, which is crucial in terms of consumer acceptance and preferences. This research aims to employ metabolomics approach for the development of a quality evaluation method for the most important shrimp species in aquaculture industry, Litopenaues vannamei, also known as the white leg shrimp. To achieve this objective, non-targeted GC/MS-based analysis and sensory evaluation were used to construct a Projection to Latent Structure (PLS) model. Sensory evaluation involving semi-trained panelists and the whole workflow for shrimp metabolome analysis were developed in this study. The constructed PLS model allowed the identification of key metabolites that are correlated to sensory attributes. The result of this research may provide a novel method for evaluating the taste quality of white leg shrimp and provide feedback for industry to improve shrimp quality.

P-37 Investigation of biomarkers of green tea and coffee consumption using metabolomics

PRESENTING AUTHOR: ***Hisami Yamanaka-Okumura, Tokushima University, Japan***

CO-AUTHORS: ***Hisami Yamanaka-Okumura, Hiroshi Tatano, Daisuke Kajiura, Akiyoshi Hirayama, Soga Tomoyoshi, Masaru Tomita***

Accurate dietary surveys are necessary in nutritional research to clarify the relationships of diet. As an alternative, biomarkers of food product consumption have been used to objectively evaluate consumed food and drinks; however, there are still no clear biomarkers of the consumption of drinks, necessitating their further screening. The present study aimed to investigate biomarkers and compare metabolites in plasma and urine after the consumption of green tea and coffee. The subjects were eight healthy men who participated in a randomized crossover trial. The drinks tested were water, green tea, and coffee (250 g each) that were consumed once a week. Metabolomics analysis was conducted on plasma samples prior the intake and repeat the process after 2 hours, also, on urine samples before and after 2 hours and 4 hours. Samples were measured by CE-TOFMS. In plasma, concentrations of trigonelline significantly increased after the consumption of coffee compared with concentrations after the consumption of the other drinks. However, metabolites weren't detected as having fluctuations as characteristic of the consumption of green tea. Furthermore, in urine, concentrations of trigonelline significantly increased after the consumption of coffee compared with those after the consumption of the other drinks. Concentrations of N-gamma-ethylglutamine significantly increased after the consumption of green tea compared with those after the consumption of the other drinks. The biomarkers of coffee consumption were trigonelline in plasma and urine and that for green tea consumption was N-gamma-ethylglutamine in urine.

P-38 Optimization of LC-MS conditions for profiling of phenolics in wine

PRESENTING AUTHOR: ***Jan Stanstrup, University of Copenhagen, Denmark***

CO-AUTHORS: ***Sara Agnolet, Nikoline Juul Nielsen, Jan H. Christensen***

When grape must is fermented, a complex mixture of metabolites is produced from the chemical moieties in grape. Due to the many possible biotransformations wine thus contain a large chemical space comprising highly volatile aroma compounds, phenolics, including anthocyanins, carbohydrates, alcohols, small organic acids, fatty acids and lipids etc. In this study, we optimized the sample preparation protocol and LC-MS conditions to obtain a broad detection coverage of phenolics in wine. The phenolics are a diverse group of compounds ranging from small phenolic acids to larger polyphenols present also as alkyl-, ester- and/or sugar conjugates. A global analysis of all phenolic classes is hence challenging because a compromise between the optimum conditions for each class of phenolics needs to be established. We investigated the effects of mobile phase conditions (pH adjustment with formic acid and ammonium formate as well as both methanol and acetonitrile) on signal intensity and peak width for a wide range of phenolics. We investigated the compromise between narrow chromatographic peaks for anthocyanins achieved at high amounts of formic acid (typically ≥ 5 %) and higher signal intensity of other phenolics achieved at lower amounts of formic acid (typically 0.1 %). Furthermore, gradient conditions and MS source parameters for both electrospray ionization modes were investigated. Sample preparation procedures were optimized so that all grape must and wine matrices from the wine making process could be analyzed with reasonable coverage and precision.

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FOOD AND NUTRITION

P-39 Metabolomics approach to identify urinary biomarkers of legume intake

PRESENTING AUTHOR: ***Pedapati S.C. Sri Harsha, UCD Institute of Food and Health, Ireland***

CO-AUTHORS: ***Roshaida Abdul Wahab, Lorraine Brennan***

Metabolomics has emerged as a key technology to identify novel food-intake biomarkers in a range of foods. In the framework of "Food Biomarker Alliance (FOODBALL)" project, the objective of this research was to identify biomarkers of legume intake by untargeted metabolomics. In this context, a randomized cross-over acute intervention study was conducted on eleven participants who consumed legumes and a control food in random order. Urine samples were collected postprandially and analysed by UPLC-QTOF-MS. The data obtained was reduced to 819 features in positive mode and 1004 features in negative mode applying filtering procedures such as data alignment and sample occurrence frequency. Robust PLS-DA models were obtained when comparing fasting samples with 4h (R2X=0.41, Q2=0.4) and 6h (R2X=0.517, Q2=0.495) post consumption of the legumes. Subsequently, the models were validated by permutation testing. The variable importance for the projection (VIP) list was created with scores ≥1.5 considered as discriminant features between the two time points. Time series plots revealed 28 features (15 in -ve mode and 13 in +ve mode) with a time response following legume consumption with 10 features demonstrating a differential time course compared to the control food. 2-Isopropylmalic acid, Asparaginyl valine and N-Carbamoyl-2-amino-2-(4-hydroxyphenyl)acetic acid were identified as specific metabolites reflecting legume intake. These metabolites were confirmed at different levels of identification based on MSMS fragmentation. Future work will validate these proposed biomarkers in a dose response study. In conclusion, the study identified novel biomarkers of food intake and developed a successful strategy for biomarker identification.

P-40 Characterization of metabolite profile from the leaves of Perilla species by HPLC/MS and GC/MS analysis

PRESENTING AUTHOR: ***Tae Joung Ha, Rural Development Administration, Korea, South***

Perilla species belonging to the Labiatae family has been frequently used in edible and medicinal plants in Asian countries including Korea, China, and Japan. The objective of this research was to determination of phenolic compound and essential oil profiles in the leaves of Perilla species including P. frutescens, P. citriodora, P. hirtella and P. setoyensis by using HPLC-PDA-MS and GC-MS analysis. A total of eighteen compositions were characterised as eight phenolics and ten essential oils of all species. Their chemical structures were elucidated as caffeic acid (1), 5'-gluco-pyranosyoxyjasmanic acid(2), apigenin-7-O-caffeoyl glucoside(3), apigenin-7-O-diglucuronide(4), scutellarein-7-O-glucuronide(5), rosmarinic acid-3-O-glucoside(6), apigenin-7-O-glucuronide(7), rosmarinic acid(8), 1-octen 3-ol(9), linalool(10), perillene(11), shisofuran(12), cis-cirtal(13), perillaketon(14), trans-citral(15), b-caryophyllene(16), a-humulene(17), and a-bergamotene(18). The individual and total compositions exhibited significant difference, especially rosmarinic acid(8) was detected as the predominant metabolite in all species. Interestingly, the predominant volatile constituents of P. frutescens and P. hirtella were perillaketone(14, 92.9 and 95.2%) and β-caryophyllene (16, 2.8 and 2.1%), respectively. While the main volatile constituents found in the oil of P. setoyensis and P. citriodora were highly dominated by shisofuran (12, 85.0%) and trans-citral(15, 46.4%), respectively.

P-41 Food-omic approach used in the characterisation of metabolite and aroma emergence during Ivory Coast cocoa beans fermentation.

PRESENTING AUTHOR: ***Mathieu Lazarus, CRIOBE, France***

CO-AUTHORS: ***Cedric Bertrand, Delphine Raviglione, Sabine Quintana, Nathalie Tapissier.***

Cocoa quality depends on numerous factors such as genetics, cultural practice or fermentation methods. Even if a great part of the aromatic development occurs during roasting, the floral and fruity aroma appears in the earliest stages of the process. In Ivory Coast, the first worldwide producer of cocoa, the company Cemoi possesses several fermentation centres. They improve the quality of the product by handling the fermentation parameters. Nevertheless, it is still possible to notice dissimilarities in cocoa beans quality. SENSOCAO project is a partnership between Cemoi and the Criobe. The main objective of the SENSOCAO project is to discover the biomarkers of the beans quality. To reach this goal, samples of cocoa from 10 fermentation centres spread all over the Ivory Coast cocoa yield area will be collected and analysed through 3 years. During the 2017 principal harvest, an experimental field test was launch to figure out the fermentative aroma generation. We collected 5 replicates per day throughout the fermentation. Afterward, an expert tasting panel was used to acquire the organoleptic profile (20 sensory descriptors) of the beans. For a large mass screening of metabolites, from 40 m/zto 1500 m/z, Headspace-SPME-GC-MS and UPLC-Qtof analysis methods were developed. Finaly,the chromatograms were integrated by XCMS and the analytical drift was corrected by the regular injection of sample pool. The combined approaches applied to the study of the kinetics fermentation allow us to propose aromatics development biomarkers of cocoa.

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POSTER SESSIONS 3 AND 4 – **Wednesday and Thursday –** all even number presenters will be at their posters.

FOOD AND NUTRITION

P-42 Metabolomic investigations into bioavailability and metabolism of raspberry ketone in diet-induced obese mice and potential implications for obesity prevention

PRESENTING AUTHOR: ***Qingli Wu, Rutgers University, United States***

CO-AUTHORS: ***Danyue Zhao, Dushyant Kshatriya, Bo Yuan, Nicholas T. Bello, James E. Simon, Qingli Wu***

Raspberry ketone (RK), a principal aroma compound, is the characteristic phenolic compound derived from red raspberry (Rubus idaeus L.). It also has anti-obesity properties demonstrated by the in vitro anti-lipogenic and in vivo weight-reducing evidences from earlier studies including ours, which involved high-fat-diet-induced obese mice fed on RK diet for 4 weeks (n=32). However, little work has been done on RK's bioavailability, metabolism, and tissue distribution, which may be correlated with its health benefits. In this study, we examined the bioavailability and metabolism of RK in both normal-weight and high-fat-diet-induced obese C57BL/6 mice. A rapid and high-throughput ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry (UPLC-QqQ/MS) method was developed for the analysis of 26 RK-related metabolites including typical microbial-derived phenolic acid metabolites (PAMs). Pharmacokinetic studies on RK were first conducted in normal mice (200 mg RK/kg body weight). Results indicated fast and efficient absorption of RK and generation of structurally-related metabolites. Moreover, in addition to PAMs, some RK-derived metabolites (e.g. vanillylideneacetone and raspberry alcohol) were also identified in the brain and white adipose tissue specimens. This is the first comprehensive study on the pharmacokinetics and microbial metabolism of RK. The present work also demonstrated that our newly-developed UPLC-QqQ/MS method was highly efficient, accurate and sensitive for analyzing RK and related metabolites in biosamples within 7 min. Currently, we are investigating whether RK and its metabolites differentially accumulate in white adipose tissues of obese compared with normal-weight mice to provide insights into the anti-lipogenic and weight-reducing mechanisms of RK.

P-43 Comparative analysis of chemical components in white ginseng, black ginseng and red ginseng

PRESENTING AUTHOR: ***Na GUO, China academy of Chinese medical sciences, China***

CO-AUTHORS: ***Xiao rong RAN, Deqiang DOU, Yan LEI***

In this study, ginsenosides and amino acids were comparatively analyzed in white ginseng, red ginseng and black ginseng. An ultra-high-performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (UPLC–Q-TOF-MS) method was developed for chemical profiling. 23 ginsenosides were fast identified by comparing the mass spectrum and/or matching the empirical molecular formula with that of known published compounds. Among them, 14 ginsenosides were further confirmed by comparisons with authentic standards. All observations acquired in both ion modes were analyzed using PCA, which indicated that the content and distribution of components were highly varied in WG, RG and BG. The results showed that during the steaming process of ginseng, ginsenosides transform into constituents of low polarity by hydrolysis, isomerization, and dehydration at C-20, and hydrolysis also occurs at C-3 or C-6. Relatively less attention has been paid to amino acids in white ginseng and red ginseng and black ginseng .And also directly simultaneous determinations of 29 amino acids were first performed on A Waters ACQUITY UHPLC system consisting of a quaternary pump with degasser and auto sampler in combination with a Waters Xevo TQ-S micro mass spectrometer (UPLC-MS/MS) with an ESI ionization source (Waters, Milford, MA, USA) in WG, RG and BG. The results showed the contents of amino acids were decreased during the steaming process. This simple and fast method provides possibility to monitor multiple components for the quality control and global evaluation of ginseng products during processing.

P-44 Gamma-ray irradiation of rodent diets alters breast cancer risk and the urinary metabolome

PRESENTING AUTHOR: ***Stephen Barnes, University of Alabama at Birmingham, United States***

CO-AUTHORS: ***Landon S Wilson, Clinton G Grubbs, Jeevan K Prasain***

60CO-irradiation of rodent diets to eliminate/reduce microorganisms has become standard practice. In models of mammary carcinogenesis in rats, such diets led unexpectedly to a 40% reduction in chemically induced-mammary tumors. 60Co irradiation also caused substantial changes in the diet metabolome with a focus on the oxidation and cleavage of unsaturated fatty acids. The goal of the present study was to examine changes in the urinary metabolome ofrats fed the irradiated diet. 24-hour urines were collected at the termination of the carcinogenesis study. Proteins were removed by methanol-induced precipitation and dried extracts subjected to nanoLC-MS/MS on a Q-TOF, collecting positive and negative ion data. Data processing involved feature alignment with XCMS, normalization, mean-centering and unit scaling, and univariate and multivariate statistical analysis using MetaboAnalyst, and pathway analysis with Mummichog. Unsupervised 3D-PCA analysis of both negative and positive ions demonstrated complete separation of the two groups. Mummichog analysis of positive ions revealed significant involvement of fatty acid metabolism, metabolism of the amino acids, lysine, methionine and cysteine, and histidine, as well as purine metabolism. Analysis of negative ions revealed significant effects on fatty acid metabolism (in particular butanoate and linoleate), as well as metabolism of the amino acids lysine, valine, isoleucine and leucine, and tryptophan. Thus, gamma-irradiation not only increased oxidation of unsaturated fatty acids in the diet, but also led to similar compounds appearingin the urine and disturbances in several other protein and energy pathways. This study stresses the importance of carefully evaluating well-meaning "improvements" in food preparation/processing.

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FOOD AND NUTRITION

P-45 Plasma metabolites associated with healthy Nordic dietary indices and risk of type 2 diabetes – A nested case control study in a Swedish population

PRESENTING AUTHOR: ***Rikard Landberg, Chalmers University of Technology, Sweden***

CO-AUTHORS: ***Lin Shi, Carl Brunius, Ingegerd Johansson, Ingvar A. Bergdahl, Bernt Lindahl, Kati Hanhineva***

Epidemiological evidence on the association of a healthy Nordic diet and future type 2 diabetes (T2D) is limited. Exploring metabolites as biomarkers of healthy Nordic dietary patterns may facilitate such investigations. In a case-control study nested in a Swedish population-based prospective cohort, we identified metabolites related to a priori-defined healthy Nordic dietary indices, i.e. the Baltic Sea Diet Score (BSDS) and the Healthy Nordic Food Index (HNFI), and evaluated their association with the risk of developing T2D. Plasma samples from 503 case-control pairs at baseline (median 7 years prior to T2D diagnosis) and from a subset of 187 pairs at 10-year follow-up were analyzed using untargeted LC-MS metabolomics. In total, 38 metabolites were associated with dietary indices, independent of several lifestyle factors. Two principal components (PC) were determined from index-related metabolites: PC1 was indicative of adherence to the indices and was stable over a 10-year period, but was not associated with T2D risk. Metabolites with high PC2 loadings were weakly related with indices, but had a stronger correlation with intakes of unhealthy foods (such as pizza, snacks and hamburgers). PC2 was also significantly associated with T2D risk. The present study did not support an association between healthy Nordic dietary indices and T2D, using either index-related metabolites or food frequency questionnaire data as exposure measurements. However, foods such as pizza, snacks and hamburgers not covered by the Healthy Nordic Food indices appeared to be more important for T2D risk in the current population.

P-46 Alterations of human urine metabolome amongst European populations at Risk-Of-Poverty

PRESENTING AUTHOR: ***Bekzod Khakimov, Associate Professor, Denmark***

CO-AUTHORS: ***Alessia Trimigno, Francesco Savorani, Leonardo Tenori, Vaiva Hendrixson, Alminas Čivilis, Marija Glibetic, Mirjana Gurinovic, Saara Pentikäinen, Janne Sallinen, Sara Garduno Diaz, Francesca Pasqui, Santosh Khokhar, Claudio Luchinat, Alessandra Bordoni, Francesco Capozzi, Søren B. Engelsen***

According to Eurostat 2016, approximately 119 million European citizens live at-risk-of-poverty (ROP). This subpopulation is highly diverse by ethnicity, age and culture in the different EU states, but they all have in common a low income, that could represent an increased risk of nutrients deficiencies due to poor nutritional habits. This study aims to investigate the human urine metabolome in the search of common biomarkers representing dietary deficiencies amongst European populations at ROP. 2732 urine samples were collected from 1391 subjects across five different European countries including the United Kingdom, Finland, Italy, Lithuania and Serbia, and analysed using 1H-NMR 1 NMR spectroscopy. The resulting urine NMR data were processed by a novel method namely SignatureMapping (SigMa) that allowed high-throughput alignment and unambiguous assignment of metabolites from exceptionally complex one-dimensional H1-NMR spectra of nearly 3000 urine samples. The resulting urine metabolome data were explored according to the study design factors including ROP, country and gender. Partitioning of the effects derived from the study design factors using ANOVA-Simultaneous Component Analysis (ASCA) revealed that country and gender effects were responsible for most of the systematic variation. The effect of the economic status, ROP versus affluent (AFF), was as expected very weak, but more pronounced in Lithuania than in other countries. Citrate and hippurate were among the most powerful ROP biomarkers. The study revealed a new knowledge on possible relationship between ROP metabolite markers, nutritional deficiencies, and life-style of European populations.

P-47 Gas chromatography-mass spectrometry analysis of headspace – solid phase microextractions for volatile metabolomic differentiation of Macrophomina phaseolina phenotypes

PRESENTING AUTHOR: ***Chathuri Udeshika Gamlath Mohottige, Mississippi State University, United States***

CO-AUTHORS: ***Richard Baird, Todd Mlsna***

Macrophomina phaseolina (MP), is important worldwidedue to its importance economically as a major fungal pathogen of commercialand agricultural crops. This plant pathogen infects crops such as soybean, corn, and sweet potatoes, and causes diseases such as charcoal rot and seedling blight. This leads to millions of dollars economic losses in the field and during storage; hence the early diagnosis of MP infection is vital in disease prevention. Following harvest and subsequent storage, the pathogen can decay food tissues. One newertoidentify MP infections in storage or is through the examinationof fungal metabolitesproducedwithin the plant-pathogen interaction such as microbial volatile organic compounds (MVOC's). Our previous studies show that the MVOC profiles of the MP fungal isolates may be varied due to the associated hosts, as well to the phenotype of the MP pathogen. In this study, MP isolated from the sweet potatoes were used to differentiate the two morphological phenotypes, namely flat (strongly pathogenic) and fluffy (low pathogenicity). The MVOC of MP were preconcentrated by using HS-SPME (Headspace- solid phase microextraction) technique and analyzed using gas chromatography coupled with triple quadrupole mass detector. Results have emphasized the possibility of using MVOC libraries to differentiate the two morphological phenotypes- MP flat and MP fluffy. Pathogen-specific volatiles may serve as biomarkers for the development of detection of Mp and other pathogen species, especially in warehouse environments. Therefore, this study may lead to developing an electronic nose the early storage detection of MP infections in sweet potatoes.

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FOOD AND NUTRITION

P-48 A comprehensive profiling method for regulatory lipid mediators using UPLC-MS/MS

PRESENTING AUTHOR: ***Jun Yang, University of California, Davis, United States***

CO-AUTHORS: ***Jun Yang, Debin Wan, Jia Sun, Bruce D. Hammock***

The regulatory lipid mediators are biologically active metabolites derived from polyunsaturated fatty acids such as the metabolites by cyclooxygenases, lipoxygenases, and cytochrome P450 enzymes. These pathways represent over 75% of pharmaceutical in mass in the world. By measuring endogenous metabolic responses to various environment stimulus, pathological processes and dietary supplements, lipidomics/metabolomics provide the most useful information about human responses. The knowledge gained from this type of research is very useful for many research fields including biomarker discovery, biotechnology advancement, discovery of novel biochemical mechanism, and assessment of clinical effectiveness. We developed a sensitive and comprehensive profiling method for the regulatory lipid mediators covering more than 150 lipid mediators including prostaglandins, leukotrienes, epoxy fatty acids, dihydroxy fatty acids, specialized pro-resolving mediators, endocannabinoids, nitro-fatty acids, poly-unsaturated fatty acids and the trace for major species of phospholipids.

P-49 Metabonomics-baesd mechanistic investigations of Chai-hu Shu Gan San, a classical TCM formula for treatment of depression

PRESENTING AUTHOR: ***Zhong-Mei Zou, Insititute of Medicinal Plant Development, CAMS &PUMC, China***

CO-AUTHORS: ***Hong-Mei Jia, Meng Yu***

Accumulating evidence suggests that the gut microbiota dysbiosis and host metabolic phenotype alterationare important factor in mediating the development of depression. Chaihu-Shu-Gan-San (CSGS) is a Traditional Chinese Medicine (TCM) prescription, which has been used for the treatment of depression and various gastrointestinal (GI) disorders in China. However, the active components of CSGS and its underlying mechanism of action remains incompletely understood. Therefore, an integrated approach combining 16S rRNA gene sequencing profile and UPLC-MS-based metabonomics strategy were performed in rat model of chronic depression to evaluate the antiderpessant effects of CSGS. Our data indicate that CSGS not noly reverses the gut microbiota dysbiosis–as indicated the decreased Firmicutes and increased Bacteroidetes levels–but also shows reverting effects on changes of host metabolic phenotye. The findings provided a comprehensive understanding of the protective effects of CSGS on depression, and further as a new methodological cue for dissecting the underlying mechanism of TCMs.

P-50 Biofluid and tissue metabolic profiling by 1D 1H NMR spectroscopy highlighting exposure to emerging drugs of abuse.

PRESENTING AUTHOR: ***Alexis Ripoche, The Institute for Global Food Security, United Kingdom***

CO-AUTHORS: ***Alexis Ripoche, Ali Yilmaz, Anna Gadaj, Tom Buckley, Stewart Graham, Mark H. Mooney***

Background: Selective androgen receptor modulators (SARMs) are of particular interest within the therapeutic drug development field due to their tissue selectivity and oral bioavailability. However, these properties provide the potential for these agents to be misused to artificially enhance performance in both livestock production and sport. One potential approach to detect SARM misuse uses metabolomics to highlight physiological responses to illegal administration. Methods: Using a rat model, we report on an in vivo exposure study used to determine the effects of three specific SARMs, namely: Ostarine, LGD4033 and RAD140. Animals were randomly allocated to four study groups (n=8 per cohort) and treated daily via oral gavage over a 17 day period with respective treatments - Control (vehicle), Ostarine (3mg/kg b.w.), LGD-4033 (3mg/kg b.w.) or RAD140 (3mg/kg b.w.). Results: Biofluid and tissues obtained throughout the study were subjected to profiling by 1D 1H NMR and raw data processed using Bayesil. Principal Component Analysis (PCA) and Partial Least Square-Discriminant Analysis (PLS-DA) demonstrate significant separation between the sample groups and across biomatrices. Using logistic regression analysis,predictive algorithms were developed for serum with an AUC (95% CI) = 0.999 (1-1) and specificity and sensitivity equal to 95% and 100%, respectively. Discussion: Putative metabolite biomarkers of SARM administration have been identified from different biomatrices highlighting novel metabolic pathways associated with their activity, providing novel insights into how these potential performance enhancing drugs work.

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POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

FOOD AND NUTRITION

P-51 Metabolic Responses of healthy rats subjected to Electro-acupuncture and Moxibustion Stimulations investigated by 1H NMR metabolomics

PRESENTING AUTHOR: ***Jingjing Xu, Xiamen University, China***

CO-AUTHORS: ***Kian-Kai Cheng, Jiyang Dong, Guiping Shen, Xiaorong Chang***

The effectiveness and molecular foundation of electro-acupuncture and moxibustion is assessed on healthy subjects for disease prevention and health care. Proton nuclear magnetic resonance (NMR) spectral analysis of multiple biological samples (cortex, stomach and liver) together with plasma hormones assay were employed to systematically investigate the metabolic profilings and their functional pathway of healthy rats subjected to either electro-acupuncture or moxibustion stimulation. Taking the Stomach meridian (SM) and the Gallbladder meridian (GM) as two studying cases, the similarities and differences in biochemical compositions due to these two interventions were obtained. When stimulating the SM acupoints, both interventions showed common features in regulations of purine metabolism/purinergic signaling, energy production of TCA cycle, glycolysis and function-connected branched-chain amino acids metabolism, while electro-acupuncture treatment was characterized in protein metabolism including alanine and aspartate metabolism, glycine, serine and threonine metabolism, pyruvate metabolism. Especially, except for universal traits shared with SM, synthesis of catecholamines in plasma was downregulated by electro-acupuncture after stimulating GM, which is distinguished with the higher excretion of catecholamines upon moxibustion to SM. These findings provides new insights into the biochemical basis of traditional Chinese medicine practices that are expected to be taken as reference index for interventional selection.

P-52 Early infant diet influences fecal and urinary bile acid excretion in healthy infants receiving breast milk, dairy milk formula, or soy formula at 3 months of age

PRESENTING AUTHOR: ***Lindsay M. Pack, Arkansas Children's Nutrition Center, United States***

CO-AUTHORS: ***Haixia Lin, Aline Andres, Kelly Mercer***

Nutrition is an important regulator of growth during the 1st year of life, and an effect of postnatal diet on subsequent growth, metabolism and obesity risk has been observed. Bile acids (BA) are metabolic signaling molecules are capable of controlling glucose homeostasis, lipid metabolism, and energy expenditure. To determine if early diets influence BA synthesis, we used targeted-LC/MS to measure total fecal BA in infants age 3 mo. receiving exclusive diets of breast milk (BM: n=16), cow's milk formula [MF: n=9], or soy formula [SF: n=15]. Total urinary BA were quantified in a second set of 3 mo. old infants [BM: n=15, MF: n=15, SF: n=15] using a method coupling enzymatic deconjugation for the removal of glycosidic- and sulfate-conjugates with targeted-LC/MS. SF group total fecal BA concentration was 2- to 4-fold higher than in BM and MF groups, $P<0.05$. Similarly, total urinary BA concentration was 20% higher in the SF group, relative to the BM and MF groups, $P<0.05$. Interestingly, the MF group had reduced fecal and urine BA elimination relative to BM and SF groups, $P<0.05$. For all diets, primary BA represented the majority of excreted BA. Secondary BA concentrations in feces and urine were significantly higher (UDCA > LCA> DCA) in both formula groups relative to the BM group. We conclude infant formula feeding alters BA synthesis, and suggest, in theory, early infant diet has a programming effect on enterohepatic BA recycling. This work was funded by USDA-ARS Project 6026-51000-010-05S; clinicaltrials.gov identifier NCT00616395.

P-53 A Combined Targeted and Untargeted LC-MS Approach to Determining Postprandial Fat Soluble Vitamin and Lipid Levels in Metabolic Syndrome and Healthy Subjects

PRESENTING AUTHOR: ***Haley Chatelaine, Human Nutrition Program, The Ohio State University, United States***

CO-AUTHORS: ***Priyankar Dey, Richard S. Bruno, Rachel E. Kopec***

Subjects with metabolic syndrome (MetS) absorb less vitamin E than healthy matched controls, despite an increased triglyceride response. This phenomenon may signify altered pathways of absorption of other lipid classes, previously uninvestigated in MetS subjects. We hypothesized that subjects with MetS (1) have decreased absorption of a biologically relevant dose of fat-soluble vitamins (FSVs; vitamins A (501 IU) and D (119 IU)) and (2) have differential postprandial lipidomic profiles, reflecting differences in lipid uptake, packaging, and/or basolateral excretion, relative to healthy matched controls. A targeted high-performace liquid chromatography-tandem mass spectrometry (LC-MS/MS) method using APCI positive ionization and stable isotopes for quantitation was adapted to quantitate retinyl palmitate and ergocalciferol in postprandial triglyceride-rich lipoprotein fractions (TRL) from MetS and control subjects after soymilk consumption. Differences in FSV absorption were calculated by comparing 0-12 hour area under the curve (AUC) concentrations normalized to ApoB-48. Results demonstrate a significant decrease in vitamin D/ApoB-48 ($p=0.05$) but no significant difference in vitamin A/ApoB-48 ($p=0.16$) when comparing MetS to healthy subjects. An untargeted liquid chromatography-quadrupole time-of-flight mass spectrometery (LC-MS) method with ESI positive ionization was used to determine lipidome profiles in the same TRL fraction. Preliminary review of lipidomics results reveal features consistent with lipids provided by the soymilk beverage (eg. linoleate TGs and phosphatidylcholines). Present results suggest that vitamin D absorption and release into the circulation is more affected by MetS than vitamin A. Continued lipidomics analysis should give more insight regarding the stage(s) of lipid absorption that differentially affect MetS, compared to healthy controls.

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FOOD AND NUTRITION

P-54 Metabolomics Analysis of Urine in hyperlipidemia Rat after Administration with hawthorn ethanol extracts

PRESENTING AUTHOR: ***Chuanqin Hu, Beijing Technology and Business University, China***

CO-AUTHORS: ***Chuanqin Hu, Jing Wang, Baoguo Sun***

The study aimed to explore the metabolic changes in urine of hyperlipidemia rats with hawthorn ethanol extracts consumption by metabolomics. The metabolic profiles from urine revealed compounds distinguishing hyperlipidemia rats from normal rats and HEE group. Totally, 21 endogenous metabolites in urine involved in amino acid metabolism, energy metabolism, glutamine metabolism, fatty acid metabolism and glycerin phospholipid metabolism were identified as potential biomarkers of urine of hyperlipidemia rats. It turns out that hawthorn was a promising functional food for hyperlipidemia. these metabolites and metabolic networks we found offer new insights into exploring the molecular mechanisms of lipid-lowering of hawthorn ethanol extracts.

P-55 Urinary metabolic differences in breastfed and formula-fed infants at 3 months

PRESENTING AUTHOR: ***Kelly Mercer, Arkansas Children's Nutrition Center, United States***

CO-AUTHORS: ***Sudeepa Bhattacharyya, Brian D. Piccolo, Aline Andres***

Body composition, energy metabolism and appetite are influenced by different infant diets. There is growing consensus that such nutritional "programming effects" may persist and influence risk for obesity, diabetes, and cardiovascular disease in adulthood. Urinary metabolic analysis is a useful tool in assessing effects of diet on early-life metabolism. In this study, urine from healthy 3 month old infants receiving breast milk [BF; n=92], dairy milk infant formula [MF; n=82], or soy-based infant formula [SF; n=86], was analyzed for untargeted metabolomics using GC-TOF MS. Statistical analyses using uni- and multi-variate methods showed several metabolites that discriminated the three diet groups. In BF infants, mono-, di- and oligosaccharides including fucose, ribose, maltose, and raffinose were significantly elevated relative to MF and SF infants. Associated pathways include galactose metabolism and the pentose phosphate pathway. Urea, amino acids and their derivatives, including phenylalanine, alanine, 4-hydroxyproline, and trimethyllysine were also elevated in BF compared to MF and SF infants,indicative of increased protein catabolism. In MF and SF infants, phenylacetylglycine, and 3,4 dihydroxybutyric acid were significantly elevated relative to BF infants, suggesting impaired energy production through the citric acid cycle. Discriminating metabolites between SF and MF infants were 3,4-dihydroxyphenylacetic acid, pinitol, and other compounds derived from soy food components. Thus, different infant diets can have profound effects on metabolism and diet-derived compounds that enter systemic circulation and are excreted in the urine. This work was funded by USDA-ARS Project 6026-51000-010-05S; clinicaltrials.gov identifier NCT00616395.

P-56 Identifying Metabolomic Profiles of Inflammatory Diets in Postmenopausal Women

PRESENTING AUTHOR: ***Fred K. Tabung, Harvard T. H. Chan School of Public Health, United States***

CO-AUTHORS: ***Liming Liang, Tianyi Huang, Raji Balasubramanian, Edward L. Giovannucci, Paulette D. Chandler, JoAnn E. Manson, Elizabeth M. Cespedes Feliciano, Kathleen M. Hayden, Linda Van Horn, Kathryn M. Rexrode***

Introduction: Dietary composition modulates inflammation. We previously showed that a food-based empirical dietary inflammatory pattern (EDIP) score is associated with circulating inflammatory biomarkers. Metabolic profiling of inflammatory diets may therefore identify diet-related mechanisms contributing to disease etiology. Methods: This baseline cross-sectional investigation studied associations between continuous EDIP scores calculated from food frequency questionnaires and 470 log-transformed metabolites as outcomes in multivariable-adjusted linear regression analyses among 1,919 Women's Health Initiative (WHI) participants. Plasma metabolites were measured with liquid chromatography tandem mass spectroscopy. Metabolite discovery was conducted among 1,109 WHI Hormone Therapy trial participants and results were replicated among 810 WHI Observational Study participants. Analyses were stratified by standard body mass index categories (BMI,kg/m2) without replication due to smaller samples. Statistical significance was defined as false-discovery rate adjusted $p<0.05$. Results: Independent of age, energy intake, BMI, physical activity, smoking; 24 metabolites were associated with EDIP score. Of these, two inversely associated metabolites were replicated - hippurate and 1,7-dimethyluric acid - related to higher fruit and vegetables intake and caffeine, respectively. Associations suggested differences by BMI category. Among normal weight women, four metabolites (hypoxanthine, C18:1SM, C34:2PC, trimethylbenzene) were inversely associated with EDIP scores. Up to 55 metabolites were identified among overweight women (mostly phospholipids and triglycerides) and 30 among obese women (mostly plasmalogen lipids, purines and pyrimidines). Conclusion: Metabolites associated with lipid metabolism may inform on the pathophysiology of inflammatory diets. The potential interaction between EDIPand adiposity on metabolomic profiles requires further study as does the linkage to disease outcomes.

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FOOD AND NUTRITION

P-57 Hypervitaminosis A: global metabolic changes in chronic high dose vitamin A in a pig model

PRESENTING AUTHOR: ***Dr Catherine L Winder, University of Birmingham, United Kingdom***

CO-AUTHORS: ***Antony Oxley, Kieran Finney, Andrew Southam, Dorsa Varshavi, Andris Jankevics, Gavin Lloyd, Warwick B. Dunn and Georg Lietz***

Vitamin A and its associated metabolites are important in multiple biological roles including vision (in the retina), immune function, gene transcription and skin health. These lipophilic unsaturated lipids include retinol, retinal, retinoic acid, retinyl palmitate and provitamins including carotenoids like carotene. Vitamin A metabolites can accumulate in mammals due to their lipophilic nature, slow metabolism and slow release from the body. In severe cases where food rich in vitamin A (e.g. liver) is consumed then acute or chronic Hypervitaminosis A can be observed. Limited knowledge is available on the global metabolic changes associated with Hypervitaminosis A and here we present a metabolic study of pigs to enhance our knowledge of low and high dose chronic Hypervitaminosis A. Pigs were provided different doses daily of retinyl proprionate (0-10,000 micrograms/kg body weight) by oral syringe with plasma and urine collected after 15 weeks. Two untargeted UPLC-MS assays (HILIC and C18 reversed phase) were applied to analyse plasma and urine followed by univariate and multivariate analysis. Significant metabolic changes were observed in vitamin A and carotenoid metabolism as expected. However, further metabolic changes were observed primarily in other areas of lipid metabolism which indicate metabolic changes in the liver and in its synthesis of lipoproteins, even at low doses.

P-58 The impact of the environment and rapseed varieties on health promoting molecules

PRESENTING AUTHOR: ***Djawed Bennouna, C2VN Faculty of Medecine of Marseille, France***

CO-AUTHORS: ***Ljubica Svilar, Célia Pontet, Frédéric Fine, Xavier Pinochet, Karl Fraser, Jean-Charles Martin.***

Rapeseed is grown for its use in the food industry, as well as its applicability in green chemistry as a biofuel.the combination of crop cultivars with environmental conditions can be a catalyst to improve the nutritional quality of plants. In order to achieve this goal, eight rapeseed varieties, grown in eight regions of France, were compared with a non-targeted metabolomics approach.The multivariate data analysis using a combination of hierarchical clustering and correlation network analysis highlighted the distance and closeness between the samples in terms of both genotypes and geographical regions. Overall a major environmental impact was observed on the polar metabolome, with different trends, depending on the varieties.In fact, some varieties were very sensitive to the environment, while others were quite resilient. The comparison of the most impacting environment has shown a dramatic effect between the balance in the molecules of interest such as phenolic compounds and glucosinolates.Lastly, the identified secondary metabolites were mapped into the KEGG pathway database to reveal the most sensitive gene targets susceptible to environmental influences. We thus identifieda glucosyl transferase encoded by the UGT84A1gene in the biosynthesis of phenylpropanoid that could be rate limiting/promoting in this pathway depending on environmental conditions. This gene could be an interesting target to investigate in order to improve the production of health promoting metabolites in rapeseed plants. The metabolomicapproach enabled us to identify and characterized the environmental influence on various cultivars of brassica napus seeds, and may help identify targets for crop improvement.

P-59 MMetabolic Signatures Associated with Western and Prudent Dietary Patterns in Women

PRESENTING AUTHOR: ***Paulette D. Chandler, Brigham and Women's Hospital/Harvard Medical School, United States***

CO-AUTHORS: ***Paulette D. Chandler, Raji Balasubramanian, Nina Paynter, Franco Giulianini, Teresa Fung, Lesley F. Tinker, Linda Snetselar, Simin Liu, Charles Eaton, Deirdre Tobias, JoAnn E. Manson, Edward L.Giovannucci, Clary Clish, Kathryn M. Rexrode***

Introduction: Dietary patterns have a complex effect on metabolism. Metabolomic profiling may identify biological mechanisms linking dietary patterns with cardiovascular disease and cancer. Methods: Evaluated the cross-sectional association between dietary patterns and metabolites in 2306 WHI participants without CVD at baseline. Used a food-frequency questionnaire to identify two dietary patterns by factor analysis consistent with previously described Western (WD) and Prudent (PD). Measured metabolites with liquid chromatography tandem mass spectroscopy (LC-MS). Completed metabolite discovery in 944 WHI Observational Study (WHI-OS) participants and replicated results among 1362 WHI Hormone Therapy trial (WHI-HT) participants. Single metabolite linear regression models of each of the 372 metabolites were created with the 2 dietary scores. Results: The PD included higher intakes of vegetables and fruits whereas the WD included higher intakes of saturated fat and meat. We identified 82 metabolites associated with dietary patterns that were discovered in the WHI-OS and replicated in WHI-HT with FDR corrected $p<0.05$. In all analyses, phosphatidylethanolamine plasmalogens and phosphatidylcholine plasmalogens, related to higher saturated fat and meat intake, were positively associated with WD and inversely associated with PD, whereas cholesteryl esters and long chain fatty acids, related to dark green leafy vegetable and fatty fish intake, were positively associated with PD and inversely associated with WD even in a BMI-matched subcohort of the 2306 participants. Conclusions: Distinct metabolite signatures are associated with Western and Prudent dietary patterns. Metabolites involved in mitochondrial fatty acid oxidation and insulin resistance have a strong inverse relationship with PD and direct relationship with WD.

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PLANT METABOLOMICS

P-60 Secondary metabolites from P. angustifolium Lodd leaves by UHPLC-QToF-MS metabolomics integrated with multivariate analysis

PRESENTING AUTHOR: ***Anuja N Patil, School of Pharmacy, University of Queensland, Australia***

CO-AUTHORS: ***Shaw Paul Nicholas, Parat Marie-Odile, Bose Utpal, Fitzgerald Melissa***

Australian native willow, (Pittosporum angustifoliumLodd.), known also as gumby gumby, has been traditionally used as a medicinal plant in Australian Aboriginal communities, to treat various skin conditions including cancer. This observation underpins our research interests in secondary metabolite fingerprinting and bioactive compound identification from the leaves of this plant. This study included cultivars of P. angustifolium Lodd. from three distinct geographic origins and their leaf extracts (Juice [GGJ] and decoction [GGD]. Plant metabolites were extracted and analysed via reverse phase ultra-high-performance liquid chromatography-mass spectrometry (UPHLC-QToF-MS) in positive ionisation mode in order to compare secondary metabolites of GGJ and GGD. To further investigate the metabolite profile of P. angustifolium Lodd. extract, we used multivariate analysis and subsequent database search to identify and monitor discriminating metabolites between two sample extraction processes. We identified 21 number of compounds from two sample groups; seven compounds were identified and purchased from a commercial source to confirm their identification LC-MS/MS. Interestingly, we have identified betulinic acid by comparing accurate mass, retention time and MS/MS fragmentation pattern, which was present as a discriminating compound in decoction extracts. Using LC-QToF-MS-based metabolomics approach, we have identified a few metabolites from P. angustifolium Lodd. plant. The putatively identified compound, betulinic acid, and other tentatively identified metabolites may potentially act as marker compounds and may be used in the future phenotypic analysis of P. angustifolium Lodd. variants.

P-61 Plant-vector-pathogen interactions: Identification of metabolome changes in individual insects infected with Candidatus Liberibacter species

PRESENTING AUTHOR: ***David R. Gang, Washington State University, United States***

CO-AUTHORS: ***Anna Berim, Ruifeng He, Xiaolan Wang, Jing Wang, Jeong-Jin Park, Nabil Killiny, Tonja W. Fisher, Judith K. Brown***

Background and Significance: Psyllids are small, phloem sucking insects that spread significant plant diseases, most particularly Zebra Chip disease of potato (spread by potato psyllid) and Citrus Greening Disease (also called Huanglongbing or HLB) of citrus (spread by Asian citrus psyllid). These diseases are caused by fastidious (so far unculturable) bacterial species belonging to the genus, Liberibacter. At the present time, tools to fight these diseases are very limited, and development of new tools, particularly that would take advantage of a knowledge of genetics of the pathogens, are hampered by the lack of ability to culture these bacteria. A critical component to this understanding occurs at the metabolic level. The bacteria are parasitic and require certain metabolites from their hosts. Learning what those requirements are may be the breakthrough that is required to culture these significant pathogens. Approach: We compared the primary metabolite and lipid profiles between Liberibacter-infected and healthy psyllids using GC-TOF-MS and UPLC-TOF-MS and also examined individual insects using MALDI-MS imaging mass spectrometry (tissue imaging), with the goal of identify changes in metabolite levels that occur upon infection and localization of those changes within the insect tissues. Results: Infection with Liberibacter and feeding on infected trees cause dramatic changes in the psyllid primary and lipid metabolome. Differences across the psyllid bodies were observed in tissue imaging results, where specific metabolites change significantly upon infection. These results suggest that infection by Liberibacter species causes major metabolic changes within the insects providing clues to infection mechanisms used by the pathogen.

P-62 Identification of triterpene saponins from Medicago truncatula using UHPLC-QTOF-MS/MS-SPE-NMR

PRESENTING AUTHOR: ***Anil Bhatia, Department of Biochemistry and MU Metabolomics Center, University of Missouri, United States***

CO-AUTHORS: ***Anil Bhatia, Feng Qiu, Dennis Fine, Daniel Wherritt, Zhentian Lei, Barbara W. Sumner, Lloyd W. Sumner***

Metabolomics represents an emerging complementary method to genomics and proteomics for studying biological system responses to various surrounding factors which lead to metabolic variations. The number one grand challenge of metabolomics is the confident identification of all the metabolites observed in the profiles. To meet this grand challenge, we have developed an integrated UHPLC-QTOF-MS/MS-SPE-NMR system for higher-throughput metabolite identifications which is providing advanced biological context and further elevating the scientific utility of plant metabolomics. This integrated method is less labor intensive and more cost effective than independent methods (LC; MS; SPE; NMR). It enables the simultaneous purification and identification of primary and secondary metabolites. In this study, metabolite profiling was performed using aqueous methanolic extracts of Medicago truncatula root and leaves samples to better understand specialized metabolism in this model legume. More than 300 metabolites from a diversity of chemical classes were successfully observed in the Medicago truncatula extracts using UHPLC-QTOF-MS/MS. The metabolites observed in UHPLC chromatogram were identified using MS/MS2 Bruker-Sumner library in Metaboscape 3.0. Metabolite identifications were predicted for those metabolites not identified through spectral matching using an in-house developed Plant Metabolite Annotation Toolbox (PlantMAT) software. The predicted metabolite identifications were then confirmed or determined de novo using 1D and 2D NMR. Approximately 100 saponins and polyphenolic glycosides have been confidently identified following UHPLC-QTOFMS/MS-SPE-NMR while noting that most of these are not commercially available. Thus, UHPLC-QTOFMS/MS-SPE-NMR has enabled greater efficiency in metabolite purification and identification, reduced the amount of a material needed, and empowered higher throughput metabolite annotations.

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POSTER SESSIONS 3 AND 4 – **Wednesday and Thursday –** all even number presenters will be at their posters.

PLANT METABOLOMICS

P-63 1H NMR-based metabolomics and transcriptomics techniques to explore salvianolic acids biosynthesis

PRESENTING AUTHOR: ***Yinghong Wang, Institute of Materia Medica (IMM), Chinese Academy of Medical Sciences & Peking Union Medical College, China***

CO-AUTHORS: ***Xia Liu, Mengxia Jin, Min Zhang, Tianqi Li, Wenyi He, Xiangju Jin, Hongyue Liu, Yan Wu, Yanan Wang, Shanshan Sun, Jinyue Zhang, Jungui Dai***

Salvia miltiorrhiza Bunge is a traditional Chinese medicine. The water-soluble phenolic acids active compounds have very important medicinal value, and the synthesis pathways of the main active ingredients are still unknown. Here, we employed nuclear magnetic resonance (NMR)-based metabolomics and transcriptomics techniques to study the biosynthesis mechanism of salvianolic acids. High-performance liquid chromatography (HPLC) combined with NMR showed an improvement over traditional techniques, and 54 metabolites were detected. The result of the multivariate statistical analysis showed that Salvianolic acid B (SAB),rosmarinic acid (RA), caffeic acid, succinate, citrate, etc., were increased in the methyl jasmonate (MeJA)-elicited group, and sucrose, fructose, glutamine, tyrosine, etc., were decreased. This phenomenon indicated that MeJA induced changes in the primary metabolic pathway, including carbohydrate metabolism, amino acid and lipid metabolism, and the secondary metabolic pathway, including the synthesis of salvianolic acids and tanshinones. The analysis of transcriptome sequencing after MeJA-induced changes showed 6991 differentially expressed genes (DEGs), which mainly included hot-spot metabolic pathways. Through the association analysis of metabolomics and transcriptomics, we found that the synthesis of RA mostly occurred through caffeic acid and 4-hydroxyphenyllactic acid bypass after MeJA treatment.This work provides a new method to elucidate the biosynthetic pathway of plant secondary metabolites.

P-64 Untargeted metabolomic approach for analysis of Angelica gigas from different regions in Korea

PRESENTING AUTHOR: ***Nahyun Kim, Forest Medicinal Resources Research Center, National Institute of Forest Science, Korea, South***

CO-AUTHORS: ***Su Jin Sim, Hyun Ji Eo, Jeong Ho Song, Mahn-Jo Kim***

Angelica gigas (Umbelliferae) is a perennial herb widely distributed in Korea, China and Japan. The roots of A. gigas have been used as an herbal medicine for prevention and treatment of diverse diseases. Since the compositional changes of most components in this plant vary with the geographical origins and affect to therapeutic efficacy, it is important to authenticate geographical origins of A. gigas. In this study, A. gigas samples were collected from 13 different regions in Korea in October, 2017, and these were analyzed by ultraperformance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF MS) using optimized analytical methods to compare the metabolite patterns. In addition, three major components including nodakenin, decursin and decursinol angelate were quantified to compare contents according to cultivated regions. The metabolite profiling data were processed by multivariate statistical analyses such as PCA and PLS-DA to determine the differences among geographical origins. To identify significant metabolites according to regional variances of A. gigas, more sophisticated multivariate statistical analyses such as metabolite selection should be performed.

P-65 Biomarkers for the Prediction of Physiological Seed Quality – Metabolite Profiling

PRESENTING AUTHOR: ***Eden Tesfu, Monsanto, United States***

CO-AUTHORS: ***Todd Brown, James Doom, Joan Wang, Stephen Karr, Kang Liu, Martin Ruebelt***

The production of seeds with high and uniform rates of germination is a key goal of the seed business, and methods to rapidly assess seed quality in production environments are critical. Current quality testing methods, while generally useful, pose critical challenges including high cost of production, longer testing period and sometimes questionable predictive ability to field performance. Our team has been looking for various quick diagnostic tools that can predict germinability of seed lots. The present study was conducted to identify metabolic properties of maize seed that are associated with germination and vigor. A number of metabolites were measured, and multivariate statistical analyses were used to identify those seed components that have a significant statistical relationship with germination and vigor. This information will be used as the basis to develop predictive models for germination and vigor and rapid, robust technologies for the analysis of seed quality.

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PLANT METABOLOMICS

P-66 Discovery of Antifungal Metabolites in Maize Cob Tissue

PRESENTING AUTHOR: ***Jeremy Winders, Mississippi State University, Institute for Genomics, Biotechnology & Biocomputing, United States***

CO-AUTHORS: ***Dr. Tibor Pechan***

Aspergillus flavus is a pathogenic fungus that causes ear rot of maize (Zea mays L.) and produces a carcinogenic secondary metabolite known as aflatoxin. A. flavus is a chronic problem in food safety as it often infects and poisons maize (both pre- and post- harvest), and accounts for an average loss of $225 million per year in US. Aflatoxin B1 is the most potent carcinogenic mycotoxin known, causing hepatocellular carcinoma, along with many other health problems. Both humans and animals are at risk from ingesting food and feed contaminated with aflatoxin. The long term goal of our NIFA funded project is to mitigate the loss in maize production due to aflatoxin accumulation while also increasing food safety and sustainability. While maize silk and kernels contributors to aflatoxin resistance have been widely investigated, maize cob rachis tissue has only received limited attention. β-GUS-tagged A. flavus bioassays demonstrated A. flavus's ability to spread through the vascular tissue of susceptible maize but is limited in resistant maize. This indicates the rachis tissue's defensive response is critical in limiting the spread of A. flavus in maize. The objective of this project is to confirm the role of maize cob metabolites in resistance against fungal pathogen, with the specific goals of identification of particular antifungal metabolites using LC-MSn technology. For the first time in cob rachis tissue, our initial research has already identified several groups of known antifungal metabolites, with statistically significant differential abundance in the resistant vs. susceptible genotypes.

P-67 Identification of the putative metabolic biomarker underlying cooked rice elongation

PRESENTING AUTHOR: ***Nnaaemeka Emmanuel Okpala, South China University of Agriculture, China***

CO-AUTHORS: ***Lixin Duan, Guoan Shen, Guiquan Zhang and Xiaoquan Qi***

Cooked rice elongation, cooked rice expansion, and water absorption have been identified as some of the parameters used in gauging rice grain quality. In the present study, we investigated the putative metabolite biomarkers associated with the variation of cooked rice elongation for Hua Jing Xian 74 (receptor), Basmati 370 (donor), and five hybrid lines resulting from a cross of these parent lines. We also investigated their cooked rice expansion and water absorption properties. After carrying out cooked rice elongation studies, metabolomics studies, correlation analyses, V-plot analyses, and thorough searches in public metabolite databases (Metlin, Massbank and KEGG), and in-house secondary metabolite database, we identified a metabolite with molecular weight of 280.25 and retention time of 6.4 min as a putative biomarker associated with cooked rice elongation in the varieties investigated. We also discovered that changes in cooked rice elongation and changes in cooked rice expansion follow a similar pattern; however, it appears that cooked rice elongation and cooked rice expansion do not affect water absorption in these rice lines. Our findings may facilitate the improvement of the cooked rice elongation of hybrids resulting from the crosses of Basmati 370 and Hua Jing Xian 74. Our results also offer interesting insight into cooked rice elongation, cooked rice expansion, and water absorption. Abbreviations: B-385_Basmati 385; HJX-74_Hua Jing Xian 74; UPLC-Q-TOF/MS_Ultra high performance liquid chromatography coupled to quadrupole time of flight mass spectrometry.

P-68 Authentication of Botanicals and Herbal Products Using UPLC/Ion Mobility QTof-MS and a Metabolomics Approach

PRESENTING AUTHOR: ***Giorgis Isaac, Waters Corporation, United States***

CO-AUTHORS: ***Bharathi Avula, Yan-Hong Wang, Jimmy Yuk, Ji-Yeong Bae, Mei Wang, Rob Plumb, Ikhlas A. Khan***

Metabolomics can be used to provide an unbiased, comprehensive qualitative and quantitative overview of the metabolites present in botanicals and dietary supplements to authenticate commercial herbal products. Fadogia agrestis is a small shrub indigenous to Africa. The aqueous stem extract containing saponins, flavonoids, etc has been used in traditional medicine as aphrodisiac. A major concern of Fadogia agrestis products is adulteration. The metabolite profiles of authentic Fadogia and Fadogia commercial products were investigated. The samples were randomized and injected three times with a set of QC pooled sample runs in both positive and negative ion mode. Details of data file format and a list of expected adducts are entered to facilitate the handling of data import followed by retention time alignment. The UPLC/IM-QTof-MS data was first aligned to correct any retention time drift between analytical runs. After retention time alignment, automatic peak detection, normalization, deconvolution, compound quantitation, identification and statistical analysis was performed. PCA clustering was observed, reflecting authentic Fadogia and corresponding commercial products. Significantly changing potential markers that differentiate between authentic Fadogia and commercial products were identified that can be used as a target markers for Fadogia authentication. The identification of potential marker compounds was based on exact mass precursor ion, theoretical isotopic distribution, retention time and high energy fragment ion. Compound identification was improved by matching 'in silico' theoretical fragmentation of a candidate compound against experimentally measured fragments ions. The potential of ion mobility for the separation of isomers and chromatographically co-eluting compounds will also be investigated.

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PLANT METABOLOMICS

P-69 Annotation of unknown phenylalanine-derived wheat metabolites in response to deoxynivalenol treatment

PRESENTING AUTHOR: ***Christoph Bueschl, BOKU University Vienna, IFA-Tulln, Austria***

CO-AUTHORS: ***Maria Doppler, Bernhard Kluger, Rainer Schuhmacher***

Fusarium graminearum is a filamentous fungus infecting small grain cereals such as wheat at flowering stage. The resulting Fusarium head blight disease is accompanied by contamination with mycotoxins such as deoxynivalenol which makes infested wheat grains unsafe for human and animal consumption. Resistance against FHB is a polygenic quantitative trait, the mechanisms of which are not fully understood to date. To investigate which of the metabolites of partly resistant wheat lines are involved in resistance, a stable isotope assisted tracer experiment with 13C-labeled phenylalanine was carried out. Flowering wheat ears were treated with deoxynivalenol, the main virulence factor of the fungus. Some 150 phenylalanine-derived metabolites were detected with this approach, most of which remained unknown. To annotate these metabolites, we combined the tracer experiment with a) a uniform 13C-labeling experiment, b) LC-HRMS/MS analysis, c) advanced sum formula generation with CSI:FingerID, d) molecular networking, and e) statistical analysis. Identified metabolites served as reference compounds for the annotation ofunknown metabolites. Analysis was concentrated on those metabolites/clusters with significant differences in metabolite levels between the deoxynivalenol treated and the control samples. The uniformly 13C-labeled samples together with the CSI:FingerID annotation allowed the generation of unique sum formulas for most of themetabolites. Furthermore, several clusters of statistically significant and structural similar metabolites were present after the individual MS/MS spectra have been correlated with molecular networking. The combination of these data evaluation approaches enabled the annotation of additional metabolites putatively involved in the plant's metabolic defense.

P-70 Characterization of the metabolic landscape of immature tomato fruit using a large-scale untargeted metabolomics approach.

PRESENTING AUTHOR: ***Maria Elena Diaz Rubio, Institute of Biotechnology, Cornell University, United States***

CO-AUTHORS: ***Philippe Nicolas, Sheng Zhang, Jocelyn K. C. Rose,Carmen Catalá.***

Tomato fruit is an economically important crop and a model system to study fruit development. Tomato fruit growth and ripening is characterized by changes in primary and secondary metabolism that result in accumulation of sugars and organic acids, volatiles, carotenoids and flavonoids. Metabolomics, combining high-throughput analytical methods and multivariate statistical analyses has beenused to evaluate changes in primary and secondary metabolites during tomato fruit ripening. However, little information exists about metabolic profiles during the stage of active fruit growth. Here we used a non-targeted metabolomics approach to determinechanges in primary and secondary metabolites in tomato pericarp tissue from fruit at 20 days after pollination, an actively growing stage. Two groups, a tomato mutant deficient in starch (n=6) and wild type fruit (n=6) were analyzed . Since we were interested in both polar and non-polar compounds, chromatographic separation was performed using both methods, Hilic and C18 reverse phase, on a Vanquish UHPLC coupled to a QE-HF mass spectrometer. All samples along with the QCs were run in negative and positive ion modes and the acquired data set was processed using Compound Discoverer 2.1. Metabolite identification was achieved with Compound Discoverer 2.1 software through mzCloud HRAM fragmentation library. Preliminary data showed significant differences ($p \leq 0.05$) between the twogroups, revealing changes in metabolic pathways caused by a lack of starch biosynthesis. Metabolite profiles of the mutant and wild type were clearly differentiated and changes in several key primary and secondary metabolites were identified.

P-71 Synchronized metabolic reprogramming by chemical elicitation: Integrative network analysis of covarianced structures of proteins and metabolites

PRESENTING AUTHOR: ***Jung-Eun Lee, Jung-Eun Lee, South Korea***

CO-AUTHORS: ***Do Yup Lee***

Direct profiling of proteins and metabolites provides unique information on biochemical network that is limitedly accessible by genome sequence and gene expression level. In addition, the reaction partner can be identified and semi-quantified in a high-throughput manner by applying compatible analytical platform, mass-spectrometry. Here, we have developed integrative analytical platform for system-wide investigation on primary and secondary metabolic network of unicellular microalga, Chlamydomonas reinhardtii. For proof-of-concept, the chemical elicitation was introduced to systematically stimulate secondary metabolism that coordinately interfaced central carbon/nitrogen metabolism. Non-targeted profiles of primary metabolites by GC-TOF-MS in combination with shotgun proteomic approach provided good predictability for the metabolic origins and routes forward secondary metabolism. The subsequent analysis focusing on secondary metabolites revealed that a range of secondary metabolites were over-produced by the elicitation in a growth phase-dependent manner. Of particular interest, many of secondary metabolites are not predictable by genome annotation-based metabolic network. Lastly, global flux estimation with 13C labeling experiment suggested potential biosynthetic path and major destination from primary carbon skeletal to complex structures of secondary metabolites. The study would be good example for metabolome-based evaluation of pathway annotation and its potential production, which can be synchronized with classical genome-based functional annotation and proteomics dynamics.

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PLANT METABOLOMICS

P-72 Stable isotope-guided metabolomics with chemoinformatics reveal key ions characteristic of plant saponins

PRESENTING AUTHOR: ***Tetsuya Mori, RIKEN, Japan***

CO-AUTHORS: ***Hiroshi Tsugawa, Feng Qiu, Yutaka Yamada, Lloyd W. Summer, Kazuki Saito, Ryo Nakabayashi***

Saponins consisting of a triterpenoid/steroid aglycon, sugars, and acyls have beneficial activities, such as anti-fungal, anti-inflammatory, and anti-oxidative to human. The wide chemical diversity produced by combinations of modifying sugars and acyl groups to various aglycones interrupts to construct profiling methods. To identify key ions, which can be used for targeted analyses, we performed metabolome analysis by liquid chromatography-tandem mass spectrometry with chemoinformatics in 12C- and 13C-labeled plants (Allium cepa, Glycine max, Glycyrrhiza giabra, G. uralensis, Medicago truncatula, Solanum lycopersicum, and Solanum tuberosum). We acquired MS/MS spectra of the samples in the positive and negative electrospray ionization mode and a ramped collision energy: 10-50 V. PlantMAT was used to predict the identity of saponins using MS/MS data, and MS-FINDER identified the elemental composition of 106 triterpenoid/steroid saponins using 12C- and 13C-labeled metabolome data. Characteristic product ions where aglycones and neutral losses indicating sugars and acyls. We identified hederagenin, glycyrrhetinic acid, and soyasapogenol B using their authentic standard compounds, suggesting that the product ion [Aglycon-H2O+H]+ and neutral losses enable us to profile these saponins and their analogues at once. In this presentation, we discuss the fragmentation mechanisms on the saponins.

P-73 Integrating seasonal chemotaxonomic based metabolomics data with DNA barcoding for the identification of South African Erythroxylaceae (coca) species.

PRESENTING AUTHOR: ***Paul Sewes Frederick Alberts, University of Pretoria, South Africa***

CO-AUTHORS: ***Jacobus Johannes, Marion Meyer***

Background: Erythroxylum and Nectaropetalum are genera belonging to the Erythroxylaceae (coca) family, with select species capable of producing highly valued “blockbuster” medicinal compounds including, atropine, cocaine, scopolamine, and tigloidine amongst others. E. delagoense, E. emarginatum, E. pictum, N. capense and N. zuluense are endemic to southern Africa. However, similar morphological characteristics between these species make for troublesome and often unreliable species identification, indicating a need for alternative identification methods. The aim of this study was to combine GC-MS- and NMR based metabolomics with DNA barcoding to evaluate chemically and genetically, the characteristics of three Erythroxylum and two Nectaropetalum species found in southern Africa. While furthermore, assessing the species grouping patterns in relation to seasonal related climatic changes. Results: This study identified trends related to species grouping patterns on a chemical and genetical basis, highlighting the discriminatory power of these combined methods. Seasonal related climatic change showed to be an important factor, to incorporate in chemotaxonomic studies. Significance: This is possibly the first study comparing species grouping patterns based on the integration of chemotaxonomy and DNA barcoding. The findings could aid in differentiating and classifying other closely related taxa.

P-74 Deep annotation strategies for non-targeted metabolomics data based on bioinformatics and high resolution mass spectrometry

PRESENTING AUTHOR: ***Xin Lu, Dalian Institute of Chemical Physics, China***

CO-AUTHORS: ***Zaifang Li, Chunxia Zhao, Yueyi Xia, Guowang Xu***

Ultra-high-performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) is a useful tool for untargeted metabolomics. The most commonly used metabolite annotation approach for untargeted metabolomics is similarity search using MS reference databases. Until now the coverage of MS database of measured reference metabolites is limited, the identification of unknown metabolites is still the main bottleneck in metabolomics study. Hydroxycinnamic acid amides (HCAAs) are a diverse class of secondary metabolites which involve in plant development and biotic/abiotic stress responses. Take HCAAs as an example, a novel metabolic profiling method based on UHPLC-HR MS was developed and further applied in the plant metabolomics studies. Computational-based simulation was used to generate a predicted in silico reference HCAAs library through pathway related HCAAs biosynthesis. In silico MS fragments were predicted under fragmentation rules and experimental MS/MS. Quantitative structure-retention relationship (QSRR) model was built to predict chromatographic retention time (tR). These predicted data were validated using a series of synthetic HCAAs compounds. The established HCAAs database with in silico fragments and the predicted tR obviously enhances the coverage, accuracy and efficiency of unknown HCAAs annotation. The developed method was used to investigate HCAAs distributions in different plant species and tissues, the identified HCAAs were far more than previously reported in the literatures. Species and tissue specific accumulation of HCCAs were observed. The results show that the developed method is useful for unknown metabolites annotation of untargeted metabolomics data.

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PLANT METABOLOMICS

P-75 Does That Berry Vary? Blueberry Volatile Screening Informs Breeding Potential for Flavor

PRESENTING AUTHOR: ***Timothy S. Johnson, University of Florida, United States***

CO-AUTHORS: ***Luis Felipe Ventorim Ferrão, Patricio Munoz, Thomas A. Colquhoun***

US consumption of blueberries has drastically increased during the last two decades and year round availability has been established owing to increasingly worldwide demand, production, and distribution. Blueberry consumers display strong purchasing likelihood for sweet berries with intense blueberry flavor. Focusing breeding efforts towards flavor is a high-risk-high-reward goal. Subjectivity of perceived flavor, complexity of the numerous genetic traits underlying flavor perception in humans, and the immense biochemical diversity and variation within fruits are all prevailing challenges. Multivariate statistical modeling for six years of consumer sensory data with tandem biochemical analysis implies six carbon alcohol and aldehyde volatile compounds influence the perception of multiple sensory traits. Accordingly, C6 volatile compounds are the initial candidates for large-scale volatile phenotype screening to assist breeding for flavor in blueberry. Liquid-liquid solvent extraction was used to investigate C6 volatile diversity in Southern Highbush blueberry germplasm varieties. The internal concentration of 19 identified volatile compounds and their individual genetic heritability scores was determined. Most volatiles identified had at least a 100-fold difference between high and low samples suggesting breeding potential is possible within existing germplasm. GWAS is ongoing for marker discovery to assist breeding efforts and future molecular elucidation of volatile compounds in blueberry fruit.

P-76 Metabolome in Different Soybean Genotypes and Distinct Metabolism in Their Seeds and Leaves

PRESENTING AUTHOR: ***Young-Gyu Kang, R&D Center AmorePacific Corporation, South Korea***

Plants are adapted to different environmental conditions and their genetic and metabolic phenotypes are determined according to different adaptations. We explored metabolome of three soybean genotypes, Glycine max Hwangkeum (elite cultivar), Glycine max Napjakong (landrace) and Glycine soja Dolkong (wild accession) to investigate their distinct metabolic mechanisms in both soybean seeds and leaves through NMR-based metabolomics approach. There were obvious differences in the primary and secondary metabolites among the soybean genotypes. In particular, different types of kaempferol glycoside among three soybean genotypes were observed in their leaves, but quercetin derivatives were found only in G. max Napjakong and G. soja Dolkong. G. max Hwangkeum contained more pinitol than other genotypes. The distinct metabolic properties of the soybean seeds were also observed among three soybean genotypes. For example, higher levels of isoflavones were observed in G. max Napjakong and G. soja Dolkong, compared with G. max Hwangkeum. Moreover, epicatechin, which is well known for main bioactive compounds, was found only in G. max Napjakong and G. soja Dolkong. These results showed that there were significant differentiations in metabolome of both leaves and seeds of soybean genotypes, demonstrating distinct metabolism to adapt toward their growing environments.

P-77 Unravelling the role of sucrose in guard cell metabolism through the use of GC-TOF-MS-based 13C-labelling analysis coupled with genome-scale metabolic modelling.

PRESENTING AUTHOR: ***Danilo M. Daloso, Universidade Federal do Ceará, Brazil***

CO-AUTHORS: ***Semidán Robaina Estévez, David B. Medeiros, Leonardo P. Souza, Wagner L. Araújo, Zoran Nikoloski, Alisdair R. Fernie***

Photosynthesis and water use efficiency, key factors affecting plant growth, are directly controlled by microscopic and adjustable pores in the leaf - the stomata. The control of stomatal aperture involves reversible changes in the concentration of osmolytes in guard cells. Sucrose has long been thought to play an osmolytic role in stomatal opening. However, recent evidence supports the idea that the role of sucrose in this process is primarily energetic. Here we have used a combination of stomatal aperture assays coupled with 13C-isotope labelling experiments and metabolic modelling to investigate the role of sucrose during light-induced stomatal opening. The isotope experiments showed a consistent 13C-enrichment in fructose and glucose indicating that during light-induced stomatal opening sucrose is degraded. We also observed a clear 13C-enrichment in Glu and Gln, suggesting a concerted activation of sucrose degradation, glycolysis and the TCA cycle. The combination of in silico with in vivo analyses also indicated that guard cells have higher anaplerotic CO2 fixation via phosphoenolpyruvate carboxylase, which was demonstrated to be an important source of malate. Collectively, our results enable us to redraw current models of the regulation of guard cell sucrose metabolism.

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PLANT METABOLOMICS

P-78 Metabolomics-Guided Phytochemical Discovery

PRESENTING AUTHOR: ***Susan J. Murch, University of British Columbia, Canada***

In any given year, between 60%-80% of new pharmaceutical introductions are isolated or derived from natural products but the traditional process of identifying, isolating and characterizing medicinally active molecules in complex plant extracts is a long and laborious process that frequently fails. The traditional plant chemical discovery pipeline starts with a botanical lead, preparation of an extract, solvent partitioning, chromatographic or physical fractionation, testing or bioassay of the crude preparation, further fractionation, more testing and repeated until a pure chemical is obtained in sufficient quantity for NMR structure analysis. Many plant chemicals are lost in this process because of solubility or stability issues. Some may not be discovered because huge amounts of plant tissue are required to extract low concentration metabolites. We have developed a new approach to phytochemical discovery by determining the differences in metabolomes of active and inactive extracts. For example, a comparison of tea metabolomes reveals putative medicinal metabolites in an active hot water extraction or a comparison of extracts of plants grown under different conditions reveals phytochemical responses to environmental stimuli. Statistical tools to eliminate false discoveries coupled with logical algorithms to identify products of enzymatic, Redox or spontaneous reactions can identify putative pathways or metabolite families. The metabolome of an average leaf contains ≈35,000 distinct phytochemicals, many of which are useful as pharmaceuticals, foods, dyes, biomaterials, and industrial chemicals. Metabolomics-guided phytochemical discovery represents a significant advancement in the discovery of useful plant chemicals for modern products.

P-79 Profiling Major Metabolites Using GC-MS and NMR in 'Small' Potato (Solanum tuberosum L.) Tubers

PRESENTING AUTHOR: ***Rong Zhou, Saskatoon Research and Development Centre, AAFC, Canada***

CO-AUTHORS: ***Ning Xu, Ken Thoms, Peter Jianfeng Zhu, Jazeem Wahab, Greg Larson, Ken Achtymichuk***

Recent health issues such as obesity and acrylamide associated with cancer in fried potato have led to consumers seeking healthier and more nutritive, non-fry alternatives. Potatoes are considered a healthy choice with balanced carbohydrates, vitamins, minerals and proteins. Presently, considerable research is being conducted to identify suitable cultivars, develop efficient production practices and examine nutritive attributes of 'Small' potato. To produce 'Small' potato, the crop is harvested prematurely to maximize the yield of tubers between 20-40 mm diameter. This project evaluated the yield potential and metabolite constituents of four potato cultivars (AAC Hamer, AC Peregrine Red, Milva, Operle) when grown under irrigation and dryland and top-killed at 10, 11, and 12 weeks from planting. This paper describes the influence of production system and top-kill timing on metabolite profile in 'Small' potato of different cultivars. Tuber tissues samples were freeze-dried and ground into a powder prior to analysis. Polar soluble metabolites were extracted using aqueous methanol. Glucose, fructose and sucrose contents were analysed using NMR, and major metabolites using GC-MS . Chemical compounds such as glucose, sucrose, fructose, citric acid, malic acid, lactic acid, acetic acid, oxalic acid, glycerol, aspartic acid, glutamic acid, proline, asparagine, and myo-inositol were identified and quantified in this study.

P-80 Analysis of NIST SRM 3291 Bilberry Extract based on multistage fragmentation and iTree, a MSn Mass Spectral Tree Library of 2,500+ Plant Natural Products

PRESENTING AUTHOR: ***Arpana Vaniya, UC Davis West Coast Metabolomics Center, United States***

CO-AUTHORS: ***Oliver Fiehn***

Identification of small molecules in untargeted metabolomics still remains a challenge, especially for natural products due to their wide diversity. Often enough MS/MS library matching does not suffice. Natural products have complex structures and require multistage fragmentation (MSn) for exhaustive structural information. Here, we analyzed NIST SRM 3291 Bilberry extract by utilizing multiple extraction solvents, MS platforms, and MS library searching. Our analysis included LC-MSn, high resolution (HR) LC-MS/MS, and ion tree analysis using iTree, an ion tree library of natural products. Reversed-phased LC-MSn data were acquired on a Thermo LTQ and Q Exactive HF in both (+) and (-) ESI modes. LC-MSn data were acquired up to MS4. Data-dependent MS/MS spectra were acquired the Q Exactive HF. We have acquired data for more than 2,500 natural product standards on the LTQ, leading to over 13,000 mass spectral trees for five molecular adducts. We used MS-DIAL version 2.82 for data processing and peak annotation. Annotations were done by matching our in-house accurate mass retention time list, nine highly-curated open source ESI-MS/MS mass spectral libraries from MassBank of North Amercia (MoNA), and NIST 17 MS/MS library. Mass Frontier 7.0 generated ion trees from LC-MSn data which were then searched against iTree, HighChem, and mzCloud. Spectral tree matching gave higher confidence and better match scores for natural product identifications than MS/MS matching alone. By combining LC-MSn, ion tree analysis, and HR MS over 100 natural products in NIST SRM bilberry extract were annotated, showing a more comprehensive analysis of natural products.

POSTER SESSIONS 1 AND 2 – **Monday and Tuesday –** all odd number presenters will be at their posters.
POSTER SESSIONS 3 AND 4 – **Wednesday and Thursday –** all even number presenters will be at their posters.

PLANT METABOLOMICS

P-81 Application of a Rapid Microbore Metabolic Profiling HILIC Approach for Analysis of Anthocyanins in Red Wine with Ion Mobility HRMS

PRESENTING AUTHOR: ***Lauren Mullin, Waters Corporation, United States***

CO-AUTHORS: ***Adam King, Robert Plumb***

Anthocyanins are phenolic pigment flavonoid compounds which are present in red wine1. These compounds are not only associated with red, blue and violet pigmentation, but also have been subject to interest as ingredients in functional foods1. Recent studies have investigated anthocyanin analysis through the use of HILIC coupled to HRMS1,2, showing some chromatographic improvements of conventional RPLC analysis for these phenolic compounds. The presented study introduces a rapid HILIC separation achieved through the use of microbore fluidics3 resulting in a 3 minute acquisition per sample. Additionally, the use of travelling wave ion mobility separation (TW-IMS) coupled with HRMS acquisition provided a gas-phase separation which resolves some chromatographic co-elutions. For example, malvidin 3-O-glucoside pyruvic acid (Vitisin A), which is produced during the alcoholic fermentation and maturation of red wine and showed variability among red wine types analyzed, was found to co-elute with a poorly resolved peak proposed to be malvidin-3-caffeoyl-glucoside. The two compounds showed both unique fragment ions and resolution in TW-IMS, and thus could be further differentiated from one another without needing to include a longer chromatographic analysis in the study. Additional anthocyanin content as well as non-targeted phenolic compounds present in red wine are also investigated in the study using PCA and OPLS-DA approaches. References: Willemse C et al. (2013) J. Chrom. A 1319:127-140 Willemse C et al. (2015) Anal. Chem. 87:12006-12015 Gray G et al. (2016) Anal. Chem. 88:5742-5751

P-82 Systematic profiling and comparison of the lipidomes from Panax ginseng, P. quinquefolius, and P. notoginseng by UPSFC/high-resolution MS and ion mobility-derived CCS measurement

PRESENTING AUTHOR: ***Xiaojian Shi, Arizona State University, China***

CO-AUTHORS: ***Xiaojian Shi , Wenzhi Yang , Shi Qiu, Jinjun Hou,Haiwei Gu, Wanying Wu, Dean Guo***

Lipidomics currently is still confronted with challenges from chromatographic separation and lipids identification. Here we report a lipidomics platform by integrating ultrahigh performance supercritical fluid chromatography/quadrupole time-of-flight mass spectrometry (UHPSFC/QTOF-MS) and collision cross section (CCS) measurement using ion mobility spectroscopy/time-of-flight mass spectrometry (IMS/QTOF-MS), aiming to enhance the profiling performance and identification reliability of lipids. The lipidomes extracted from three congeneric Panax species (P. ginseng, P. quinquefolius, and P. notoginseng) by methyl tert-butyl ether are comprehensively profiled and compared by use of this platform. A potent UHPSFC/QTOF-MS approach was developed on a 1.7-μm particles packed Torus 2-PIC column using CH3OH (in CO2) as a modifier and CH3OH/0.2 mM ammonium acetate as the makeup liquid, enabling the well resolution of six lipid subclasses by both positive and negative MSE modes. In contrast to the reversed-phase chromatography, “normal-phase” like elution order and better resolution of polar lipids and some lipid isomers were achieved by UHPSFC separation. Pattern recognition chemometric analysis of 60 batches of Ginseng samples ultimately unveiled 24 lipid markers, of which triacylglycerols were the most important. Aside from the automated MS database searching against HMDB and LIPID MAPS, the application of CCS retrieval or CCS prediction improved lipid identification by reducing the possible hits. In conclusion, this integral platform can significantly improve the chromatographic separation and the reliability of lipids identification in lipidomics studies. It is the first report that systematically compares the lipidomic difference of three reputable Panax species, providing information for their quality control in addition to ginsenoside analysis.

P-83 How plants became the greatest chemists - metabolomics-enabled development of genome-scale models of plant specialized metabolism

PRESENTING AUTHOR: ***Bernd Markus Lange, Washington State University, United States***

CO-AUTHORS: ***Jordan J. Zager, Sean R. Johnson, Amber N. Parrish, Iris Lange, Narayanan Srividya***

The plant kingdom is characterized by an enormous chemical diversity, which is in part enabled by the occurrence of anatomical structures (glandular trichomes, resin ducts, laticifers, and others) and specialized cell types. The secretions stored in these structures play vital roles in plant defenses and other interactions with the environment. Much progress has been made to obtain transcriptomes of secretory cell types, which has enabled the identification and subsequent characterization of genes involved in the biosynthesis of various classes of specialized metabolites accumulated therein. We have developed online spectral resources to capture plant chemical diversity. We have incorporated multi-omics data sets in genome-scale models, which have tremendous potential to shed light onto the biochemical processes that facilitate the accumulation of specialized metabolites. Our studies indicate that the flux distribution through the metabolic network of secretory cell types, regardless if they are photosynthetic or non-photosynthetic, shares commonalities across plant lineages. We also present examples of how flux distribution differs in various secretory cell types, and provide experimental and computational evidence for the contribution of cellular respiration, fermentation, and unique redox biochemistry. Taken together, these studies are giving us the first direct insights into the bioenergetic processes that drive flux in secretory cell types of plants.

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PLANT METABOLOMICS

P-84 How can modern crop development consistently improve maize yield? Evaluating maize phenotypic variance, heritability, and yield relationships at multiple biological scales across agronomically relevant environments

PRESENTING AUTHOR: ***Mohamed Bedair, Monsanto, United States***

CO-AUTHORS: ***Sarah L. Tucker, *Frank G. Dohleman, Dmitry Grapov, Lex Flagel, Sean Yang, Kimberly M. Wegener, Kevin R. Kosola, Ryan A. Rapp, Steven C. Halls, Kevin C. Glenn, Michael A. Hall, Edwards Allen, Elena A. Rice***

A key challenge to improve an integrative phenotype, like yield, is the interaction between the broad range of possible molecular and physiological traits that contribute to yield and the multitude of potential environmental conditions in which they are expressed. The present study systematically collected data on 31 phenotypic traits (including yield), 83 annotated metabolites, and nearly 22,000 transcripts from a set of 57 diverse, commercially relevant maize hybrids across three central USA corn-belt environments. Although significant variability in measured characteristics created a complex picture of how groups of traits interact and how they combine to produce yield, overall, replicate measurements of phenotypic traits and gene expression were more consistent across environments, while metabolite levels showed low repeatability. Genetics had the largest effect on whole-plant phenotypic traits, phenology, plant height and kernel row number. Environmental and residual factors had the largest effect on yield and yield components, biomass, roots, and nitrogen traits. Phenology traits and nitrogen content showed the most consistent correlation with yield across hybrids and environments. This analysis reveals that continued improvement of maize yields requires a strong understanding of baseline variation of plant characteristics across commercially-relevant germplasm to drive strategies for optimizing germplasm interactions with environmental variables

P-85 Comparative Metabolomics and Hormone Profiling of Grafted Melon

PRESENTING AUTHOR: ***Maria Dolores Camalle, Ben-Gurion University of the Negev, Israel***

CO-AUTHORS: ***Ondřej Novák, Aaron Fait, Noemi Tel-Zur***

Grafting, an ancient technique used extensively in the horticulture of perennial plants, is currently finding application in the production of vegetable and other annual crops. However, the full potential of the technique for improving breeding and selecting elite rootstock will not be realized unless the complex mechanisms underlying graft compatibility are elucidated. In the current study, we sought to determine the factors affecting graft in/compatibility in melon grafted onto pumpkin rootstocks as a model system. To this end, we conducted comparative metabolomic and hormone profiling of scion-rootstock phloem sap of melon Kiran (Ki) (Cucurbita melo L.) grafted onto two compatible rootstocks, TZ and Shimshon (Sh), and one less compatible rootstock, 53006 (53). Plants were grown in net houses in Israel's hot, dry Arava valley. For these plants, a PCA model of scion-rootstock phloem sap for all graft combinations revealed good separations between 'compatible' and 'less compatible' combinations. Compatible combination Ki/TZ showed the best performance in terms of nitrogen (assimilation and metabolism) and carbon metabolism, but the less compatible Ki/53 combination exhibited delayed assimilate transport across the graft. Hormone analysis suggested a role for auxin and cytokinins in graft compatibility. Taken together, our results indicate that an integrated analysis of metabolomic and hormone data can lead to a better understanding of the graft compatibility mechanism.

P-86 LC-MS and MALDI-MS untargeted metabolomics of Solanum lycopersicum infected by Phytophthora infestans

PRESENTING AUTHOR: ***Paula Galeano Garcia, Universidad de los Andes, Colombia***

CO-AUTHORS: ***Fábio Neves dos Santos, Samantha Zanotta, Nicholas Vinícius Silva, Marcos Nogueira Eberlin, Chiara Carazzone***

Tomato crops suffer attacks of various pathogens that cause great production loss. Late blight, caused by Phytophthora infestans, is one of the most devastating pathogen to tomato because of its difficult control. Our aim was discriminate infection times and identifies major metabolites biomarker candidates in late blight of tomato plants. We applied metabolomics based on LC-MS and metabolic profiling by MALDI-MS associated with multivariate data analysis to analyze tomato plants of Santa Cruz Kada cultivar infected by P. infestans. MALDI-MS profiles of metabolites and multivariate data analysis are able to detect late blight in infected tomato plants. Metabolomics based on LC-MS discriminates infection times in asymptomatic infected tomato plants. The metabolites that discriminate infection times were putatively identified by database. MALDI-MS analysis can therefore be used as a rapid and effective method to detect late blight in asymptomatic tomato plants offering a suitable tool to guide the correct management and application of sanitary defense approaches. LC-MS based metabolomics seems also to provide a suitable tool to identify major biomarkers of late blight disease.

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PLANT METABOLOMICS

P-87 Metabolite profiling, pathway analysis, and bioactivity correlations between leaf and stem for plant species belonging to the Asteraceae, Fabaceae, and Rosaceae families

PRESENTING AUTHOR: ***Sunmin Lee, Konkuk University, Korea, South***

CO-AUTHORS: ***Dong-Gu Oh, Sarah Lee, Digar Singh, Ga Ryun Kim, Jong Seok Lee, Choong Hwan Lee***

Fifty-one species form three plant families, named Asteraceae, Fabaceae, and Rosaceae were subjected to metabolite profiling by gas chromatography time-of-flight mass spectrometry (GC-TOF-MS) and ultrahigh-performance liquid chromatography quadrupole orbitrap ion trap tandem mass spectrometry (UHPLC-Q-orbitrap-MS/MS) with multivariate analysis. In partial least squares discriminant analysis (PLS-DA), the leaves and stems of 51 species clustered depending on each part and phylogenetic family. The pathway analysis further highlighted the relatively higher proportions of amino acids, fatty acids, and genistein in Fabaceae family, higher proportions of catechin, and ellagic acid derivatives in Rosaceae family, and higher levels of kaempferol derivatives, and organic acids in Asteraceae family than in the other two families. Regardless of family, the aromatic amino acids, branch chain amino acids, chlorogenic acid, flavonoids, and phenylpropanoids related with shikimate pathway were found in leaves, whereas amino acids such as proline, lysine, and arginine and fatty acids were found mainly in stems. In addition, the antioxidant activity using DPPH and tyrosinase inhibition activity were determined to investigate their effects on the part of plant. The leaves were shown the highest antioxidant activity, correlating with its highest levels of phenolic compounds while the stems were shown the highest tyrosinase inhibition activity. Hence, this work suggests that metabolite profiling including multi-parallel approach was an efficient tool for finding the different metabolic states of each plant parts and understanding the correlation between metabolites and bioactivities in accordance with plant parts.

P-88 Identification of Ozone-Induced Metabolic Response in Maize via NMR

PRESENTING AUTHOR: ***Elizabeth K Eder, Pacific Northwest National Laboratory, United States***

CO-AUTHORS: ***Jessica M Wedow, Elizabeth K Eder, Eric D Walter, Robert P Young, David W Hoyt, Elizabeth A Ainsworth***

Ozone is a greenhouse gas and considered the most damaging air pollutant to plants. Ozone enters leaves through the stomata and reacts to produce other reactive oxygen species (ROS) initiating a cellular response that ultimately reduces photosynthesis. The specific ozone-induced ROS and the antioxidant metabolites produced to quench ROS are largely unknown and vary among plant species, and even genotypes within a species. Very little is known about how maize responds to acute ozone exposure. To address this gap, maize was planted in growth chambers and grown for 3 weeks with sufficient water and nutrients. After 3 weeks, plants were exposed to 200 ppb ozone and sampled after 0,1,5, and 24 hours of ozone exposure. Leaf material was taken for nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) from the youngest fully expanded leaf. EPR results showed the concentration of hydroxyl radicals in the leaves are higher under elevated ozone concentrations. Untargeted metabolomics was performed with 1H-NMR and resulted in identification of 40 metabolites. Ascorbate and glutathione, the most abundant antioxidant metabolites in plants, were identified following initial ozone exposure. Ascorbate increased from 0 to 24 hours of exposure, while no effect on glutathione concentrations was observed. NAD+ was also significantly increased after ozone exposure. This improved understanding of the nature and lifetime of ozone-induced ROS and antioxidant responses in maize will assist in modeling the fate of ozone and could help identify strategies for improving tolerance in C4 crops.

P-89 Investigating the natural variation of pennycress metabolome, an emerging crop for aviation biofuel applications.

PRESENTING AUTHOR: ***Tyler Swanson, BioDiscovery Institute University of North Texas, United States***

CO-AUTHORS: ***Fan Yang, Cintia Airas, Ana Alonso***

Pennycress (Thlaspi arvense L.), a member of the Brassicaceae family, is a native plant to Eurasia but can be found in temperate regions around the world. This species is particularly interesting for the oil composition of its mature seeds, which is ideally suited for aviation biofuel. Because it requires minimal agricultural inputs it can be grown on marginal land. Additionally, as a winter annual it can be integrated with current commodity crop systems with little to no disruption. However, for this crop to become a viable source of jet fuel, its oil production must be increased. The goal of this study is to find biomarkers positively or negatively correlating with oil content to boost the breeding and/or metabolic engineering process. For this purpose, we studied the natural variation in the metabolome of 11 pennycress accessions from around the world. Biomass components - oil, protein, starch and cell wall – were sequentially extracted and quantified from two different stages of developing embryos. The quantification of intracellular metabolites such as sugars, amino acids, organic acids and phosphorylated compounds was achieved by liquid chromatography tandem mass spectrometry. The present research focuses on integrating the results of these analyses and attempts to highlight the correlations between certain intracellular metabolites and oil composition and accumulation. Ongoing experiments are expanding this study to a total of 20 pennycress accessions. The long-term goal of this research is to use the biomarkers that we identified to enhance oil production in pennycress using rational breeding and/or metabolic engineering.

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PLANT METABOLOMICS

P-90 Phenolic compounds shape fungal endophyte diversity in Rubiaceae leaves

PRESENTING AUTHOR: ***Fernanda Rezende de Castro Moretti, University of North Texas, BioDiscovery Institute, United States***

CO-AUTHORS: ***Humberto Castillo Gonzalez, Jason C. Slot, Priscila Chaverri, Ana Paula Alonso***

Rubiaceae is a large family of plants that englobe species with great importance in food and pharmaceutic industries. Coffea is a genus in this family with significant economic relevance, but it is susceptible to the pests and pathogens that decrease productivity. It is known that a diverse endophyte community strengthens plant resistance to biotic and abiotic factors, enhances nutrient availability, and plays a role in important functions of the host metabolism. Studies on the microbiome of other Rubiaceae species could reveal crucial information about plant health and fitness. The hypothesis of this study is that plant secondary metabolites interfere with endophytic diversity, and vice versa. This idea was tested on young and mature leaves from different Rubiaceae species collected in Costa Rica. First, the biochemical diversity of leaf extracts was assessed with untargeted metabolomic profiling using gas chromatography and mass spectrometry. Additionally, the phenolic content -which are known molecules for plant defense -was quantified with targeted metabolomics using liquid chromatography tandem mass spectrometry. Finally, fungal colonies were isolated from the leaves and counted, disclosing the diversity of the endophytic community. Results from the metabolomics experiments and colony evaluation revealed significant differences between maturation stages and species, underlying a correlation but also interactions between secondary metabolites and endophyte diversity. We anticipate that this work will pave the way for the application of fungal endophyte strains to produce natural compounds and to develop biocontrol strategies.

P-91 Metabolomics approach for investigating metabolic changes in seedlings of Arabidopsis GCR1 mutant

PRESENTING AUTHOR: ***Seung-A Baek, Incheon National University, Korea, South***

CO-AUTHORS: ***Soon Kil Ahn, Kil Won Kim, Jaehyuck Choi, Jinho Kim, Jaegyoon Ahn, Jae Kwang Kim***

G-Protein Coupled Receptor (GPCR) is an important cell membrane receptor that transfers extracellular signal to inside of a cell. There are over 800 GPCRs in humans, and they are conserved in eukaryotes. In Arabidopsis, there is strong putative GPCR gene, GCR1, its metabolic pathway and ligand were not studied well. To confirm the roles of GCR1, metabolites were investigated such as chlorophylls, carotenoids, glucosinolates, lipophilic and hydrophilic compounds in gcr1 knock-out mutant and wild type of A. thaliana. In seedling grown for 10 days, total 69 of metabolites were detected using gas chromatography time-of-flight mass spectrometry (GC-TOFMS), gas chromatography quadrupole mass spectrometry (GC-qMS) and high performance liquid chromatography (HPLC). The content levels of total pigments, phytosterols, polycosanols, amino acids and organic acids in gcr1 mutant were lower than wild type. On the other hand, total tocopherols had high level in gcr1 mutant. In the statistics results, principal component analysis (PCA) showed the distinguishable differences between the mutant and wild type (54.2 % of the total variance in principal component 1). The t-test showed that 25 metabolites have a difference ($p<0.05$) between two plants. Among them, serine and glycine were the most contributable metabolites with p-value of less than 0.001. These results will be useful to determine roles of GCR1 and find its ligand in plants and other eukaryotes.

P-92 Fluorescently targeted analysis of tissue-embedded single cells by optical fiber-based laser ablation electrospray ionization mass spectrometry

PRESENTING AUTHOR: ***Sylwia A. Stopka, The George Washington University, United States***

CO-AUTHORS: ***Rikkita Khattar, Beverly J. Agtuca, Christopher R. Anderton, Ljiljana Paša-Tolić, Gary Stacey, Akos Vertes***

Cell-to-cell variations are observed in biological systems due to the stochastic expression of transcripts, proteins, and metabolites. To explore cellular heterogeneity, analytical techniques that can report composition with single cell resolution and under in situ conditions are needed. This can be achieved by microsampling using mid-IR laser pulses delivered through an optical fiber that has an etched tip comparable in size to that of a single cell. Fiber-based laser ablation electrospray ionization mass spectrometry (LAESI-MS) is an emerging technique that allows for the direct sampling of tissue-embedded single cells at ambient conditions. Furthermore, integrating it with fluorescence microscopy allows for the selection of subpopulations of rare cells, e.g., excretory idioblasts from Egeria densa and specialized nitrogen fixing cells in Glycine max (soybean) root nodules infected by rhizobia. Over 100 E. densa leaf cells were analyzed and significant differences were observed between epidermal and excretory idioblast cells that otherwise would have been masked by the population average. For example, metabolites including malate, citrate, glutamate, and kaempferol/isomers were detected at much higher abundances in the epidermal cells, whereas coumaroylcorosolic acid/isomers and azukisaponin I/isomers were localized only in idioblasts. Metabolite level fluctuations across all cells were observed, which revealed the presence of cellular subpopulations based on medians, whereas the magnitude of metabolic noise was expressed as the statistical range. Within a soybean root nodule, bacteroid cells that are capable of biological nitrogen fixation are interspersed among neighboring uninfected plant cells, and can be selected for analysis by fiber-LAESI-MS using GFP fluorescence.

POSTER SESSIONS 1 AND 2 – Monday and Tuesday – all odd number presenters will be at their posters.
POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

MICROBIOME

P-93 Untargeted LC-MS Metabolomics Differentiates between Virulent and Avirulent Clinical Strains of Pseudomonas aeruginosa

PRESENTING AUTHOR: ***Tobias Depke, Helmholtz Centre for Infection Research, Germany***

CO-AUTHORS: ***Janne Thoeming, Susanne Haeussler, Mark Broenstrup***

The opportunistic Gram-negative pathogen Pseudomonas aeruginosa is a major threat for patients suffering from cystic fibrosis, pneumonia, or wound infections. Different P. aeruginosa strains can cause acute infections or persist chronically in predisposed individuals. In the clinical setting, knowledge about the virulence of a given strain is needed as early as possible after the diagnosis as it can inform therapeutic decisions as well as the prognosis of the infected patient. Because the P. aeruginosa genome is highly conserved, it is more promising to differentiate strains based on functional genomics data. Compared to other pathogenic bacteria, P. aeruginosa relies quite strongly on secondary metabolites for the exertion and regulation of its virulence. These small molecules, e.g. phenazines, rhamnolipids, homoserine lactones and alkylquinolones, can be detected and quantified by liquid chromatography coupled to mass spectrometry and differential abundance of these metabolites can be identified. We applied an untargeted LC-MS metabolomics approach on cell extracts of P. aeruginosa clinical strains and used multivariate statistics and machine learning approaches to identify sets of metabolites that differentiate between virulent and avirulent/persistent strains whose pathogenic potential was determined by the use of the Galleria mellonella virulence assay. Among the differentially abundant metabolites are, e.g., alkylquinolone quorum sensing signal molecules but also metabolites less directly linked to virulence along with previously undescribed compounds. We were able to generate and initially validate predictors based on the metabolomics data that are under investigation as putative biomarkers for the differentiation between virulent and avirulent strains of P. aeruginosa.

P-94 Metabolic adaptations of Pseudomonas aeruginosa to the cystic fibrosis lung

PRESENTING AUTHOR: ***Sarah K Davies, Imperial College London, United Kingdom***

CO-AUTHORS: ***Volker Behrends, Emma Walmsley, Natasha Wierre-Gore, Jane C Davies, Huw D Williams, Sean Kassen, Jake G Bundy***

Pseudomonas aeruginosa is an opportunistic environmental pathogen that forms chronic infections in the lungs of cystic fibrosis (CF) patients. The majority of infecting isolates are environmental strains, rather than a result of patient-to-patient transmission, so each newly established infection can in a sense be thought of as representing a separate experiment in evolution. In order to increase the chances of successful treatment, it is important to know how P. aeruginosa adapts to the lung during chronic infection. We know that genetic adaptation occurs during long-term infection. Next generation sequencing has transformed our ability to obtain genome sequences, but these data cannot be easily interpreted to reveal the metabolic changes associated with adaptation. Metabolomics provides an intermediary complex biochemical phenotype, in an untargeted fashion. We have used untargeted 1H NMR profiling of growth media (metabolic footprinting, or exometabolome data) to study the effects of long-term chronic infection on the metabolism of P. aeruginosa CF clinical isolates, for both matched and unmatched isolates, for different cohorts collected at different times and in different countries. Footprinting represents the most direct (and hence most interpretable) exchange of nutrients between cells and the environment. We have also complemented this with NMR and GC-MS based analysis of endometabolomes for some strains. We have identified a number of complex metabolic adaptions, representing a clear signature of P. aeruginosa changing its metabolic capability to allow it to grow more successfully in the CF lung environment.

P-95 Intracellular metabolite profiling and the evaluation of metabolite extraction solvents for syngas-fermenting Clostridium carboxidivorans

PRESENTING AUTHOR: ***Jungyeon Kim, Korea University, South Korea***

CO-AUTHORS: ***Kyoung Heon Kim***

Clostridium carboxidivorans, which ferments syngas to biofuels such as biobutanol and biohexanol, has been receiving increased attention. Although many metabolic bottlenecks such as slow growth and production rates during fermentation of syngas using the microorganism have been reported, no study has been done on the intracellular metabolisms of the microorganism which suggest clues for the metabolic engineering. In this study, we exploited metabolomic approaches to unveil intracellular metabolic status of C. carboxidivorans cultivated on CO-containing syngas. A total of 82 intracellular metabolites were identified and relatively quantified from C. carboxidivorans by gas chromatography/time-of-flight–mass spectrometry. In comparison with glucose media, CO-containing syngas media allowed higher levels of fatty acid synthesis and lower levels of sugar and amine production of the microorganism. Due to the different intracellular metabolite profiles in CO media compared to in glucose media, it was essential to optimize extraction solvents for extraction of intracellular metabolites from C. carboxidivorans cultivated in CO media. The evaluation of four extraction solvents revealed the mixture of water-isopropanolmethanol (2:2:5, v/v/v) to be the best extraction solvent, which showed the highest extraction capability and reproducibility. This is the first metabolome sampling study on a microorganism fermenting syngas containing CO and CO2.

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MICROBIOME

P-96 Determination of 42 chiral amino acids in biological samples using high-throughput and comprehensive LC-MS/MS: Investigation of D-amino acids produced by intestinal microbiota

PRESENTING AUTHOR: *Takanari Hattori, Shimadzu Corporation, Japan*

CO-AUTHORS: *Akihiro Kunisawa, Shuichi Kawana, Shinichi Kawano, Yoshihiro Hayakawa, Junko Iida, Eiichiro Fukusaki, Mitsuharu Matsumoto*

Metabolites produced by intestinal microbiota are absorbed constantly from the intestinal lumen and carried to systemic circulation; they play a direct role in health and disease. D-Amino acids are one of the low-molecular-weight metabolites and have different physiological functions from L-amino acids. However, there are limited reports concerning D-amino acids produced by intestinal microbiota. In this study, a comprehensive LC-MS/MS method for 42 chiral amino acids in biological samples was developed. Then, the feces and plasma obtained from germ-free (GF) mice and ex-germ free (Ex-GF) mice were analyzed by this method to investigate the effect of intestinal microbiota on D-amino acids. Nexera X2 system coupled with LCMS-8060 (Shimadzu Corporation, Japan) was used to analyze 42 chiral amino acids in the extracts. The separation was achieved on CROWNPAK CR-I(+) and CR-I(-) (Daicel Corporation, Japan). The mobile phase consisted of a mixture of acetonitrile, ethanol, water and TFA. As a result of the analyses of the feces of GF mice and Ex-GF mice, 7 and 14 D-amino acids were detected, respectively. Seven D-amino acids (D-Ala, D-Gln, D-allo-Ile, D-Leu, D-Lys, D-Phe and D-Ser) were detected only in Ex-GF mice, showing that these D-amino acids are produced by intestinal microbiota. In plasma, 3 and 6 of D-amino acids were detected from GF mice and Ex-GF mice, respectively. Both fecal and plasma D-Ala concentration in Ex-GF mice were significantly higher than those in GF mice, indicating that D-Ala produced by the intestinal microbiota transferred to the blood. Detailed results will be presented in the poster.

P-97 Application of GC/MS, LC/MS/MS, and HPLC-profiling Based Metabolomics to the Analysis of Gut-microbiota Dependent Metabolome in Rats Treated with Green Tea Polyphenols

PRESENTING AUTHOR: *Jun Zhou, University of Georgia, United States*

CO-AUTHORS: *Lili Tang, Jia-Sheng Wang*

Our previousmetagenomics analysishas demonstrated that green tea polyphenols (GTPs) could modify gut-microbiota community structure and enzyme orthologs in SD rats. Here metabolomics analysis was conducted to explore the changes of the gut-microbiota dependent metabolisms in gut andpotential health outcomes.Six groups of SD female rats (n = 13) were administered with drinking water containing 0%, 0.5%, and 1.5% GTPs (wt/vol) for 6-month. At each treatment level, 1group of rats were sacrificed after 3-month, and the other rats were sacrificed after 6-month. Colorectal contents were collected for analysis via HPLC-profiling, GC/MSand LC/MS/MS metabolomics analyses.Of note, GC/MS metabolomics captured 2668 features.Principal component analysis (PCA) found that nearly 90% of data variance wasexplained with PC1 and PC2. Two-way ANOVA showed that GTPs-administration caused dose-dependent changes of 53 metabolites, time-dependent changes of 14 metabolites, and dose × time dependent changes of 39 metabolites. A group of key metabolites were quantitated—the elevated components were niacin (8.61-fold), 3-phenyllactic acid (2.20-fold), galactose (3.13-fold), mannose (2.05-fold), pentadecanoic acid (2.15-fold), and lactic acid (2.70-fold); the reduced components were cholesterol (0.29-fold), cholic acid (0.62-fold), deoxycholic acid (0.41-fold), trehalose (0.14-fold), glucose (0.46-fold), fructose (0.12-fold). ViaSelected Reaction Monitoring mode,LC/MS/MS showed that the metabolisms of amino acids were generally suppressed. HPLC-profiling demonstrated decrease of bile constituents, butno remarkable alterations of short chain fatty acids were found. Results of our comprehensive metabolomics analysissuggested that GTPs can effectively modify gut-microbiota dependent metabolisms of energy, bile constituents, and micronutrients in rats.

P-98 Metabolic responses of the Clostridium difficile induced by Lactobacillus rhamnosus gg

PRESENTING AUTHOR: *Han-Gyu Park, Soongsil University, Korea, South*

CO-AUTHORS: *Da-Hee Ahn, Won-Suk Song, Yun-Gon Kim*

Clostridium difficile is known as causing C. difficile infection (CDI) which is the most prevalent nosocomial infection. For therapy of CDI, metronidazole is commonly used and oral vancomycin is suggested when metronidazole is ineffective. Eventually, such antimicrobial therapy cause alteration of microbiome that was related with CDI pathogenesis in colon. In addition to chemotherapy, probiotics which have a beneficial effect on the host is studied for therapy in CDI by inhibiting C. difficile. Lactobacillus species have been studied as such probiotics. Among them, Lactobacillus rhamnosus gg (LGG) which was isolated from healthy human has potential to treat and prevent CDI by secreting metabolites such as lactate. However, it is still unclear what metabolic pathway changes in C. difficile are induced by LGG. Understanding how C. difficile responds to LGG in can improve understanding of antibacterial mechanism in metabolomics perspective. Thus, the aim of this study was to track metabolic pathway changes in C. difficile by quantitatively analyzing the metabolites. We measured growth curves of LGG and C. difficile, respectively. To the observe metabolic responses of C. difficile, the bacteria was co-cultured with LGG in transwell and then intracellular metabolite of C. difficile was analyzed using LC-MS/MS. As a results, we observed quantitative change of metabolites and based on the data, we confirmed the alteration of metabolism.

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POSTER SESSIONS 3 AND 4 – **Wednesday and Thursday –** all even number presenters will be at their posters.

MICROBIOME

P-100 Influence of gastric bypass surgery on microbiota-associated metabolic changes

PRESENTING AUTHOR: ***Ulrike Elisabeth Rolle-Kampczyk, Helmholtz Centre for Environmental Research, UFZ, Germany***

CO-AUTHORS: ***Sven-Bastiaan Haange, Nico Jehmlich, Constantin Hintschich, Mohammed Hankir, Florian Seyfried, Dirk Wissenbach, Jean Froment, Wiebke Fenske, Martin von Bergen***

Background: The commensal microbiota plays an important role in host metabolism. Through acting on different receptors and downstream intracellular signaling cascades, bile acids exert powerful influence on a range of metabolic processes including glucose, energy and lipid homeostasis, as well as immune responses. Roux-Y-Gastric Bypass (RYGB) surgery causes superior and sustained weight loss in severely obese subjects. However, the metabolic consequences of such an intervention in the gut compared to chronic food restriction have so far not been elucidated in detail. Methods: High-fat diet-induced obese male Wistar rats were randomized to either RYGB or sham (control) surgeries. Post-operatively, animals were maintained on standard chow and the sham group was chronically food restricted so that it weighed the same as the RYGB group. The microbiome was analyzed at multiple sections of the intestinal tract as well as the metabolome (180metabolites) including bile acids (20). 16S rRNA gene sequencing and metaproteome analysis was also performed. Results: We observed striking differences in the microbiota community structure between the RYGB and the sham groups. Correspondingly, using targeted metabolomics, a differential profile of amino acids and bile acids was also found: in the cecum, aromatic and branched chain amino acids were lower, whereas in the colon, conjugated and secondary bile acids were higher in the RYGB group. Conclusion: Weight loss through RYGB and chronic food restriction has a markedly different influence on microbiota and their associated metabolites along the intestine. Further investigations are necessary to determine the functional consequences.

P-101 Lipid signatures associated with glycopeptide, lipopeptide and lipoglycopeptide cross-resistance and the β-lactam "seesaw effect" in MRSA

PRESENTING AUTHOR: ***Libin Xu, University of Washington, United States***

CO-AUTHORS: ***Kelly M. Hines, Tianwei Shen, Adam Waalkes, Kelsi Penewit, Elizabeth A. Holmes, Stephen J. Salipante, Brian J. Werth***

Glycopeptides (GP), lipopeptides (LP), and lipoglycopeptides (LGP) are key antimicrobials for managing methicillin-resistant Staphylococcus aureus (MRSA) infections, but resistance and cross-resistance to these cell envelope-active antimicrobials have emerged. We hypothesize that lipidomic changes in the cell membrane reflect specific resistance mechanisms to GP/LP/LGP. Using the MRSA strain N315, we selected for strains with reduced susceptibility to vancomycin (VAN-8), daptomycin (DAP-1), and dalbavancin (DAL-0.5), as well as two non-cell wall-active antimicrobials, moxifloxacin (MOX-32) and doxycycline (DOX-2) in vitro. Whole genome sequencing revealed mutations in cell envelope stress-response genes in DAP-1 and DAL-0.5. Susceptibility profiling of the N315-mutants revealed overall cross-resistance to individual GP/LP/LGP in VAN-8, DAP-1, and DAL-0.5, as well as the "seesaw effect" with the β-lactams (32- to 256-fold reduction in their MICs) in VAN-8 and DAP-1. Subsequently, untargeted lipidomics were carried out using HILIC-ion mobility-mass spectrometry. In principal component (PC) analysis of the data, MOX-32 and DOX-2 mutants cluster closely to the parent strain, but the VAN-8, DAL-0.5, and DAP-1 mutants all separated from the parent strain along PC1, mainly due to changes in phosphatidylglycerol (PGs) and diglycosyldiacylglycerol levels. DAP-1 was further separated from VAN-8 and DAL-0.5 along PC2 due to elevated abundance of lysyl-PGs. Importantly, several individual species of PGs with long-chain fatty acids correlated with the observed seesaw effect in VAN-8 and DAP-1 and the absence of seesaw effect in DAL-0.5. This study suggests that lipid signatures may serve as biomarkers for cross-resistance to GP/LP/LGP and the "seesaw effect" with β-lactams in GP or LP-resistant MRSA.

P-103 Foodomics research based on Aspergillus species isolated from Korean traditional nuruk

PRESENTING AUTHOR: ***Jang-Eun Lee, Korea Food Research Institute, Korea, South***

CO-AUTHORS: ***Jeong Hyun Yun, Jae Ho Kim***

Nuruk is a traditional Korean fermentation starter used to produce starch-based alcoholic beverages. Nuruk, also called 'Gokja' in Korea, contains naturally occurring and multiplying microorganisms such as wild fungi, yeast, and lactic acid bacteria. Aspergillus oryzae has been found to be safe for brewing and is a representative strain of Nuruk. However, there is also an opportunity for contamination by A. flavus that produces aflatoxin, which is a secondary metabolite and carcinogen. There have been many attempts to determine whether fermented foods are safe from aflatoxins. However, classifying A. flavus from A. oryzae has been extremely difficult because they are 99% genetically similar. Thus, in order to confirm the safety of Nuruk and alcoholic beverages made with Nuruk, (1) the total aflatoxin contents of 61 Nuruk and alcoholic beverages were analyzed, (2) a metagenomic analysis aimed at identifying microbial community differences was performed in both groups containing 14 high- and 3 low-aflatoxin Nuruks, and (3) a metabolomic approach was used to understand the correlation between microbial metabolites and the aflatoxin-producing Aspergillus species isolated from Nuruk. In conclusion, the safety of alcoholic beverages was not affected by <356.1 ppb of total aflatoxin in Nuruk. Furthermore, some microbial metabolites were selected via the metabolomics analysis as candidates related to aflatoxin production. The results from the present study will be used as basic data for securing the safety of traditional Nuruk and alcoholic beverages.

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MICROBIOME

P-104 Mass spectrometry reveals diversity of malyngamide biosynthetic pathway in cyanbacterium Okeania hirsuta

PRESENTING AUTHOR: ***Nathan A Moss, Scripps Institution of Oceanography, University of California San Diego, United States***

CO-AUTHORS: ***Lena Gerwick, William H. Gerwick***

Malyngamides are a class of drug-like molecules isolated from marine macroalgal and cyanobacterial assemblages from tropical locations around the globe. Over 40 analogs have been published in the four decades since the first malyngamide was discovered, and they present a diverse bioactivity profile in human disease-relevant assays. “Type A” malyngamides typically feature two distinctive chemical moieties: a 14-carbon methoxylated fatty acid tail, and a head group containing a six-carbon hexanone ring. LC/MS/MS analysis of a 3000-sample fraction library of cyanobacterial samples, followed by mass spectral networking, indicated numerous library sources and analogs of malyngamide molecules within the collection. Subsequent genome sequencing of cultured Okeania hirsuta assemblages resulted in the discovery of a bioinformatically predicted 65 kb polyketide synthase/non-ribosomal peptide synthetase (PKS/NRPS) malyngamide biosynthetic gene cluster. Stable-isotope labeled feeding studies, followed by 13C-NMR analysis of purified malyngamide analogs, revealed unusual features in the biosynthesis of this molecule. First, a novel loading octanoyltransferase abstracts an octanoate moiety from the acyl carrier protein used in fatty acid synthase and transfers it to the first ketosynthase module of the malyngamide pathway, and second, a mutated ketoreductase domain enables formation of a C-C bond by way of an intramolecular Claisen condensation, ultimately forming the hexanone ring characteristic to malyngamides. Ultimately, characterization of these non-canonical genes and pathway enables assignment of a plausible model for the biosynthesis of all type-A malyngamides, and paves the way for discovery of new analogs relevant to human health.

P-105 Bovine milk oligosaccharides act as prebiotics in a simplified model of the gut microbiota

PRESENTING AUTHOR: ***Louise M. Arildsen Jakobsen, Aarhus University, Denmark***

CO-AUTHORS: ***Henrik J. Andersen, Ulrik K. Sundekilde, Dennis S. Nielsen, Hanne C. Bertram***

Advances within molecular biology techniques have led to improved approaches to study the gut microbiota and prebiotic effects. Potential prebiotics include bovine milk oligosaccharides (BMO) and galacto oligosaccharides (GOS), but there is still insufficient knowledge about their effects on the composition and activity of the gut microbiota.The literature mainly describes mechanistic studies with mono-cultures to investigate whether potential prebiotics are fermentable by the genus or species of interest (often species of Bifidobacterium or Lactobacillus), but mono-cultures lack the competitive nature of a complex ecosystem such as the gut microbiome. On the other hand, 16S studies on complex ecosystems provide useful information about the effects of a prebiotic treatment on compositional changes in major groups of bacteria, but lack the detailed elucidation of the underlying mechanisms, e.g. activity, cross-feeding, etc. Here we combine metabolomics with analysis of bacterial composition to study effects of BMO and GOS in a modified version of a co-culture model of the human gut microbiota. By applying qPCR and 1H NMR metabolomics a robust measure of bacterial growth combined with an exploratory metabolomics approach to better elucidate detailed mechanistic interactions is obtained. The co-culture experiments show that there is a possible advantage of combining BMO and lactose or GOS in prebiotic formulations as this reduced the numbers of Clostridium perfringens with a simultaneous increase in B. longum. Associating the growth of bacterial species with the metabolic fingerprint of mono- and co-cultures will contribute to a better understanding of the symbiotic interactions between gut bacteria.

P-106 Characterization of Gut Metabolome between Young and Old Mice

PRESENTING AUTHOR: ***So-Young LIM, Chonnam National University, Korea, South***

CO-AUTHORS: ***Young-Shick HONG***

Since the gut microbiota varies with age and is extensively involved in human health and disease, gut metabolome would likely be influenced by aging. However, gut metabolite perturbations associated with aging were not reported. Here we reports the metabolic phenotyping of various intestinal tissues including duodenum, jejunum, ileum, cecum and large intestine as well as plasma and feces from old mice (22-month-old) compared with young mice (4-month-old), through 1H NMR-based metabolomics approach. The reductions in the levels of most amino acids and glucose in feces of old mice might reflect alterations of gut microbiota compositions with age. The largest metabolite perturbations were observed in colon tissues between old and young mice, indicating most profound effects of aging and aging-related gut microbial alterations on colon tissues. Moreover, different metabolic patterns in duodenum, jejunum, ileum and cecum with age were found, which explained distinct role of each intestinal tissues. This study highlights the metabolic perturbations in various intestinal tissues with age and their distinct roles in host metabolism.

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MICROBIOME

P-107 A comparison of the hindgut metabolome and microbiome of Anoplocephala perfoliata infected and non-infected horses: an abattoir study

PRESENTING AUTHOR: ***Rachael Hough, University of Liverpool, United Kingdom***

CO-AUTHORS: ***Alessandra Frau, Anita Lucaci, John Kenny, Jane Hodgkinson, Debra Archer, Chris Probert***

Tapeworm is a common gastrointestinal parasite of horses worldwide. Anoplocephala perfoliata is the most pathogenic tapeworm species. There is evidence that parasites and intestinal microbiota interact. However, a study comparing the microbiome of horses infected and not-infected by tapeworm is yet to be carried out. The aim of this work was to compare the metabolome (functional microbiome) and microbiome of hindgut contents from horses infected with A. perfoliata (TP) and non-infected controls (CO). To allow diagnosis of A. perfoliata by the current gold standard (counting of worms in the gut) colon contents were collected from 51 horses (TP n=21, CO n=30) killed at an abattoir for non-experimental purposes. Faecal egg counts (FEC) were performed on rectal contents to control for other gastrointestinal parasites. The volatile organic compound (VOC) metabolome of colon and rectal contents were characterised by headspace solid-phase microextraction gas chromatography mass spectrometry (HS-SPME-GCMS). Samples of colon contents were sequenced using Illumina MiSeq platform targeting the 16S rRNA gene. Preliminary data analysis has shown that medium (20-49 worms) and high (50+ worms) TP burdens were able to describe variation in the VOC profile of rectal contents (R2=0.06, p=0.03; R2=0.10, p=0.003 respectively, PERMANOVA). Furthermore, low TP burdens (1-19 worms) were not able to describe variation in the VOC profile (R2=0.04, p=0.15, PERMANOVA). Metabolome data analysis is to be continued and analysis of 16S rRNA data will be performed. It is hypothesised that microbiome sequencing results will reflect VOC profile observations.

P-108 Magnesium lithospermate B changes gut microbiome in diabetic nephropathy mice model

PRESENTING AUTHOR: ***Jia Liu, Shanghai Institute of Materia Medica, China***

CO-AUTHORS: ***Jing Zhao, Kai Wang, Qingli Zhang, Jianhua Shen***

Magnesium lithospermate B (MLB) is a marketed drug for angina in China, but extremely low oral bioavailability limits its clinical application to intravenous route. Paradoxically, oral administration of low dose of MLB was proved to alleviate kidney injury in diabetic nephropathy (DN) rats,making the pharmaceutical mechanism obscure. Recent years, kidney-gut axis becomes a new pathogenesis of renal damage, representing a non-classical pathway for pharmacological intervention. To verify whether the kidney-gut axis was targeted by MLB, streptozotocin-treated DBA/2J mice (STZ mice) were used to model DN and treated orally with MLB (STZ-MLB mice) for 8 weeks. The urine albumin (ALB) was quantified to mirror kidney function. Feces were collected to quantify 16S rRNA gene sequences to identify the alterations gut microbial population. The partial least squares discrimination analysis (PLS-DA) revealed that the overall composition of gut microbiome of mice changed by STZ and MLB. The results revealed that the therapeutic effect of MLB on hyperglycemia-induced renal injury might be attributed partially to its capability to modulate the disordered gut microbiome in diabetic condition. However, we have to do more study to find the link between the changes of gut microbiome and the host metabolism.

P-109 Metaorganism Metabolomics: Hydra as a tool for understanding the role of bacterial metabolites in shaping the metabolic landscape of the host

PRESENTING AUTHOR: ***Danielle M. M. Harris, International Max Planck School for Evolutionary Biology, Germany***

CO-AUTHORS: ***Philippe Schmitt-Kopplin, Thomas C. G. Bosch***

Changes to the resident microbial community of animal hosts influence host behaviour. This is true even of the animals with the earliest extant nervous system: the cnidarians. Recently our lab has revealed that a secreted bacterial metabolite influences the neuron-dependent contractile behaviour of the cnidarian Hydra vulgaris. Of the five main bacterial colonizers of Hydra, only one species produces the active compound, with the efficacy of the effect increasing when the entire microbial community is present. My goal is to identify the metabolites involved by leveraging our knowledge about the system before proceeding to classical fractionation protocols. Utilizing untargeted FT-ICR-MS, we have revealed, for the first time, the Hydra metabolome. By identifying which biochemical pathways are impacted when Hydra is grown with and without its species-specific bacterial community, we demonstrate how the bacterial community shapes the metabolic landscape of the metaorganism (the host plus all of its associated microbiota). By extracting the metabolome of each of the five main colonizing bacteria, along with the collective metabolome of the whole bacterial community, we have effectively created a “meta-metabolomic” database: the metabolome of a metaorganism, its individual host and microbial community constituents, and the individual bacterial species within that community. Three of the eight treatments contain the biologically active compound of interest, allowing for comparative compound identification. By understanding how microbial metabolites influence the metabolic landscape of the whole metaorganism, we are closer to understanding how bacteria influence the behaviour of their animal hosts.

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MICROBIOME

P-110 The succinate as the biomarker used for identification of succinate dehydrogenase inhibitors MOA

PRESENTING AUTHOR: ***Zhihong Hu, China Agricultural University, China***

CO-AUTHORS: ***Pengfei Liu***

At present, metabolomics has become a powerful tool for the study of the mode of action (MOA) of pesticides. There have been reports on the MOA revelation of herbicides, insecticides, and fungicides with pattern recognition of metabolic fingerprints of organism treated by pesticides. However, little has been reported so far on the research of fungicides MOA using biomarkers. Based on GC-MS and Student's t test, we compared and analyzed the situation of metabolic profile fluctuations of pathogens treated with different fungicides and obtained succinate as the specific biomarkers for succinate dehydrogenase inhibitors (SDHIs). After being treated by different MOA fungicides at EC50, the succinate content of Botrytis cinerea was found significantly upregulated specifically in the boscalid (succinate dehydrogenase inhibitor) treatment, whereas unchanged in carbendazim, thiophanate-methyl, kresoxim-methyl, cystromalazol, and imazalil treatments or down-regulated in pyrimethanil, cyprodinil, and fludioxonil treatments. In addition, the succinate content of Botrytis cinerea, Rhizoctonia solani and Colletotrichum theobromicola treated by SDHIs carboxin, fluopyram, and boscalid all significantly increased. It is presumed that SDHIs specifically inhibits the succinate dehydrogenase-catalyzed reaction of succinate to form fumarate, resulting in the accumulation of succinate. Based on this, succinate could be used as a specific biomarker for recognizing the inhibition of succinate dehydrogenase activity, which is characterized by a significant up-regulation of the content. The method established in this study to screen biomarkers and use them to identify the MOA of fungicides is fast and reliable. The specific changes in biomarker content can be used for high-throughput identification of pesticide MOA.

P-112 NMR-based metabolic profiling of plasma and fecal samples unravels biomarkers of gut condition in farmed salmon

PRESENTING AUTHOR: ***Violetta Aru, University of Copnehagen, Denmark***

CO-AUTHORS: ***Violetta Aru, Bekzod Khakimov, Elvis Chikwati, Alex J Torres, Aleksei Krasnov, Trond Kortner, Åshild Krogdahl, Søren Balling Engelsen***

The fish immune system is intimately related to the microbiome and functionality of the gastrointestinal tract. Optimal intestinal and digestive functions have been demonstrated to be essential prerequisites for the production of healthy and robust fish. Occasionally, the aquaculture industry reports compromised gut function in farmed salmons. Previous research suggests that the increasing levels of plant ingredients in feeds can be an important contributing factor. In this work, a NMR metabolomics approach has been applied to investigate the metabolome of plasma and fecal (pyloric caeca and distal intestine) samples obtained from 120 smolts. Salmons were collected from 6 different farming sites in Norway in December 2017. Histological examination of the intestinal tissues was carried out to determine the health status of the fish. Plasma samples and fecal extracts were measured by 1H-NMR spectroscopy. Standard operating procedures, developed for the high-throughput analysis of human plasma samples, were applied for the first time to analyze salmon plasma. Several metabolites were identified including cholesterol, several w-3 fatty acids (i.e. eicosapentaenoic and docosahexaenoic acids), phospholipids (i.e. phosphatidylcholine and phosphatidylethanolamine), organic acids (i.e. lactic acid) and amino acids (i.e. alanine and phenylalanine). Amongst the identified metabolites, lactic acid was found to be particularly abundant in the plasma of salmons with gut inflammation. The analysis of the fecal samples evidenced similar metabolic composition for pyloric caeca and distal intestine specimens. In particular, amino acids (i.e. methionine), organic acids (i.e. lactic acid) and sugars (i.e. b-glucose) were found to be the main components of the fecal samples.

P-113 Regulation of metabolism in divergent eukaryotic pathogens lacking conventional transcriptional control

PRESENTING AUTHOR: ***Malcolm McConville, University of Melbourne, Australia***

CO-AUTHORS: ***Simon Cobbold, Martin Blume, Joachim Kloehn, Julie Ralton, Fleur Sernee, Chris Tonkin***

Protists are evolutionarily divergent single-celled eukaryotes that include a number of important human pathogens, such as Plasmodium spp, Toxoplasma gondii and Leishmania spp. The parasitic protists typically have complex life styles, and are exposed to markedly different nutrient environments in their insect vector and mammalian hosts. Comparatively little is known about metabolic regulation in these organisms and how they adapt to rapid fluctuations in carbon source availability. Remarkably, and in contrast to many model prokaryotic/eukaryotic organisms, most of the parasitic protists lack conventional transcriptional regulation (some lack transcription factors altogether). We have utilized a combination of genetic and 13C-labeling studies to investigate regulation of carbon metabolism in Plasmodium falciparum, Toxoplasma gondii and Leishmania spp. We show that all three pathogens constitutively express most enzymes in central carbon metabolism under variable nutrient conditions and are highly dependent on metabolic futile cycling for maintenance of balanced metabolic fluxes. In particular, we show that Toxoplasma gondii and Leishmania spp are dependent on coexpression of enzymes involved in glycolysis and gluconeogenesis for growth on glucose-replete conditions and pathogenesis in animal models. In contrast, Plasmodium falciparum is dependent on the expression of metabolite phosphatases to regulate key fluxes in lower glycolysis. We propose that the greater dependence of these parasites on metabolic regulation, compared to their mammalian hosts, opens up new opportunities for developing selective therapeutic options.

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MICROBIOME

P-114 Quantification of Short-Chain Fatty Acids in Serum, Feces and Cecum

PRESENTING AUTHOR: ***Fan Fei, McMaster University, Canada***

CO-AUTHORS: ***M. Kirk Green***

Short chain fatty acids (SCFAs) and their levels in feces, cecum and serum are known to be tightly linked to microbiome activity. Measurement of SCFA levels has become increasingly important in current microbiome research. Current observations and correlations to microbiome composition have been largely reliant on the measurement of fecal/cecal SCFAs, and it is unclear how these relate to the circulatory levels of SCFAs. Traditional extraction methods require the use of hard to handle solvents (e.g. ether), large sample volumes, or complex extraction techniques (e.g. the use of hollow fiber supported liquid membrane for serum extraction). Here, a simple liquid extraction technique using propyl formate will be presented that is able to work with 50 µL or less serum samples. The same extraction method has been evaluated on fecal samples with the goal of achieving a uniform extraction method across different types of samples. A direct comparison of SCFA levels in serum and fecal samples collected from the same test subjects has also been evaluated to examine system correlations.

P-115 Untargeted LC-MS Metabolomics of >2000 Fecal Samples Reveals Association between Pseudomonas spp. Metabolites and Gastrointestinal Health

PRESENTING AUTHOR: ***Alan K. Jarmusch, University of California, San Diego, United States***

CO-AUTHORS: ***Alan K. Jarmusch, Daniel McDonald, Ricardo Da Silva, Emmanuel O. Elijah, Julia M. Gauglitz, Paul Wischmeyer, Rob Knight, Pieter C. Dorrestein***

The role of our gut's impact on health is recognized; however, we are just beginning to understand the interplay between microbial composition, metabolome, and disease. The task of inventorying metabolites was undertaken prior to ascertaining any metabolome-microbiome association. Metabolites known to be present in feces were detected, e.g bile acids. Molecular networking via GNPS assisted in the annotation of previously unannotated MS signals. Drugs were detected in ~39% of all samples. Samples from the USA had both the greatest prevalence (43.8%) of drugs as well as the greatest number per sample (0.90) compared to the UK and Australia. Gastrointestinal diseases (IBD, IBS, SIBO and C. difficile infection) were explored in greater detail; 575 individuals reported one or more of the GI diseases. Bacterial metabolites (quinolones, pyochelin, phenzines, and rhamnolipids) of Pseudomonas aeruginosa and other Pseudomonas spp. were detected in ~8.8% of all samples (188/2129). Approximately 1/3 of the samples (62/188) which contained Pseudomonas spp. metabolites originated from individuals reporting GI disease. Prior literature reports positive cultures of Pseudomonas spp. from fecal samples, but it is not believed to be a typical component of the normal gut microbiota. ~12% of individuals with GI disease have detectable metabolites (62/575). Pseudomonas spp. might be an understudied microorganism involved in GI disease, while certainly not responsible for all cases. We are currently exploring the microbiome information to better understand the relationship between detectable levels of such molecules and reported GI disease.

P-116 Untargeted Lipidomics Approach in the Characterization of Coronavirus 229E-Infected Huh7 Cells: lipid interaction of host cell and virus

PRESENTING AUTHOR: ***Bingpeng YAN, The University of Hong Kong, Hong Kong***

CO-AUTHORS: ***Kong Hung Sze, Hin Chu, Dong Yang***

Coronavirus infections in humans (Human coronavirus 229E [HCoV-229E]) are mainly associated with upper respiratory tract infections and exhibit common cold-like syndrome. On the other hand,the body immune system aims to stop the 'invader' from entering your body in the first place and fight the infection induced by the foreign invader. To perform comprehensively insight for monitoring virus infection and host cell response, an establishing ultrahigh performance liquid chromatography-mass spectrometry (UHPLC–MS) based untargeted lipidomics approach was applied to define intracellular and extracellular lipids discrimination of 229E virus infection compared to a control group. As a result, 20 intracellular lipids with significantly higher level and 2 extracellular lipids with lower level were identified in virus infection group, which mainly were glycerophospholipids and polyunsaturated fatty acids. Subsequently, pathway analysis was applied to investigate global affection at metabolites molecular level after virus infection. The results revealed that the linoleic acid metabolism and arachidonic acid metabolism were heavily perturbed, which were presumably related to inflammation signal transduction and immune system activation. In addition, the linoleic acid and arachidonic acid also exhibited a strong anti-viral effect in cell incubating assay, which in line with immune system fight infection and finally clear out the virus. In conclusion, the LC-MS-based lipidomics method was successfully explored lipids difference after virus infection, which demonstrated that not only host cell produced pro-inflammatory signal through regulating these lipids level, but also suggested that virus may adjust membrane lipid remodelling for better replication.

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MICROBIOME

P-117 Interactions between commensal Escherichia coli dans the host : impact on the metabolic profile of the host

PRESENTING AUTHOR: *Cecile CANLET, INRA Toxalim Metatoul-AXIOM, France*

CO-AUTHORS: *Marie Tremblay-Franco, Unai Escribano-Vazquez, Claire Cherbuy*

Escherichia coli is a common and widespread inhabitant of human gastrointestinal tract, the prevalence in human gut is more than 90%. The objective of this work is to better understand the interplay between commensal E. coli and the host in physiological conditions and if a High Fat Diet (HFD) modifies E. coli impact. We have mono-colonizedprevious germ-free (GF) mice with two different E. coli commensal strains: i) the CEC strain, able of reinforcing the mucus barrier, ii) the characterized probiotic strain Nissle1917. GF, monoxenic and conventional mice were fed a standard diet or a HFD. Urine, plasma and caecal samples have been analyzed using 600 MHz proton NMR spectroscopy. NMR data were subjected to multivariate statistical analyses, Principal Component Analysis (PCA). PCA score plots showed a clear separation between the two diets for the three matrices. For urine and caecal samples, with standard diet, metabolic fingerprints are different between axenic, monoxenic and conventional mice. The two groups of monoxenic mice are not discriminated with a standard diet, but are well separated with a HFD for urine and caecal samples. For plasma samples, the four groups of mice cannot be discriminated using PCA analysis with standard diet or HFD. These data reveal that the metabolomic trajectory of the host is more sensitive to bacterial status over a HFD. The identification of discriminant metabolites between groups is in progress. The metabolic pathways disrupted by the bacterial status will be studied using the MetExplore software.

P-118 Factors affecting the bacterial composition and metabolites on the cuticle of two Amazonian ants species living in the same nest

PRESENTING AUTHOR: *Caroline Birer, University of Pittsburgh, France*

CO-AUTHORS: *Corrie S. Moreau, Niklas Tysklind, Gregory Genta-Jouve, Lucie Zinger, Christophe Duplais*

Bacteria associated with the cuticle of ants are generally studied for their role in defense against pathogens especially in the clade of fungus-growing ants. However, we have little understanding of cuticular bacterial diversity and function on a larger taxonomic scale or what host and environmental factors shape the community composition. Here, we use high-throughput 16S rRNA gene sequencing to study how host and nest material influence the cuticular microbiota of two Amazonian ant species Camponotus femoratus and Crematogaster levior that frequently nest together in what are commonly called ant gardens. This unique interaction inside the roots system of epiphytic plants, allows us to study species interactions and bacterial transmission of two distantly related ants living in a shared nest. Our results show that the majority of bacterial microbiota on the cuticle is acquired from the nest, some OTUs are specific to each species likely representing the core cuticular microbiota and shared OTUs between the two species are rare. In a second step, we conducted a mass spectrometry metabolomics analysis of metabolites on the cuticle of ants to perform correlations between bacterial OTUs and m/z ion mass. We find both positive and negative interactions between the bacterial community and the metabolites from the cuticle of these co-living ants, although we are not entirely sure how to interpret these results. Overall our results show how chemical and biological compositions may be impacting each other highlighting overall an untapped complexity of the cuticle of ants.

P-119 Integrated Metabolome-Microbiome Analyses to Evaluate the Preventing Effects of Short-term Green Tea Supplementation for UltravioletB-induced Erythema

PRESENTING AUTHOR: *Eun Sung Jung, Konkuk University, Korea, South*

CO-AUTHORS: *Jong Il Park, Hyunjoon Park, Wilhelm Holzapfel, Jae Sung Hwang, Choong Hwan Lee*

In this study, we aimed to investigate comprehensive skin metabolomics study towards evaluating the preventing effects of erythema formation of green tea extract (GTE) or its ingredients viz., epigallocatechin gallate (EGCG), caffeine, and theanine supplementation for 7 days prior to single ultraviolet (UV) B irradiation (2.5 MED, 188 mJ/ cm2). Further, established an integrative cecum metabolome-microbiome model for correlating with skin metabolome. Among GTE, EGCG, caffeine, and theanine supplemented groups, only GTE supplemented group showed significant inhibition effects of erythema formation induced by UVB irradiation. Skin metabolome changing patterns highly reflects those of skin phenotype. Single UVB irradiation increased most of amino acids, organic compounds, nucelobases, and lysophospholipids, whereas decreased saccharides and fatty acids in skin. While completely reverse metabolic change patterns were only founded in prior GTE supplemented group, particularly, trans-urocanic acid, fatty acids, lysophospholipids, and cholesterol. Cecum metabolome and microbiome modulations were mild by single UVB irradiation and varied accordance with each supplemented compound. According to biplot principal component analysis, relatively high association of Clostridium butyricum with UVB irradiated group was observed. The most influential bacteria distinguishing prior GTE supplemented group from control were Bifidobacteria and Lactobacillus spp. Those of bacteria also showed high positive correlation with skin metabolites. From this study, we suggested that short-term supplementation of GTE prior to UVB irradiation could effectively prevent erythema formation on skin through modulation of skin metabolome and direct/indirect influences of cecum metabolome-microbiome changes.

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MICROBIOME

P-120 Comprehensive Metabolite Profiling for interpretation of the Solid state and Submerged Fermentation of Aspergillus oryzae KCCM 12698

PRESENTING AUTHOR: ***Su Young Son, Konkuk University, South Korea***

CO-AUTHORS: ***Summin Lee, Digar Singh, Na-Rae Lee, Dong-Yup Lee, Choong Hwan Lee***

Aspergillus oryzae has been commonly utilized to make miso, koji, and rice wine in food fermentation industry. However, the metabolomics of A. oryzae during fermentation in various culture conditions are not well-understood.Therefore, the present work investigated time resolved (0, 4, 8, 12, 16 day) secondary metabolite profiling for A. oryzae KCCM 12698 under solid-state and submerged fermentation (SSF and SmF).Metabolite profiling was carried out using the ultrahigh performance liquid chromatography – linear trap quadrupole – Electrospray ionization – ion trap – mass spectrometry (UHPLC – LTQ – ESI – IT – MS) followed by multivariate analyses. We showed the relatively higher levels of coumarins and oxylipins under SSF, while terpenoids were more abundant under SmF. Moreover, the antimicrobial activities of extracts from SSF and SmF were measured and indicated that the SSF extracts showed higher activities as compared to SmF, with higher production of active secondary metabolites such as ketone-citreoisocoumarin, pentahydroxy-anthraquinone, hexylitaconic acid, saturated fatty acids, and oxylipins. This study highlights the pre-eminence of a metabolomic foundation regarding the growth parameter and active compound production for A. oryzae under the mostly applied to industrial cultivation states. Furthermore, current work contains the potentials for screening and MS-based metabolites helpful in fermented foods such as Koji mold.

P-121 Influence of lincosamides antibiotics on fecal and plasma metabolite profiles in rats

PRESENTING AUTHOR: ***Oliver Schmitz, Metanomics GmbH, Germany***

CO-AUTHORS: ***Sabina Ramirez-Hincapie, Christina Behr, Hennicke Kamp, Volker Strauss, Tilmann Walk, Michael Herold, Bennard van Ravenzwaay***

In this study, an antibiotic-mediated alteration of the gut microbiome was carried out. Clindamycin or lincomycin were administered orally to Wistar rats. With the aim of identifying metabolites derived from the gut microbiome, metabolite profiles of feces and plasma were assessed and compared. As these antibiotics have an almost negligible systemic toxicity, the metabolomics findings in the plasma of treated animals is, most likely, exclusively related to changes in the gut microbiome. Taurocholic acid and cholic acid were both highly increased in feces indicating an accumulation of these primary bile acids in the gut which is likely caused by the disruption of microbial communities and an increase of bile production via the liver derived from a lack of secondary bile acids. Additionally, 7-alpha-dehydroxylating bacteria can convert the small amount of secondary bile acids to yield cholic acid and taurocholic acid and have been shown to be favored after antibiotic treatment. However, the reduced level of cholic acid in plasma indicates that this metabolite is not being reabsorbed from the gut into the portal vein presumably because of its limited aqueous solubility and precipitation in the protonated form in the intestinal tract. Bile acid composition may be greatly affected by microbial community structure and function. The results of this metabolome analysis show that antibiotic treatment has a great impact on the bile acid metabolism. Since bile acids have been hypothesized to be related with several diseases, investigation of these metabolites is of high relevance in the field of toxicology.

P-122 A Metabolic Pattern of Influenza A Virus Infected Sus scrofa: Establishment of the pig as new infection model for bacto-viral infections

PRESENTING AUTHOR: ***Daniel Schultz, University of Greifswald, Germany***

CO-AUTHORS: ***Karen Methling, Michael Lalk***

Introduction: Virus infections of the upper respiratory tract in combination with secondary bacterial infections can lead to severe lung infections. The aim of the current project KoInfekt is to elucidate the host-pathogen interactions establishing the pig as an animal infection model due to high genetic and physiological similarities to human beings. Material and Methods: Animal experiments were done on the Federal Research Institute for Animal Health (Isle of Riems, Germany). A group of 25 pigs were infected with Influenza A virus (H1N1, Germany) and samples were collected over 31 days. For metabolic analysis tissues samples (lung, spleen), biofluids (blood plasma, BAL) and feces were collected and analyzed by a combination of 1H-NMR, GC-MS and LC-MS/MS. Results: Extraction protocols for pig fecal material was established for analysis of host and gut microbiota digestion processes analyzed by 1H-NMR and GC-MS. For eicosanoid detection of tissue and biofluid samples the extraction steps were optimized for a LC-MS/MS method working on dynamic MRM. Discussion: Perturbations in the eicosanoid profile of Influenza A virus infected pigs were detected. The analysis of the fecal metabolites enables an overview about the gut microbiota, which is linked to the host immune response and the interplay of the host and the bacteria community. This is the first step for the metabolic analysis of bacto-viral co-infections, which play an important role in human and animal health.

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POSTER SESSIONS 3 AND 4 – **Wednesday and Thursday –** all even number presenters will be at their posters.

MICROBIOME

P-123 Characterization of grass fermentation by microbial consortia using HPLC/MS

PRESENTING AUTHOR: ***Sanchao Liu, Army Research Lab, United States***

CO-AUTHORS: ***Matthew Perisin, Elliot Gerlach, Christian Sund***

Cellulose, nature's most abundant polymer, is considered among the most promising sources for biorenewable feedstocks. Although it is possible to use a single microbe for direct biochemical production, it is hard to optimize the process of biomass breakdown and chemical production with a single organism. Microbial consortia have the benefit of combining the metabolic capabilities of more than one microbe and provide more efficient bio-transformations. In this study, fermentation of reed canary grass under different conditions was investigated and the fermentation products were monitored by HPLC and MS. Different fungi and media were tested for their ability to breakdown grass and the production of chemicals. It is found that while different fungus provide different ability for grass breakdown and produced different sugars, it is the media which have the most impact on the final chemical production. With the combinations of bacteria (Clostridium acetobutylicum (Cac)), fungi and media, we are able to design a consortia system in which grass can be effectively converted into useful chemicals as directed.

P-124 Interactions between Environmental Chemicals PCBs and Gut Microbiota Modulate Intermediary Metabolism Pathways of the Host

PRESENTING AUTHOR: ***Julia Yue Cui, University of Washington, United States***

CO-AUTHORS: ***Sunny Lihua Cheng, Dongfang Wang, Xueshu Li, Hans-Joachim Lehmler, Daniel Raftery, Haiwei Gu, Julia Yue Cui***

Background: Polychlorinated biphenyls (PCBs) have been suggested to contribute to metabolic syndrome, but the mechanisms remain poorly understood. We hypothesize that PCBs and gut microbiota interact to reduce beneficial microbial metabolites and exacerbate PCB-induced toxicities. Methods: Three-month-old female C57BL/6 conventional or germ-free mice were orally exposed to vehicle (corn oil, 10ml/kg) or an environmentally relevant PCB mixture (i.e. the Fox River Mixture, 6mg/kg or 30mg/kg) once daily for 3-days (n=5 per group). Intermediary metabolic metabolites (LC-MS), hepatic transcriptome (RNA-Seq), and gut microbiota (16S rRNA sequencing) were determined. Metaboanalyst (http://www.metaboanalyst.ca) and Pearson's correlation analysis were used for muti-omic data integration. Results: In serum and liver, lack of gut microbiota increased the basal levels of palmitic acid and glycine. The increase in glycine corresponds to increased hepatic mRNA of Agxt2, which converts glyoxylate to glycine, but decreased hepatic mRNA of Gatm, which converts glycine to creatine. In liver, PCBs dose-dependently increased the host-derived choline metabolite dimethylglycine in a gut microbiota-dependent manner. This corresponds to a dose-dependent PCB-mediated decrease in Proteobacteria (Alphaproteobacteria class) and Firmicutes (Bacteroidia and Erysipelotrichi classes) phyla, which are known to degrade choline. In serum, PCBs dose-dependently increased serine and methionine in a gut microbiota-dependent manner. The anti-obesity genus Allobaculu positively correlated with serum mannose, whereas the Delftia genus positively correlated with serum glycine, serine, and methionine. Conclusion: The present study was the first to identify that gut microbiota critically modifies PCB-mediated disruption of amino acid and carbohydrate metabolism pathways in liver and serum, which may exacerbate PCB-induced multi-organ toxicities.

ECOLOGY AND ENVIRONMENT

P-125 Air Pollution and Metabolomics in Maternal Serum

PRESENTING AUTHOR: ***Qi Yan, UCLA School of Public Health, United States***

CO-AUTHORS: ***Zeyan Liew, Xin Cui, Karan Uppal, Dean Jones, Beate Ritz***

Background: Studies have shown that maternal exposed to ambient air pollution can increase the risk of a variety of adverse birth outcomes and neurodevelopmental disorders.Assessing environmental metabolomic profiles using high-resolution metabolomics(HRM) is a novel tool to investigate air pollution exposure history and related biological mechanism. Objective: The aim of this study is to comprehensively profile metabolomics in the blood of women in mid-pregnancy and identify perturbations in metabolites and metabolic pathways associated with air pollution exposure. Methods: We retrieved stored mid-pregnancy maternal serum samplesforwomen living in the Central Valley of California, a region with high particulate air pollution exposures. We developed measures of air pollution exposure at the mothers birth address. By using HRM, we identified significant metabolites and pathways within 99 exposed mothers and 62 unexposed mothers. Partial least squares discriminant analysis (PLS-DA) and support vector machine (SVM) were used to select significant metabolic features. Pathway and network analyses were done by using WGCNA and mummichog. Results: A set of 3917 metabolic features were used for discriminant analysis and pathway analysis. We have identified 25 metabolic biomarkers which can properly classify the air pollution exposure status with over 80% accuracy. Pathway analysis showed that air pollution exposure associated with leukotriene metabolism, amino acid metabolism, and pyrimidine and purine metabolism pathways. Conclusion: We found that maternal exposed to air pollution during critical pregnancy period could potentially affect inflammation and oxidative stress-related metabolism, which may then contribute to the development of neurodevelopmental disorders in the offspring.

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ECOLOGY AND ENVIRONMENT

P-126 Metabolomics of EDC exposure: assessing impacts on cellular and circadian metabolism

PRESENTING AUTHOR: ***Lisa N Bottalico, University of Pennsylvania, United States***

CO-AUTHORS: ***Dania Malik, Saikumari Krishnaiah, Arjun Sengupta, Yool Lee, Amita Sehgal, Aalim Weljie***

The rising global incidence of diabetes and obesity comes at a significant economic and public health cost. Environmental pollutant exposures may play a role. Endocrine disrupting chemicals (EDCs) are environmental contaminants that disrupt hormone action, causing developmental and reproductive abnormalities. Mechanistic and epidemiological studies link EDC exposure to development of metabolic diseases. EDCs have been found to disrupt energy homeostasis in various tissues, interfering with hepatic energy metabolism, adipose physiology, and pancreatic beta cell functioning. In parallel, the circadian clock is a critical contributor to energy homeostasis and regulates glucose and lipid metabolism. Circadian disruption is also strongly associated with metabolic disease. The aim of the current study was to assess potential overlaps between circadian control of cellular metabolism and EDC-induced metabolic disruption utilizing global metabolomics analysis. The effect of bisphenol-A on global cellular metabolism was assessed utilizing mouse clock models of hepatic and adipose physiology. MMH-D3 (hepatocyte) and 3T3-L1 (adipocyte) cell lines containing circadian clock reporter genes Per2-dLuc and Bmal1-dLuc were analyzed using metabolic profiling. Aqueous phase cellular metabolites (340) were profiled utilizing a HILIC-based LC approach followed by ESI-QqQ-MS with multiple reaction monitoring. Lipidomics analysis of cellular organic phase metabolites was conducted utilizing high resolution LC-ESI-qTOF-MS. 82 aqueous phase cellular metabolites were significantly altered in response to BPA exposure in 3T3-L1, with pathways analysis revealing effects on bioenergetic metabolism, purine metabolism and amino acid biosynthesis. Future directions include high-temporal resolution sampling followed by global metabolomics to assess the effect of EDCs on circadian cycling cellular metabolites.

P-127 Characterization of Environmental Pollution & Ecosystem Health of an Australian Multi-Commodity Port and the Surrounding Ecosystem using Metabolomics-Based Approaches

PRESENTING AUTHOR: ***David J. Beale, CSIRO Land and Water, Australia***

CO-AUTHORS: ***Avinash Karpe, Joey Crosswell, Andy Steven***

Understanding the complex interactions between biological systems and environmental changes, natural or anthropogenic, is a major research challenge. While traditional environmental and organism health monitoring techniques (e.g. chemical monitoring and bioassays) provide some insight, they are often unsuitable for assessing subtle changes in ecosystem and organism's physiology associated with low-level exposure(s). This work presents the application of omics-based approaches for the assessment and characterization of bacterial community interactions with their environment through a system metabolome analysis. As such, the impact of several anthropogenic factors arising from point/non-point pollution sources at a multi commodity marine port (Gladstone, Australia) and, its surrounding ecosystems were studied using sediment samples from onshore (n=5) and offshore (n=4) sites. Sediment samples were analysed for trace metals, organic carbon, polycyclic aromatic hydrocarbons (PAH), emerging chemicals of concern (ECC) and sterols. Similarly, the biological and biochemical interactions between the reef and its environment were analysed using next-generation sequencing of the bacterial community and community metabolic profiling. The multi-omics data indicated stresses on the bacterial community at all the sampled sites. Especially, elevated metabolic rates were observed for fatty acid synthesis and the intermediates of the shikimate pathways, leading to the production of quinic acid-like metabolites and mycosporine-like amino acids. Such information provides an early warning sign of ecosystem degradation and demonstrates the value of a multi-omics for ecological assessments, in which a more detailed perspective of physical and chemical contaminants and, their impact on the community bacterial biome is obtained.

P-128 A GC/MS based metabolic profiling of liver tissues in mice by instillation of fine particulate matter (PM2.5)

PRESENTING AUTHOR: ***Shi Chunzhen, Beijing Technology and Business University, China***

A method based on gas chromatography-mass spectrometry (GC-MS) was established to analyze the changes of intracellular metabolites and study the toxic mechanisms of different concentrations of particulate matter (PM2.5) effecting the liver tissues in mice. Nasal drip experiments of PM2.5 suspensions (0, 7.5, 20, 37.5g/L) for mice were carried out, and the intracellular metabolites in liver tissues were extracted, pretreated and analyzed. Principal component analysis (PCA) and partial least-squares discriminant analysis (PLS-DA) were used for pattern recognition, and an obvious distinction among different conditions was found. According to the PLS-DA loading diagram and variable important factor (VIP) value, 10 potential biomarkers in liver were determined with significant differences between four different concentrations of PM2.5. With the increase of PM2.5 dropping, the concentrations of purines in mice increased significantly, the concentrations of amino acids decreased, and the concentrations of the beta-hydroxybutyric acid decreased. Metabolic pathway analysis indicated that the cell tissues of different target organs were in the state of oxidative stress. The glycolysis pathway and tricarboxylic acid cycle were disordered, urea cycle was enhanced, amino acid catabolism was activated, the decomposition of fatty acids was reduced, the purine metabolism disordered and insulin secretion was abnormal. This study could provide a new perspective and theoretical basis for the further analysis on toxic mechanisms of PM2. 5.

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ECOLOGY AND ENVIRONMENT

P-129 Lipid profiling of coral exposed to herbicide for biomonitoring and health risk assessment

PRESENTING AUTHOR: ***Chuan-Ho Tang, National Museum of Marine Biology and Aquarium, Taiwan***

CO-AUTHORS: ***Shu-Han Shi, Ching-Yu Lin, Wei-Hsien Wang***

Coral is commonly selected as a bioindicator to detect a variety of adverse factors using pan-type biomarkers. Omic technologies have recently been applied to model the systemic changes in an organism to improve the biomonitoring. Membrane lipids create a dynamic cell structure based on the physiological state, which offers a distinct lipid profile to specifically detect environmental threats and assess the associated health risk. Glycerophosphocholine (GPC) profiling of coral Seriatopora caliendrum exposed to environmentally relevant levels of Irgarol 1051 was therefore performed to demonstrate the potential of a lipidomic methodology for biomonitoring. The lipid variations were well modeled based on the exposure dose and the coral photoinhibition levels to develop a quantitative model. The predominant lipid changes correlated with the photoinhibition can be related to the consequence of blocking the photosynthetic electron flow based on the associated physiological roles. The lipid metabolic alterations further predict the coral health risks associated with altered physiological conditions. In this study, the lipidomic methodology is demonstrated as a potential tool for environmental monitoring and assessment.

P-131 Metabolomic analyses of Namibian Desert soil to determine the cause of the mysterious fairy circles.

PRESENTING AUTHOR: ***Jan Willem Hurter, University of Pretoria, South Africa***

CO-AUTHORS: ***J.J.M. Meyer, D.A. Baranenko, A.V. Kind***

Circular barren patches, known as fairy circles (FCs) occur throughout Namibia, devoid of vegetation and surrounded by a matrix of grasses. Several hypotheses regarding the origin and maintenance of FCs have been proposed, none widely accepted. Soil physical properties and chemical constituents were investigated to determine whether a variance in properties and itsmagnitude is present. Results obtained from physical property analyses indicated greater hydrophobicity in soil collected from FCs than matrix soil. Extracts prepared from FCs and matrix soil, and from locations where Euphorbiaceae are decomposing (DP), were analysed by GC-MS and NMR to determine the cause of hydrophobicity. To identify discriminative signals from GC-MS and NMR data, PCA-plots, OPLS-groups, and S-plots were created to aid compound identification. Specific compoundswere present in both FC and DP soil samples and not in matrix soil. NMR based metabolomics indicated similar concentrations of ester, phenol, alkene and aromatic functional groups within FC and DP soil, while differences between FC and matrix soil were greater. From these results, it is deduced that there's a definite difference in physical properties and chemical constituents of FC and matrix soil. Causes of these differences can be concluded to be initiated by decomposing Euphorbiaceae species, as indicated by compounds (e.g. 1-(4-Acetamidoanilino)-3,7-dimethylbenzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile) present in FC and DP soil samples, previously identified in a variety of plants' latex (Jessica et al. 2016). Decomposing Euphorbiaceae causes hydrophobicity in soil upon decomposition and is implied toalterthe structural nature of soil organic matter at its location. Jessica,A,Rao,MRK,Anthony,J,Prabhu,K,Johnson,WMS,Balasubramanian,BS,Sundaram,L,Dinakar,S_2016_'The_GC-GS_Study_of_One_Ayurverdic_Preparation_Katakakhadiradi_Kashayam' International_Journal_Pharmaceutical_Sciences_Review_and_Research_vol.39,no.2,pp.216-224

P-132 Ultra high performance tandem mass spectrometric determination of F2α-isoprostanes in urine and cell medium after exposure to particles

PRESENTING AUTHOR: ***Jutta Lintelmann, HelmholtzZentrum München, Germany***

CO-AUTHORS: ***Jutta Lintelmann, Sebastiano di Bucchianico, Stefanie Kasurinen, Sebastian Öder, Xiao Wu, Jerzy Adamski, Ralf Zimmermann***

F2-isoprostanes (F2-IsoPs) are a series of prostaglandine-like compounds formed via non-enzymatic, radical initiated oxidation of arachidonic acid. F2-IsoPs turned out to be robust biomarkers of lipid oxidation and thus of oxidative stress in different organisms. We developed and applied a UHPLC-MS/MS method for the determination of four F2α-IsoPs, and prostaglandine F2α in urine and cell medium. The method includes a solid phase extraction on a weak anion-exchange cartridge (StrataX-AW, Phenomenex) applying 1 ml urine and 5 – 6 ml cell medium. Resulting extracts are separated on a core-shell column (Kinetex XB-C18, 1.7 µm, 150 x 2.1 mm I.D., Phenomenex) using a methanolgradient (0.1 % formic acid) at 0.2 ml/min and 35°C. Analytes were detected by a QT 4000 mass spectrometer with electrospray ionization in negative ion mode. Method validation resulted in recoveries between 54 % and 64 % without internal standard correction (deuterated F2α-IsoPs); repeatability and reproducibility were good with coefficients of variation from 0.7 % to 7.2 %. No matrix effect was observed (recoveries of spiked extracts ≥ 90%); and detection limits were around 0.07 ng/ml. The method was used to determine F2α-IsoPs in urine of individuals exposed to various levels of particulate matter (PM2.5) and in cell medium of A549 and THP-1 cells. The cells were exposed to carbon concrete particles, concrete particles and Printex particles in an air-liquid interface. In urine, all F2α-IsoPs in different concentrations were found. The medium samples analyzed until now showed a significant increase of mainly prostaglandin F2α after exposure to particles.

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ECOLOGY AND ENVIRONMENT

P-133 Falcon Hatch Failure: A Metabolomics Study

PRESENTING AUTHOR: ***Katrina A Doenges, University of Colorado, United States***

CO-AUTHORS: ***Richard Reisdorph, Kevin Quinn, Xing Zhang, Nichole Reisdorph***

In 2015, a falcon breeding facility in Colorado had difficulties with their Anatum Peregrine Falcon (Falco peregrinus anatum) eggs reaching maturity and hatching. Upon inspection, the eggs were either not properly fertilized, had under-developed embryos, or reached a mature size but did not hatch. A preliminary metabolomics study was proposed to investigate why the eggs were not viable. Metabolites from heart tissue, brain fluid, mutes, nesting materials and quail feed were searched against a Pesticide Database (Agilent), to see if pesticides potentially played a role in the egg failures. From this search, six environmentally relevant pesticides of interest were selected to investigate further—Atrazine, Glyphosate, Metolachlor, Paraquat, Pendimethalin and Spiromesifen. Falcon eggs are precious, therefore a secondary study was conducted to determine if chicken eggs from a single source could be used as a surrogate species to study falcons. The results show that there are approximately 11,000 metabolites in common between chicken and falcon brain fluid samples, and approximately 11,500 metabolites in common between the heart tissues. However, approximately 10% of the total metabolites detected in these two tissues are unique to each species, indicating that chickens may not be a suitable surrogate. Future studies will focus on investigation of the six pesticides of interest, identification of unknown metabolites and comparison of additional falcon and chicken embryo tissue samples.

P-134 Using UHPLC-Q-TOF/MS based lipidomics approach to determine effects of ZnO-particles exposure on the rat plasma

PRESENTING AUTHOR: ***Yi-Ru Chen, Institute of Environmental Health, College of Public Health, National Taiwan University, Taiwan***

CO-AUTHORS: ***Hao-Jan Liang, Tsun-Jen Cheng, Ching-Yu Lin***

Previous studies have shown that ZnO exposure may lead to metal fume fever and pulmonary inflammation symptoms in the human. Numerous animal studies also demonstrated that exposure to ZnO-particles are associated with pulmonary inflammation and injury. Our recent studies showed ZnO-particles lead to perturbation of metabolites in the bronchoalveolar lavage fluid and lung tissues in a rat inhalation model. Since few studies focus on systemic lipid changes after ZnO-particles exposure, untargeted lipidomic approach will be used to measure changes of lipidome from the blood of rats inhaled ZnO-particles in this study. Several classes of lipids including lysophospholipids, monoacylglycerols, phospholipids, sphingomyelins, ceramides, diacylglycerols,triacylglycerols were tested in our UHPLC-QTOF/MS system. All the lipid standards were successfully detected by our platform. We further used rat plasma samples to test the analytical performance, more than 1000 features were detected and further extracted from the spectra raw data of rat plasma. Above 80% features showed coefficients of variation below 20%, demostrating good analytical reproducibility of the system. Plasma samples from rats inhaled 250nm ZnO particles or the filtered control for 24 hours were analyzed by this UHPLC-QTOF/MS system. Different lipidome from the exposure and control rats were observed by multivariate statistical analysis. Lipids associated with ZnO exposure will be identified. The final goal of this study is to examine effects of ZnO-particles exposure on the plasma using lipidomic approach and further investigate more comprehensive mechanisms of effects of ZnO-particles.

P-135 Heteronuclear 2D J-resolved spectroscopy for metabolite fingerprinting and flux in earthworm coelomocytes

PRESENTING AUTHOR: ***Corey M. Griffith, University of California, Riverside, United States***

CO-AUTHORS: ***Cynthia K. Larive***

Earthworms (Eisenia fetida) are vital members of the soil environment. Because they are sensitive to contaminants, monitoring their metabolism may be a useful indicator of soil health and ecotoxicity. Metabolomics has been used to evaluate the impacts of toxicants on whole-earthworm extracts and coelomic fluid; however, the utility of coelomocyte metabolomics has not been assessed. Coelomocytes are free-moving, hepatocytic and immune cells within the earthworm coelom that could provide an alternative method for assessing toxicant mode of action and monitoring the impact of environmental insults on earthworms. Coelomocytes may be a more relevant pool to monitor toxicity since these cell types are designed to combat stress. Additionally, this assay provides an opportune medium to introduce labeled compounds (e.g., glucose-U13C) to study metabolic flux in earthworms and further elucidate the effects of stressors on biochemical pathways. Herein, we describe a toxicity test to monitor metabolic perturbations in coelomocytes using nuclear magnetic resonance (NMR). Heteronuclear 2D J-resolved spectroscopy disperses heteronuclear scalar coupling into a second dimension, reducing spectral complexity and providing the means to quantify 13C-labeled isotopomers. Additionally, projection in the first-dimension sums 1H-12C and 1H-13C coupling into a single, 1H-12C resonance, allowing us to study metabolite flux and fingerprinting in single NMR experiment. These methods provide the prospect for high-throughput of the effects of environmental stressors on earthworm metabolism.

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ECOLOGY AND ENVIRONMENT

P-136 NMR-based metabolomics contributing to saving critically endangered species

PRESENTING AUTHOR: ***Miki Watanabe, Cincinnati Children's Hospital Medical Center, United States***

CO-AUTHORS: ***Stuart J. Bauer, Lindsey E. Romick-Rosendale, Erin Curry, Terri L. Roth***

Sumatran rhinoceroses and polar bears are facing urgent and unique challenges. NMR-based metabolomics was used to better understand factors contributing to the declining populations of these critically endangered species. The Sumatran rhinoceros is affected by hemochromatosis and its population is less than a hundred rhinos worldwide. To investigate the metabolic changes during the development of hemochromatosis and potentially increase our understanding of susceptibility differences, the serum metabolome from the Sumatran rhinoceros was investigated by NMR-based metabolomics. A comparison of the 43 serum metabolomes of three zoo rhinoceros showed two distinct groupings, healthy (n=30) and unhealthy (n=13). A total of eighteen altered metabolites were identified in healthy versus unhealthy samples. Given the impending crisis facing wild polar bears due to diminishing sea ice, zoos are striving to develop a self-sustaining, population; however, few cubs are produced each year. The ability to non-invasively determine pregnancy in polar bears will help illuminate the cause of reproductive failure in zoo bears and provide a means of monitoring the impact of climate change on reproductive processes of wild bears. To detect unique metabolic changes distinct to pregnancies, 53 fecal metabolites were measured in over 800 dried fecal samples from 3 control bears, 3 pseudo-pregnant bears and 10 pregnant bears collected throughout the year . This longitudinal study with large sample size allows us to study data mining challenges due to individual variabilities in basal metabolite levels. Metabolomics studies in these and other endangered species may help with the preservation of our preciouse wildlife populations.

P-137 Activation of Fungal Isolate, Pestalotiopsis microspora Secondary Metabolite Gene Clusters for Discovery of Anti-infectives Against Drug-Resistant Pathogenic Bacteria

PRESENTING AUTHOR: ***Cassandra N Naphen, University of North Carolina Greensboro, United States***

CO-AUTHORS: ***Lindsay K Caesar, Huzefa A. Raja, Nicholas H Oberlies, Nadja B Cech***

Annually in the US, methicillin-resistant Staphylococcus aureus (MRSA) causes over 80,000 severe infections and 11,000 deaths. MRSA possesses an arsenal of virulence factors that are controlled by a regulatory system known as the quorum sensing system. Targeting virulence factor producing pathways is a promising strategy for anti-infective therapy, which is expected to allow the host organism to clear the infection without the need of antimicrobial agents that lead to the development of resistance. There is particular interest in targeting the signal biosynthesis component of the quorum sensing, which is highly conserved among gram positive pathogens. The only known inhibitor of signal biosynthesis in Gram positive pathogens the natural product ambuic acid, which is produced by the Ascomycete fungus, Pestalotiopsis microspora. With silent secondary metabolite biosynthetic gene clusters in the laboratory settings, it is important to consider variables that will effectively enhance and promote silent secondary metabolite production as well as the up-regulate metabolite production. We observe significant changes in metabolite production accompanying variations in the media used to culture P. microspora. Changes in culturin the increased productionof known components and/or of new metabolites with antimicrobial and antivirulence activity. Using untargeted metabolomicswe seek to measure the effects of culturing techniques to induce changes in metabolite production in the fungus, P. microspora. The influence of different growth conditions is tracked with biological testing, using our laboratory's novel mass spectrometry-based bioassay, which specifically identifies compounds that target quorum sensing signal biosynthesis.

P-138 Untargeted metabolomics analysis of enrichment reactor cultures performing enhanced biological phosphorus removal (EBPR)

PRESENTING AUTHOR: ***Nay Min Min Thaw Saw, Singapore Center for Environmental Life Science Engineering, Singapore***

CO-AUTHORS: ***Rogelio E. Zuniga Montanez, Guanglei Qiu, Pipob Suwanchaikasem, Sara Swa Thi, Stefan Wuertz, Rohan B. H. Williams***

Enhanced biological phosphorus removal (EBPR) is a bioprocess for removal of phosphorus (P) from wastewater, facilitated by activity of polyphosphate accumulating organisms (PAOs). PAOs are currently unculturable and require enrichment reactors to permit study of their genomic, biochemical and physiological properties. Despite extensive metagenome and some metatranscriptome analysis of such PAO enrichment communities, their direct metabolic characterisation remains unexplored. Here we present a complete analytical protocol for untargeted metabolomics analysis of microbial communities from two laboratory scale activated sludge reactors enriched for PAO bacteria Candidatus Accumulibacter phosphatis and Tetrasphaera spp., respectively. Extra- and intra-cellular metabolites were extracted using five methods and analysed by ultraperformance liquid chromatography mass spectrometry (UPLC-MS). Uniformity of mass features distribution across the retention time axis and number of differentially expressed mass features between anaerobic and aerobic stages demonstrated that optimal extraction was biomass specific. For the Ca. Accumulibacter phosphatis enriched culture, a methanol-water (1:1 v/v) mix was the best extraction solvent, and a mixture of methanol/chloroform/water (2:2:1 v/v) for the Tetrasphaera spp. enriched culture. Subsequently we have obtained n=19 time points across a P-release/P-uptake cycle experiments with three replications. Principal component analysis demonstrated a defined reproducible trajectory of extracellular metabolites over the EPBR cycle. Using affinity propagation clustering, we identified 124 and 175 clusters of extra- and intra-cellular metabolites, respectively, that demonstrated distinct profiles over time. Our approach provides provide direct surveys of the metabolic state of PAO-enriched EBPR communities, and these data build the foundation for ongoing integrative omics studies of these complex microbial communities.

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ECOLOGY AND ENVIRONMENT

P-139 A cell-culture based lipidomics approach to assess the biological impact of exposure to environmental surface waters

PRESENTING AUTHOR: ***Huajun Zhen, US EPA National Exposure Research Laboratory, United States***

CO-AUTHORS: ***Jonathan Mosley, Drew Ekman, Tim Collette, Daniel Villeneuve, Gerald Ankley, Paul Bradley, Quincy Teng***

Environmental surface waters often contain a variety of chemical contaminants from different sources including wastewater treatment plants, concentrated animal feeding operations, agricultural runoff and other human-related activities. Exposure to these contaminants may pose a threat to the health of humans and wildlife (e.g. fish). We have reported the use of cell-culture based exposures in monitoring the ecotoxicological effects of chemicals in the aquatic environment. Our previous focus has been mainly on changes in polar and semi-polar metabolites (i.e. the metabolome) following exposure. Here, we extend this approach by focusing on changes in endogenous lipophilic compounds (i.e. the lipidome) of cells in response to contaminant exposure. Two human cell lines (HepG2 and LN229) and one fish cell line (ZFL) were exposed to surface water collected from 8 streams nationwide. These sites were selected from a larger study encompassing 38 sites that cover a variety of land-use types and contamination statuses. After a 48-hr exposure, the lipidome of control and exposed cells was profiled with Ultra-High Performance Liquid Chromatography coupled to a Q-Exactive Orbitrap Mass Spectrometer (Thermo Scientific). We analyzed the perturbed pathways of lipid metabolism and identified potential biomarkers following exposure to environmental contaminants. In addition, we used partial least-squares regression models to explore covariances among measured chemicals in the surface waters and the lipidomics dataset, providing insights into ecological impacts and thus determining potential relevance of environmental contaminants.

P-140 Use of Bronchoalveolar Lavage Fluid Lipidome to Characterize Naphthalene Toxicity in Mice

PRESENTING AUTHOR: ***Shang-Ting, Lin, Institute of Environmental Health, College of Public Health, National Taiwan University, Taiwan***

CO-AUTHORS: ***Shang-Ting Lin, Ping-Chun Hsieh, Hao-Jan Liang, Sheng-Han Lee, Ching-Yu Lin***

Naphthalene, listed by the US Environmental Protection Agency as suspected or highly potent carcinogens, widely exists in the environment. Previous studies demonstrated that naphthalene induced acute respiratory toxicity in mice. Acute exposure to naphthalene caused swelling and vacuolation of airway epithelial cells-Clara cells and changed cell membrane integrity with dose-response relationship. Studies on changes of lipids in the respiratory system may help us understand naphthalene induced airway injury. Bronchoalveolar lavage fluid (BALF) molecular constituents provide valuable information when studying toxicology of the lungs. Few studies examined the lipid components of BALF due to technical limitation. In this study, we aim to examine the respiratory effects of naphthalene by measuring BALF lipids. 7-week male ICR mice were treated with 100 or 200 mg/kg naphthalene or the control by intraperitoneal injection. After 24 hours, the mice were scarified, and BALF was collected and extracted for further lipid analysis. Phosphorylcholine-containing lipids including phosphatidylcholine and sphingomyelin were analyzed by ultra-performance liquid chromatography tandem mass spectrometer (MS) followed by partial least squares discriminant analysis (PLS-DA). The PLS-DA result demonstrated that lipidome of low dose, high dose and control group were clear separated. The effects of low and high doses are different, which suggested different mechanisms were involved. The critical lipids were identified and linked with possible bio-functions. In conclusion, MS-based lipidomics provide a powerful platform to reveal BALF lipid perturbation caused by naphthalene in order to understand the mechanisms and develop biomarkers.

P-141 Sex-base differences in metabolic and immunological responses of mussel haemocytes during Vibrio sp. infection

PRESENTING AUTHOR: ***Ming Li, Auckland University of Technology, New Zealand***

CO-AUTHORS: ***Thao V. Nguyena, Andrea C. Alfaroa, Fabrice Merienb, Tim Younga, Roffi Grandiosaa, Ming Li***

Massive mortalities due to pathogens are routinely reported in bivalve cultivation practices, with significant economic consequences for the global aquaculture industry. However, host-pathogen interactions and infection mechanisms that mediate these interactions are poorly understood. In addition, gender-specific immunological responses have been reported for some species, but the reasons for such differences have not yet to be elucidated. In this study, we used a GC/MS-based metabolomics platform and flow cytometry approach to characterize metabolic and immunological responses in haemolymph of male and female mussels (Perna canaliculus) experimentally infected with Vibrio sp. Sex-based differences in immunological responses were identified, with male mussels displaying higher mortality, oxidative stress and apoptosis after pathogen exposure. However, central metabolic processes appeared to be similar between sexes at 24 h post injection with Vibrio sp. DO1. Significant alterations in relative levels of 43 metabolites were detected between infected and non-infected mussels. These metabolites are involved in major perturbations on the host's innate immune system. In addition, there were alternations of seven metabolites in profiles of mussels sampled on the second day and mussels that survived seven days after exposure. These metabolites include itaconic acid, isoleucine, phenylalanine, creatinine, malonic acid, glutaric acid and trans-4-hydroxyproline. Among these, itaconic acid could be considered as an important biomarker for Vibrio sp. DO1 infection. These findings provide new insights on the mechanistic relationship between a bivalve host and a pathogenic bacterium, and highlight the need to consider sex as a biological variable in future immunological studies.

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ECOLOGY AND ENVIRONMENT

P-142 Utilising metabolomics to discover an adverse outcome pathway in a multi-trophic Daphnia-algae system

PRESENTING AUTHOR: ***Jelena Sostare, University of Birmingham, United Kingdom***

CO-AUTHORS: ***Martin Jones, Nadine Taylor, Jinkang Zhang, Ulf Sommer, Mark Viant***

The adverse outcome pathway (AOP) framework structures knowledge of how chemicals perturb biological systems, and is gaining popularity in chemical risk assessment. While the number of draft and OECD-endorsed AOPs is growing steadily, almost all have focused on single organism toxicology. Ecotoxicology is far more complex, with ecosystem heath dependent on several factors including the interactions between species. A challenge, therefore, is to evaluate and ensure that AOPs capture and manage toxicity data derived from pollutant-impacted multi-species ecosystems. Recently, using metabolomics, we discovered that zinc oxide nanoparticles (ZnO NPs) decrease sulfonated lipids in Daphnia magna, which are chemical signalling molecules (kairomones) excreted by these freshwater crustaceans that induce their algal prey to form inducible defences (spines and multicellular structures to avoid predation). Here we sought to discover a chain of 'key events' that leads to this perturbation using targeted metabolite analysis of sulfonated lipids and related sulfur biochemical pathways, and transcriptomics (RNAseq). The results showed that in Daphnia blind guts (their digestive gland) sulfonated lipids occurred at high concentration and decreased significantly upon ZnO NP exposure, suggesting this tissue may serve as a kairomone production site and target for NPs. Furthermore, ZnO NPs altered glutathione levels and globally suppressed sulfur metabolism, most notably PAPS (3'-phosphoadenosine-5'-phosphosulfate), which is required for sulfonated lipid production. This study partially reveals the mechanistic basis for the NP-induced perturbation to Daphnia-algae signalling, demonstrating the utility of metabolomics for discovering toxicity mechanisms and facilitating the development of a multi-trophic AOP.

P-143 Environmental exposure induced metabolic profiling alterations in a migration panel between Los Angeles and Beijing

PRESENTING AUTHOR: ***Xinchen Lu, Peking University, China***

CO-AUTHORS: ***Xinghua Qiu, Yan Lin, Yifang Zhu***

Migration provides individuals experiencing different environmental exposure episodes naturally, which offers a chance to attribute physiological response to certain exposure factors. To reveal the mechanism of health effect under environmental exposure, we designed a migration panel study between Los Angeles and Beijing, two cities with different exposure levels. Specifically, serum samples were collected from 27 summer school exchange students three times in total, one week before their departure (LA1), and at least four weeks respectively after their arrival to Beijing (BJ) and return to Los Angeles (LA2). The metabolic profiling was obtained through untargeted metabolomics approach with a high resolution TOF-MS. Combining with a series of statistical methods like Mann-Whitney test and Orthogonal partial least squares discriminant analysis, we screened prospective markers that changed significantly between LA and BJ. The significant decrease ($p<0.001$, $FC>1.2$) of indole and its precursor tryptophan in BJ reflected the induction of AhR and CYP by higher levels of pollutants. The decreased cortisol ($p<0.001$, $FC>1.5$) was potentially due to the accelerated consumption of CYP3A, supplying further evidence of enhanced CYP activity and related xenobiotics elimination in BJ. On the other hand, the increase of IL-8 and IL-10 from cytokine analysis suggested the oxidative stress during the process. Higher levels of uric acid ($p<0.01$, $FC>1.1$) along with its precursors implied the upregulation of antioxidation under the environmental exposure in BJ. Generally, this study illustrated that metabolomics offered an effective tool to assess the overall health impact under subchronic exposure.

P-144 Metabolmics responses of Western clawed frogs (Silurana tropicalis) and Wavy-rayed lampmussels (Lampsilis fasciola) exposed to naphthalene sulfonic acids

PRESENTING AUTHOR: ***Vimal K. Balakrishnan, Environment and Climate Change Canada, Canada***

CO-AUTHORS: ***Ryan Prosser, Patricia L. Gillis, Sarah J. Wallace, John Toito, Quintin Rochfort, Valerie S. Langlois***

Under the Chemicals Management Plan (CMP), the Government of Canada is analyzing the risks presented by approximately 20,000 priority chemicals to the Canadian environment. Naphthalene sulfonic acids (NSA) are a set of CMP priority compounds that are used in surfactants for fuel rod cell production, jet fuel, oil lubricants, laundry detergents, and emulsifiers, but little is known about their chronic toxicity to aquatic species. In this study, Western clawed frogs (Silurana tropicalis, a model species) and the freshwater mussel (Lampsilis fasciola, an at-risk species) were exposed to sub-lethal concentrations of calcium dinonylnaphthalene sulfonate (CaDNS). Mussel hemolymph was non-destructively sampled prior to extraction with a cold solvent mixture, while S. tropicalis embryos were flash frozen and homogenized prior to extraction. These extracts were then assessed using both targeted and non-targeted metabolomics approaches; extracts were analyzed by LC-MS-MS and LC-QToF-MS, and the resulting data treated using Agilent Mass Profiler Professional software (including pathways analysis). In targeted metabolomics, amino acids such as histidine, leucine, phenylalanine, and kynurenine were assessed, as were compounds in the Tricarboxylic acid (TCA) cycle (e.g., lactic acid, pyruvic acid, etc.). Data will be compared to parallel microarray studies that were conducted on the same samples to gain insights into Adverse Outcome Pathways (AOPs).

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ECOLOGY AND ENVIRONMENT

P-145 Stress-induced release of glutathione compounds creates an eco-evolutionary opportunity for restoring TORC1 regulation

PRESENTING AUTHOR: ***Wenying Shou, Fred Hutch Cancer Research Center, United States***

CO-AUTHORS: ***Robin Green, Wenyun Lu, Lin Wang, Josh Rabinowitz***

A central player in coupling nutrient availability to growth control is TORC1, and its misregulation is implicated in human diseases such as cancer and obesity. To understand how cells adapt to TORC1 misregulation, we evolved a lysine auxotroph (lys-) of S. cerevisiae under lysine limitation where mis-regulated TORC1 is known to contribute to poor survival. As expected, increased affinity for lysine evolved across all populations. Surprisingly, even though the growth medium contained no sulfur-containing organic compounds (organosulfurs), we repeatedly observed a sub-population evolving organosulfur auxotrophy (orgS-). Metabolite analysis revealed that upon lysine limitation, live orgS+ rapidly increased the release rate of glutathione-based compounds, which supported the growth of orgS-. Limited but not abundant glutathione conferred orgS- with a frequency-dependent fitness advantage over orgS+ via autophagy. Thus, growth misregulation can initiate novel ecological interactions, which can subsequently restore regulation in a subpopulation via the evolution of cross-feeding.

P-146 Gut Check On Air Pollution: Effects Of Biodiesel Ultrafine Particles On Human Gut Microbiota

PRESENTING AUTHOR: ***Jiangjiang (Chris) Zhu, Miami University, United States***

CO-AUTHORS: ***Kundi Yang, Flora Xu, Monica Rahman, Jingyi Cao***

Emerging evidence has highlighted the need for scientists, physicians, and policy-makers to understand the adverse health effect of air pollution substances, such as ultrafine to overall human health, through their complicated modulation of human gut microbiota. Therefore, this study will focus on deciphering the interactive modulating effects of gastrointestinal (GI) UFPs exposure to gut microbial composition and functions, then eventually to enable systematic evaluation of the impact of UFPs to host health. Due to the increasing importance of biodiesel to our society, and the vastly lacking information to their health effect, the UFPs generated from combustion of both petrodiesel (B0) and petrodiesel/biodiesel blend (80:20 v/v, B20) in a representative light-duty diesel engine were used in this study. An in vivo murine model with both male and female groups was applied for investigating gut microbial population and diversity changes during frequent UFP exposure. Multi-omics approaches, including targeted and untargeted metabolomics, and microbiome analysis were applied for evaluating the UFP-induced gut microbial population and functional changes. We discovered that the concentration and relative abundance of bacterial metabolites from the host gut were depended on the percentage biodiesel in the fuel blends (B0 vs. B20). Correlation analysis was conducted to obtain the relationship between UFPs composition and gut microbial metabolic profiles. Bacterial cellular oxidative stress and their metabolic signatures, such as the decreased concentration of nucleotides and lipids and increased concentrations of carbohydrate, energy and vitamin metabolites are examined via metabolomics approaches. Furthermore, the altered metabolites are correlated to microbial composition changes.

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ECOLOGY AND ENVIRONMENT

P-149 Environmental Cadmium Exposure Induces Urinary Metabolite Profile Alterations in General Pregnant Women

PRESENTING AUTHOR: ***Han Li, Huazhong University of Science and Technology, China***

CO-AUTHORS: ***Yi Tang, Yang Peng***

Cadmium (Cd) is a well-recognized hazardous toxic heavy metal. It is already known that high-level Cd exposure has adverse effects on human health. However, little is known about the health effects of low-level environmental Cd exposure on pregnant women. The aim of this study is to assess the urinary metabolic changes in pregnant women with low-dose environmental Cd exposure, and to identify effective biomarkers. Urine samples from 246 pregnant women were collected in the first trimester of pregnancy, and the urinary Cd concentrations were quantified using inductively coupled plasma mass spectrometry (ICP-MS). The urinary metabolomics was analyzed by ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS/MS). Cd-related metabolic biomarkers were investigated by comparing the samples of the first and third tertiles of Cd exposure classifications using a partial least-squares discriminant (PLS-DA) model. Five potential biomarkers were identified, including L-cystine, L-tyrosine, dityrosine, histamine and uric acid, and these biomarkers were related to oxidative stress effect and nephrotoxic effect induced by Cd exposure. The results showed that low-level environmental Cd exposure could cause metabolite profile alterations in pregnant women which might be associated with adverse health outcomes. Our findings provide new insight into the early molecular events of Cd exposure.

P-150 Metabolite pools in the marine microbiome across a basin-wide oceanographic gradient in the North Pacific

PRESENTING AUTHOR: ***Katherine R Heal, University of Washington, United States***

CO-AUTHORS: ***Katherine R. Heal, Laura T. Carlson, Angela K. Boysen, Bryndan P. Durham, E. Virginia Armbrust, Anitra E. Ingalls***

Metabolites of the marine microbiome are responsible for fueling microbial metabolism and maintain community interactions. Yet, there are few holistic measurements of these metabolite pools that capture the full suite of compounds or allow the discovery of unknown metabolites. Untargeted metabolomics provides a relatively unbiased lens through which we can begin to assess the standing stocks of small compounds that likely fuel heterotrophic activity in the surface ocean. Here we present targeted and untargeted metabolomes of particulate material (the microbiome) in the North Pacific Ocean and explore their latitudinal distributions. This analysis reveals several nitrogen-containing compounds that have not been previously recognized as abundant components of the marine organic carbon pool. We were surprised to find that the nitrogen-rich compatible solute glycine betaine was present in high concentrations across the entire sampling transect despite changing community composition and nitrogen availability. Some individual compounds showed distinct patterns between oceanographic regimes, including homarine, an abundant molecule that contributed up to 0.8% of the total particulate carbon pool but whose role in the microbial loop is almost completely unknown. Glutamic acid and glutamine showed opposite patterns in the oceanographic regimes, suggesting differences in community-level nitrogen assimilation. This study offers a new window into particulate carbon composition in oceanographic research, reveals important unappreciated metabolites that may fuel the microbial loop, and suggests an altered community-level nitrogen assimilation capacity over the North Pacific transition zone.

P-151 Metabolomic Response of Fluorescent Protein and Their Cell Signalling Capacity to Environmental Conditions

PRESENTING AUTHOR: ***Elif Tufce Aksun Tumerkan, University of Exeter, College of Life and Environmental Sciences, Penryn Campus, United Kingdom***

Fluorescent proteins (FPs) are unique in that they are self-sufficient in forming chromophores with a visible wavelength from 3 amino acids sequence within their own polypeptide structure. Currently, more than 150 different FP-like proteins have been found in marine organisms. With rising interest in cell biology, FPs have used as biosensor indicators and probes in pharmacology and cell biology. Using fluorescent proteins in genetically encoded metabolite sensors has many advantages than chemical probes for metabolites such as easily introduced into any cell or organism in any sub-cellular localization and giving chance to fixing to fluoresce of different colours or characteristics. These favourable sensors have some visualization problem cellular signal. The intracellular mechanism such as proteins, lipids, and nucleic acids or some diagnostic or prognostic sub-cellular structures can cause the fluctuation of cell signalling differences.Since fluorescent proteins obtained from nature, the species that use for purification of FPS has also impressed the signalling mechanism. Their own ecological properties such as symbiotic relationship and living conditions have the impact on FPs efficiency. It is known that the dynamicity of detector that used for reading fluorescence and the level of background fluorescence are key parameters for the quality of the fluorescent signal.In this study, it was aimed to clarify the metabolomic response of fluorescent protein signalling capacity to different environmental conditions such as pH, temperature and target cell property. Also, better understanding of reasons that cause to signal fluctuations offers the potential for tailoring FPs selection and application to specific uses.

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ECOLOGY AND ENVIRONMENT

P-152 Effects of atmospheric CO2 level on the metabolic response of resistant and susceptible wheat to Fusarium graminearum infection

PRESENTING AUTHOR: ***Miroslava Cuperlovic-Culf, National Research Council of Canada, Canada***

CO-AUTHORS: ***Miroslava Cuperlovic-Culf, Martha M. Vaughan, Karl Vermillion, Anu Surendra, Jennifer Teresi, Susan McCormick***

Diseases of agricultural crops caused by fungi have devastating economic and health effects. Fusarium head blight (FHB) is one of the most damaging diseases of wheat and other small grain cereals. FHB contamination of wheat has been steadily increasing over the last decade leading to an increase in risk of mycotoxin contamination in food and feed. Rising atmospheric CO2 concentration and associated climate changes are thought to have contributed to this increase in FHB. However, our understanding of the mechanisms behind the influence of CO2 levels on the wheat's defense response against Fusarium graminearum infection, and the spread of FHB remains limited. In this study, the defense response of wheat plants grown at ambient (400 ppm) and elevated (800 ppm) CO2 was evaluated and compared. Plant and fungal metabolites play a major role in defense and virulence with significant differences in metabolic response in resistant and susceptible plants. NMR spectroscopy performed in this work have provided detailed metabolite information leading to metabolic markers of susceptibility and resistance in wheat at different CO2 levels. Fusarium infection-induced metabolic changes under different conditions are discussed in the context of metabolic network and resistance. This work shows that the effect of CO2 level increase is different in the susceptible and resistant wheat making the resistant wheat more susceptible to particular fungal strain and also leading to increase in DON production through change in the several known resistance related metabolic pathways that will be discussed in the presentation.

P-153 Metabolic and Physiological Responses of Halanaerobium Growth and Persistence in Hydraulically-Fractured Shale Ecosystems

PRESENTING AUTHOR: ***David W. Hoyt, EMSL - Pacific Northwest National Laboratory, United States***

CO-AUTHORS: ***Anne E. Booker, Tea Meulia, Elizabeth K. Eder, Mary S. Lipton, David W. Hoyt, Michael J. Wilkins***

Bacterial Halanaerobium strains become the dominant microbial community member in produced fluids across geographically distinct hydraulically fractured (HF) shales. Halanaerobium is not native to the subsurface, but is inadvertantly introduced during the drilling and fracturing process. The accumulation of biomass in pipelines and shale formations is detrimental due to possible corrosion and bio-clogging that could negatively impact oil and gas recovery. Here, we used Halanaerobium congolense strain WG8 isolated from Utica Shale to identify metabolic and physioloigical responses to growth under high-pressure subsurface conditions. Laboratory incubations confirmed the capability of strain WG8 to grow under pressures representative of the subsurface (21-48 MPa). Shotgun proteomic measurements identified higher abundances of proteins associated with the production of extracellular polymeric substances (EPS), and utilization of 1,2 propanediol when strain WG8 was grown under pressure, where hydrogenase proteins were less abundant. Confocal laser scanning microscopy and scanning electron microscopy indicated that EPS production was associated with greater cell aggregation and attachment to shale surfaces under high pressure conditions. NMR measurements of fermentation products revealed changes in strain WG8 central carbon metabolism under high pressure growth. Twice as much ethanol, acetate and propanol were generated per cell under high pressure conditions, while hydrogen production almost completely ceased. These metabolic shifts were associated with carbon flux through 1,2 propanediol in response to slower fluxes of carbon through stage 3 of glycolysis. Overall, these results revealed the potential for bio-clogging and corrosion (via organic acid fermentation products) associated with persistent Halanaerobium growth in deep, hydraulically-fractured shale ecosystems.

P-154 A 2-year NMR-based metabolic profiling study of Chinese cabbage (Brassica rapa subsp. pekinensis) grown under a long-term organic farming system

PRESENTING AUTHOR: ***Yasuyo Sekiyama, Food Research Institute, NARO, Japan***

CO-AUTHORS: ***Yasuyo Sekiyama, Seishi Ikeda, Masahiro Mitsuboshi, Jun Kikuchi, Yuuzou Kioka, Katsunori Noguchi***

The long-term application of organic fertilizers may improve soil quality and enhance nutrient cycling, thereby contributing to future agricultural production. An examination of the influence of long-term organic management on soil microbial communities, the diversity of plant microbial symbionts, and the plant metabolome could increase our understanding of plant health and sustainable agroecosystems. Here, we report a 2-year nuclear magnetic resonance (NMR)-based metabolic profiling study of Chinese cabbage (Brassica rapa subsp. pekinensis) grown under an organic farming system established in 1987 (Ibaraki, Japan). A replicated three-block experimental design was used for each treatment: inorganic fertilizer (control; consisting of ammonium sulfate, calcium superphosphate, and potassium chloride), rapeseed meal, steamed bone meal, and fish meal. Chinese cabbage (cultivar Kigokoro 85, Takii & Co., Japan) was grown in 2015 and 2016 after crop rotation with soybean. The tightly formed heads were sampled and separated into three parts: leaf blades, petioles of fully developed leaves, and the central part of the head. The 1H-NMR spectra of metabolites soluble in a D2O-based buffer were measured and subjected to multivariate or univariate analysis. The difference in the metabolic profile was greater between organs and years than fertilizer applications. The sugar content was higher in the fully developed leaf blades and petioles in 2016, but higher in the core of the head in 2015. Effects of the application of rapeseed meal and steamed bone meal were observed in both 2015 and 2016. Details of the metabolic changes will be presented.

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ECOLOGY AND ENVIRONMENT

P-155 Workflow for the targeted and untargeted detection of small metabolites in fish skin mucus

PRESENTING AUTHOR: ***Silvio Uhlig, Chemistry Section, Norwegian Veterinary Institute, Norway***

CO-AUTHORS: ***Haitham Tartor, Søren Grove, Anja B. Kristoffersen, Lada Ivanova***

The skin mucus of fish is in permanent contact with the aquatic environment. Data from the analysis of the chemical composition of skin mucus could potentially be used for monitoring the health status of the fish. Knowledge about mucus composition or change in composition over time could also contribute to understanding the aetiology of certain diseases. The objective of the present study was the development of a workflow for non-invasive sampling of skin mucus from farmed salmon (Salmosalar) for the targeted and untargeted detection of small metabolites. Skin mucus was either scraped off, wiped off using medical wipes, or the mucus' water phase was absorbed using the same type of medical wipes that was used for the wiping method. Following a simple filtration step, the obtained mucus samples were subjected to hydrophilic interaction chromatography coupled to high-resolution mass spectrometry. Post-acquisition processing included the targeted analysis of 86 small metabolites, of which up to 60 were detected in absorbed mucus. Untargeted analysis of the mucus samples from equally treated salmon revealed that the total variation of the metabolome was lowest in absorbed mucus and highest in the scraped mucus. Thus, future studies including small-molecule metabolomics of skin mucus in fish would benefit from a sampling regime employing absorption of the water phase in order to minimize the bias related to the sampling step. Furthermore, the absorption method is also a less invasive approach allowing for repetitive sampling within short time intervals.

P-156 The influence of drought on the soil microbiome: an ecosystem metabolomics perspective

PRESENTING AUTHOR: ***Claudia M Boot, Colorado State University, United States***

CO-AUTHORS: ***Sarah Evans***

Drought has a profound impact on microorganisms that inhabit soil ecosystems. As water disappears from the extracellular matrix, water potential stress accumulates for anything living in soils. Microorganisms tolerate drought stress either compensating for shifts in osmotic potential by accumulating compatible solutes or combatting matric and/or osmotic stress by going dormant. There are known molecular signatures for each of these strategies in the small molecule profiles of cultured microorganisms, yet detection of these strategies and concrete links to microbial responses in ecosystem-level studies remain elusive. We used an ecosystem metabolomics approach to study the molecular signatures of microbial drought stress in a Kansas grassland. Following four weeks of drought treatment, chlorofrom fumigtated soils were extracted with water, concentrated, and extracts were profiled using HILIC-UPLC-MS (Waters Xevo G2 Q-TOF). Data were analyzed using XCMS for feature detection and alignment, RAMclustR for spectral deconvolution and ion clustering, and MS Finder for molecular formula generation and annotation. We found that a small subset of the total molecular profile was responsive to drought and annotation and characterization of responsive metabolites is ongoing. Responsive molecules were evenly distributed in terms of normalized oxidation state and elemental ratios, indicating drought tolerance mechanisms are not chemically conserved with representatives across compounds classes. The specifics of microbial drought tolerance mechanisms may have far reaching implications for ecosystem-level functions such as global biogeochemical cycling, as well as efforts to improve agricultural production on marginal or arid lands, as microorganism are key players both cases.

MODEL ORGANISMS

P-157 Parallel metabolomics and lipidomics in Caenorhabditis elegans

PRESENTING AUTHOR: ***Michel van Weeghel, Laboratory Genetic Metabolic Diseases, Academic Medical Center, Amsterdam, Netherlands***

CO-AUTHORS: ***Reuben L. Smith, Marte Molenaars, Arwen W. Gao, Mia L. Pras-Raves, Angela C.M. Luyf, Antoine H.C. van Kampen, Georges E. Janssens, Frédéric M. Vaz, Riekelt H. Houtkooper***

The round worm Caenorhabditis elegans is frequently used to investigate metabolism and has been instrumental in the elucidation of key regulators of metabolism-related traits such as ageing. Most methods for measuring metabolites in C. elegans are based on gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy. These methods have drawbacks, including the large number of worms needed and the limited number of metabolites that can be measured accurately. Recently, we made significant advances in quantifying over 600 metabolites from three major metabolite classes, namely fatty acids, amino acids and phospholipids, in a sample of approximately 500-2000 worms. The current study improves on these findings and provides a detailed step-by-step protocol for the detection and quantification of >100 "polar" and >1000 "non-polar" metabolites from a single sample containing 500-1500 worms. Our method allows accurate and reproducible detection of both metabolites and lipids in a single sample using a combined metabolome/lipidome extraction protocol, which covers >90% compared to dedicated traditional extraction methods. We developed a bioinformatics pipeline for the annotation, processing, and semi-quantitative analysis of the metabolome/lipidome data, making it suitable for semi-high-throughput screening. Finally, we used the pipeline to define the metabolome/lipidome in C. elegans treated with RNAi against enzymes in key metabolic pathways including the TCA cycle, glycolysis, pentose phosphate pathway, nucleotide metabolism, and fatty acid synthesis. As such, our current method provides ample opportunities that help to define how metabolism is involved in (patho)physiological processes, including aging.

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MODEL ORGANISMS

P-158 Metabolomic profiling with 1H NMR spectroscopy of sterile male of Mediterranean fruit fly Ceratitis capitata

PRESENTING AUTHOR: ***Andre Padilla, CBS, France***

CO-AUTHORS: ***Fadhel S., M'saad Guerfali M., Roumestand C., Marzouki W., Hamden H., Saidi M.***

The sterile insect technique (SIT) is among the most environmentally conservative insect pest control method ever developed for the suppression or eradication of a number of insect pests such as the Mediterranean fruit fly Ceratitis capitata. Radiation-induced mutations have deleterious effects for reproductive male cells, while males remain sexually competent once released in the field. Characterization of the metabolic shifts associated with gamma irradiation exposure in sterile medfly males would be helpful identifying the perturbated metabolites that have a primordial role in the mating process. In this work, a metabolomic study was performed to characterize the global metabolic changes induced by gamma irradiation of medfly sterile males treated at 0, 70, 90, 110 and 145 Gy. We utilized an NMR-based metabolomic approach in combination with multivariate analysis (PCA) to profile the metabolites. The integral data was found to cluster into four different groups representing untreated males (0 Gy) as the first group, the second group includes males treated at 70 and 90 Gy, the third group included males treated at 110 Gy and the last represents males treated at 145 Gy. The results show the PCA score plots for all treated males taken together with PC1 explaining 35.7% of the variation, PC2 explaining 15.9% of the variation and PC3 explaining 11.6% of the variation between groups. Marked disturbances in the metabolites of somatic cells can lead to biological malfunctions by diminishing the competitiveness and mating behavior of the irradiated males, decreasing ipso facto the effectiveness of the SIT program.

P-159 The role of liver metabolism in determining plasma metabolite changes during short-term fasting in the laboratory rat

PRESENTING AUTHOR: ***Kalyan C. Vinnakota, Dept. of Defense Biotechnology High Performance Computing Software Applications Institute, Telemedicine and Advanced Technology Research Center, United States***

CO-AUTHORS: ***Venkat R. Pannala, Martha L. Wall, Shanea K. Estes, Irina Trenary, Tracy P. O'Brien, Richard L. Printz, Jaques Reifman, Masakazu Shiota, Jamey D. Young, Anders Wallqvist***

The liver—a central metabolic organ that integrates whole-body metabolism to maintain glucose/fatty-acid regulation and detoxify ammonia—is susceptible to injuries induced by drugs and toxic substances. Increasingly, plasma metabolite profiles are being investigated for their potential to detect liver injury earlier than current clinical markers. Because plasma metabolite profiles are affected by the nutritional state and the physiological state of the animal,we sought to predict plasma metabolite changes originating in the liver during short-term fasting. We used a constraint-based metabolic modeling approach to integrate central carbon flux measurements and physiological flux boundary conditions into a genome-scalemodel of rat liver metabolism. We then measured plasma metabolite profiles in rats fasted for 5-7 or 10-13 h to test our model predictions. Our model correctly predicted two-thirds of the observed directions of change (an increase or decrease) in plasma metabolites, which is better than an expected correct prediction of one third of the observed directions of change by random chance. These findings suggest that changes that originate in other organs contribute at least in part to the changes unaccounted for by the model. Our approach provides a mechanistic model for identifying the liver-specific sources of plasma metabolite changes that occur during physiological perturbations.

P-160 Metabolic Alterations in Retina and Retinal Pigment Epithelium during Aging

PRESENTING AUTHOR: ***Kristine Tsantilas, Department of Biochemistry, University of Washington, United States***

CO-AUTHORS: ***Jonathon D. Linton, Martin Sadilek, Matthew Campbell, Mariya Sweetwyne, Jeremy Whitson, Peter Rabinovitch, David Marcinek, Connor S.R. Jankowski, James B. Hurley***

The underlying molecular mechanisms behind age-related vision loss are not fully understood and the role of metabolism is particularly under-studied. We are analyzing the effects of aging on metabolism in two ocular tissues: retina and retinal pigment epithelium (RPE). Metabolic features of the retina and RPE are specialized and interdependent. The glycolytic retina produces lactate, which can be taken up by the RPE and used as fuel by RPE mitochondria. We sought to determine how the baseline metabolism and interactions of retina and RPE change with aging by analyzing metabolic flux in mouse retinas and mouse RPE-choroid complexes (eyecups). Mouse retinas and eyecups were incubated in U-13C-glucose. The tissues and incubation media were collected for quantification of 13C-labeled metabolites by selected ion monitoring with gas chromatography coupled to mass spectrometry. The metabolite panel included amino acids, glycolytic and TCA cycle intermediates. Several metabolites exhibited changes in retinas, eyecups and exported metabolites of young (4 month-old) and old (28 month-old) mice. The ratios of M3 lactate to M2 citrate (M3Lac/M2Cit) were used as a measure of flux through glycolysis and the TCA cycle and succinctly encapsulate our overall findings. M3Lac/M2Cit in retinas decreases with age whereas in eyecups it increased with age. This showed that old retinas are less glycolytic than young retinas and old eyecups are more glycolytic than young eyecups – suggesting an age-induced metabolic shift. We will utilize shorter and longer durations of isotopically enriched metabolite incubation to fully characterize the metabolic changes in these tissues.

POSTER SESSIONS 1 AND 2 – Monday and Tuesday – all odd number presenters will be at their posters.
POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

MODEL ORGANISMS

P-161 Metabolomic Profiling of Pancreatic Cancer Cell Line Lysates and Conditioned reveals a dynamic process of metabolite production and release

PRESENTING AUTHOR: ***Eunice Murage, The University of Texas at MD Anderson Cancer Center, United States***

CO-AUTHORS: ***Johannes F Fahrmann, Jennifer Dennison, Michella Capelo, Corin Emery, Peng Wang, Chuan Yih Yu, Samir Hanash***

Background Metabolic signatures have utility for monitoring disease development, progression and regression. We have assessed the dynamics of metabolite production and release into the extra-cellular space in pancreatic adenocarcinoma (PDAC) through in depth metabolic profiling of cell line lysates and conditioned media with a particular focus on polyamine metabolism. Experimental Design A multi-assay, untargeted metabolomic approach was utilized to evaluate a broad spectrum of metabolites in total cell lysates and longitudinally collected conditioned media (baseline, 1, 2, 4, and 6 hours of conditioning) for 11 pancreatic cancer cell lines. Intracellular metabolite levels were correlated with rates of accumulation in conditioned media, the exometabolome. Results Approximately 650 structurally annotated metabolite ions were identified in lysates; whereas 350 annotated metabolites ions were identified in conditioned media. Approximately 140 of these were found to be overlapping between lysates and conditioned media. Eight of eleven polyamines metabolites were positively identified. In particular, two polyamines detected in lysates of all pancreatic cancer cell lines, exhibited positive rates of accumulation (area units/hour/100µg protein) in conditioned media. Intrestingly, the two polyamines were inversely correlated to each other highlighting an intrinsic heterogeneity in polyamine metabolism in PDAC. Transcriptomic data analysis (Badea dataset) revealed that mRNA expression of enzymes responsible for the synthesis of these polyamines were significantly ($P<0.05$, paired T-test) elevated in PDAC as compared to adjacent control tissue. Conclusion Using a systems approach, we have identified two polyamines one of which was significantly elevated in PDAC cell lines that may provide a means to monitor the disease.

P-162 Unraveling the Antimicrobial Mechanism of Action of Gallic Acid

PRESENTING AUTHOR: ***Ethan Lowry, University of Auckland, New Zealand***

CO-AUTHORS: ***Silas Villas-Boas, Simon Swift***

Gallic acid (GA) is a weak phenolic acid found abundantly in plants all over the world with known roles in UV protection and antimicrobial pathways. While GA's physicochemical properties have been well established in literature, its bioactivity has not been well defined. Its abundance and low cost makes it an attractive additive for antimicrobial applications, but a more thorough understanding of how it works--both on its own and in conjunction with other compounds--would aid in finding an application.This study aims to use metabolomics to further develop our hypothesis regarding GA'santimicrobial mechanism of action using Escherichia coli ATCC 25922as a model organism. Briefly, E. coli was grown (in M9 minimal medium with 4 g/L glucose supplement and 1 g/L casamino acid supplement) to a steady state growth phase in a Labfors Infors 4 biofermentor. Intracellular and extracellular samples were harvested under three conditions: pre-challenge steady state, immediately after challenge (transition state), and post-challenge steady state. Metabolites in each phase were analysed using GC-MS, and relative abundance and pathway analyses were used to further elucidate the mechanism of action.Future studies will compare metabolite regulation of this challenge with control challenges using antimicrobials with mechanisms of action related to our hypothesis.

P-163 Metabolomic characterization of Wfs1-deficient mouse

PRESENTING AUTHOR: ***Rando Porosk, University of Tartu, Estonia***

CO-AUTHORS: ***Anton Terasmaa, Riina Mahlapuu, Aigar Ottas, Ursel Soomets, Kalle Kilk***

Wolfram syndrome 1 (DIDMOAD) is a rare autosomal recessive disease characterized by diabetes insipidus, diabetes mellitus, optic atrophy, deafness and neurodegeneration. Mutations in the WFS1 gene can lead to endoplasmic reticulum stress and unfolded protein responses in cells, but the pathophysiology at whole organism level is poorly understood. In this study, heart, liver, kidneys, and pancreas and bodily fluids, blood and urine, of 2- and 6-month old Wfs1 knockout (KO), heterozygote (HZ), and wild-type (WT) mice were analyzed by untargeted and targeted metabolomics using liquid chromatography–mass spectrometry. We found significant perturbations in the metabolism of pancreatic and heart tissue before the onset of clinical signs such as glycosuria that precedes hyperglycemia and thus implies a kidney dysfunction before the onset of classical diabetic nephropathy. The glucose use and gluconeogenesis in the KO mice are intensified in early stages, but later the energetic needs are mainly covered by lipolysis. Furthermore, in the liver and blood of younger mice, we detected hypouricemia, which in time turns to hyperuricemia. In summary, we show that the metabolism in Wfs1-deficient mice markedly differs from the metabolism of WT mice in many aspects.

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MODEL ORGANISMS

P-164 Metabolomics analysis suggests a predominant role of diet on cardiac metabolism in PFK-2 over-expression mouse model

PRESENTING AUTHOR: ***Albert Batushansky, Oklahoma Medical Research Foundation, United States***

CO-AUTHORS: ***Satoshi Matsuzaki, Maria Newhardt, Melinda West, Timothy Griffin, Kenneth Humphries***

The healthy heart can use different nutrients to meet energetic demands. However, with diabetes the heart increases reliance on fatty acid at the expense of glucose. This phenomenon - metabolic inflexibility - is an important contributor to heart pathologies in diabetic patients, and it's important to be studied. The irreversible conversion of fructose-6-phosphate to fructose-1,6-biphosphate is a key regulatory step of glycolysis, and it's allosterically activated by fructose-2,6-bipshosphate that is produced by phosphsofructikinase-2 (PFK-2). The goal of this study is to determine how PFK-2 activity affect cardiac metabolism. To achieve this, control (C) and transgenic (constitutively active PFK-2 in the heart) mice were fed either a low-fat (LFD) or high-fat (HFD) diet (one week, n=4 per group). Sacrificed animals were used for whole-heart extraction, followed by metabolic profiling using GC-MS. Annotated metabolites (51) were subjected to principal component analysis and displayed broad differences between all groups. This suggests a contribution of both PFK-2 over-expression and diet to these changes. Comparative analysis of metabolites content relative to C/LFD group revealed that the broadest changes occurred in the transgenic/HFD group, including exclusive metabolites that levels decreased significantly (Dunnett's test, $p \leq 0.05$) compared to C/LFD: citrate (1.3-times), malate (1.3-times), fumarate (1.4-times); and metabolites with levels that increased significantly: palmitate (1.3-times), stearate (1.4-times). Correlation-based network analysis was applied to explore a systematic effect of PFK-2 over-expression under the two diets. The results showed remarkable differences between all groups, specifically suggesting a predominant effect of diet on cardiac metabolism.

P-165 A metabolomic survey of marine microorganisms

PRESENTING AUTHOR: ***Angela Boysen, University of Washington, United States***

CO-AUTHORS: ***Katherine R. Heal, Bryndan P. Durham, Laura T. Carlson, E. Virginia Armbrust, Anitra E. Ingalls***

Marine microorganisms drive the global carbon cycle and sustain the marine food web. The flow of energy and matter in marine microbial communities is in part governed by small molecules, or metabolites. In marine systems, metabolites are the basis for microbial interaction and evidence of environmental acclimation. Despite their importance, many quantitatively and functionally significant metabolites have been challenging to identify due to the biological and chemical diversity of seawater communities. Here we use both targeted and untargeted LC-MS metabolomics to characterize the metabolites produced by over 35 representative marine microbes, ranging from heterotrophic bacteria to cyanobacteria to eukaryotic phytoplankton. All organisms were grown axenically and harvested during mid- to late-exponential phase. This dataset sheds light on how phylogenetic and functional diversity manifests in the metabolites produced by various organisms, and allows identification of metabolites that could serve as biomarkers of particular groups of organisms in mixed microbial communities. Additionally, abundant metabolites produced by these representative organisms are likely some of the critical currencies driving microbial processes. Ultimately, this dataset is a powerful database for interpreting metabolomics data from the marine environment.

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POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

MODEL ORGANISMS

P-167 **Targeted Metabolomics analysis using LC-MS for determining metabolic changes in canines in response to two different diets**

PRESENTING AUTHOR: ***Robin Aubrey Torbjörn Moore, University of Helsinki, Finland***

CO-AUTHORS: ***Dr. Anna Hielm-Björkman (PI), Johanna Anturaniemi , Vidya Velagapudi***

Anecdotal evidence suggests that a raw, high-fat, low-carbohydrate diet lowers the overall risk of common diseases in canines. In this experiment, our objective was to provide quantitative evidence for how dietary choice is linked to metabolic changes in canines, specifically differences between raw diets (high-fat, low carbohydrate) and kibble (low-fat, high carbohydrate). To achieve this, a cohort of 22 pet dogs (same breed) were divided into two groups, and were fed a diet of commercially available raw and kibble diets, respectively, over a period of 4-5 months. Blood and urine samples were collected at baseline and at the end of the trial, and 102 key metabolite concentrations were measured using targeted LC-MS. Changes over the trial period between and within the two dietary cohorts were analysed using statistical analysis to determine significant changes between the targeted metabolite concentrations. So far, we have determined that key metabolites such as homocysteine and methionine of the methylation pathway, which have vital roles for detoxification and immune function in both canines and humans, can be found in significantly differing concentrations between the two diet cohorts. Previous studies have indicated that the changes observed in these metabolites may be lead to abnormal liver and kidney function, and may indicate insufficient uptake of B vitamins. In conclusion, certain canine breeds eating a raw, high-fat, low-carbohydrate diet may be less prone to suffer metabolic responses associated with abnormal liver and kidney function and insufficient B-vitamin uptake. However, the underlying causes for this need to be further studied.

P-168 **Metabolic phenotyping of adipogenesis in a human cell strain**

PRESENTING AUTHOR: ***Florian Miehle, Helmholtz Zentrum München, Germany***

CO-AUTHORS: ***Janina Tokarz, Cornelia Prehn, Gabriele Möller, Jerzy Adamski***

Question Obesity and Type 2 Diabetes have increased dramatically during the last decades. Both diseases are mostly characterized by an excess of white adipose tissue (WAT). Adipocytes, the main constituent of this tissue, control energy balance by storing triacylglycerols during periods of energy excess and mobilizing them upon energy deprivation. Much is already known about the involved metabolic and regulatory pathways in adipogenesis, especially from analyses using the murine cell model 3T3-L1. However, in the humans the exact molecular mechanisms are not fully characterized yet. In order to elucidate in the human system the adipogenesis pathways, we analyzed the metabolite levels of differentiating human SGBS cells using a targeted metabolomics approach. Methods A human cell strain derived from subcutaneous WAT of a patient with Simpson-Golabi-Behmel syndrome (SGBS) was differentiated to mature adipocytes. Cells of different stages of adipogenesis were analyzed by targeted metabolomics using the Biocrates AbsoluteIDQ p180 kit quantifying metabolites from five different compound classes (acylcarnitines, amino acids, biogenic amines, hexoses, phospho- and sphingolipids). Results and conclusion Throughout the differentiation process from preadipocytes to adipocytes, the SGBS cells showed significant alterations in the metabolite status of amino acids, biogenic amines, lysophosphatidylcholines, and phosphatidylcholines. The results increase the knowledge about the metabolic pathway dynamics of human adipogenesis.

P-169 **BDE-47 induced Alterations in Mitochondrial Function and Glucose metabolism.**

PRESENTING AUTHOR: ***Daciana Margineantu, Clinical Reaserch and Human Biology Division, Fred Hutch, United States***

CO-AUTHORS: ***Kusum Chawla, Yunjia Lai, Mona El-Badawi, Seamus Hughes, Oliver Fiehn, David Hockenbery***

BDE-47 is a dominant congener of environmentally persistent polybrominated diphenyl ethers (PBDEs) widely used as flame retardants in manufacturing until recently. Bioaccumulation and evidence of potential endocrine disruption in animal studies prompted the need to understand the effects of BDE-47 exposure at a cellular and organism level. A number of in vitro studies demonstrated dose-dependent alterations of mitochondrial function, induction of oxidative stress, and cell death. In order to uncover potential biomarkers of metabolic dysfunction caused by BDE-47, we have used global metabolomics analysis of extracellular media from mouse transformed hepatocytes (TAMH), primary hepatocytes, and mouse plasma and urine samples. Data from transformed hepatocytes shows skewed changes in TCA cycle metabolites (decreased secretion of oxoglutarate and citrate with dose dependent increase of fumarate or succinate) suggesting complex metabolic adaptations to reduced mitochondrial function. Surprisingly, BDE-47 exposure of primary hepatocytes caused a large accumulation of sugar polyols (xylitol, arabitol) and other glucose derived metabolites (xylulose, galactinol, gluconic, and saccharic acids) and less pronounced changes in TCA cycle metabolites. Given the more resilient respiratory and survival phenotype of primary hepatocytes we attribute this signature to increased metabolic flux via the pentose phosphate pathway. Increased NADPH synthesis is required to counteract the oxidative stress caused by BDE-47 exposure and inhibition of this pathway sensitizes cells to BDE-47 and other mitochondrial toxicants. Plasma xylitol and 3-hydroxybutyrate accumulation after in vivo exposure along with glucosuria point to systemic alterations in glucose metabolism, potentially linking prolonged exposure to BDE-47 to the onset of diabetes.

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MODEL ORGANISMS

P-170 In vivo dynamic metabolomics of C. elegans by HR-MAS

PRESENTING AUTHOR: ***Goncalo J Gouveia, University of Georgia, Portugal***

CO-AUTHORS: ***John Glushka, Arthur S. Edison***

NMR metabolomic studies are typically comparative, using extracted metabolites that represent snapshots in time of a given organism, mutant or treatment. This type of approach relies heavily on rigorous experimental designs and quality controls to minimize variance added through sample preparation and extraction. However, metabolism is inherently dynamic, which can prove to be a challenge for traditional static metabolomic approaches. One such example, is the change in metabolism that occurs during the development of a synchronized Caenorhabditis elegans population from eggs to adults with eggs. The time resolution necessary to capture these metabolic changes with extraction-based approaches is logistically difficult and labor intensive. Here we describe an extraction-free, NMR High-Resolution Magic Angle Spinning (HR-MAS) alternative, that can continuously monitor and measure metabolic changes in vivo of a single synchronized population of C. elegans. Using this approach, we can directly monitor specific metabolites and their fluctuations throughout the course of the developmental cycle. Further, we are developing new computational tools to model these anabolic and catabolic changes to specific life stages within a growing C. elegans population.

P-171 Metabolic Response in Rabbit Urine to Occurrence and Relief of Unilateral Ureteral Obstruction

PRESENTING AUTHOR: ***Guiping Shen, Xiamen University, China***

CO-AUTHORS: ***Zhenzhao Wang, Jianghua Feng***

Ureteral obstruction will lead clinically to hydronephrosis, which may further develop into partial or complete loss of kidney function and even cause permanent histological damage. However, there is little knowledge of metabolic responses during the obstructed process and its recoverability. In this study, a complete unilateral ureteral obstruction (CUUO) model was established in rabbit, and 1H NMR-based metabolomic analysis of urine was used to reveal the metabolic perturbations caused by CUUO and the metabolic recovery after CUUO was relieved. The gradually decreased levels of 3-hydroxykynurenine, 3-methylhistidine, creatinine, guanidoacetate, meta- & para-hydroxyphenylacetate and phenylacetylglycine and the gradually increased levels of acetate, alanine, citrate, glycine, lactate and methionine in urine could be regarded as the potential biomarkers for the occurrence and severity of ureteral obstruction. And the reduced levels of 3-methylhistidine, creatinine, guanidoacetate, hippurate, meta-hydroxyphenylacetate and methylguanidine and the elevated levels of 2-aminoisobutyrate, acetylcholine, citrate, lactate, lysine, valine and α-ketoglutarate in urine compared with the obstructed level could characterize the metabolic recovery of ureteral obstruction. The involved biochemical pathways in ureteral obstruction and relief were correspondingly depicted. Our results demonstrated the practicability and availability of recovering renal functions of the patients with severe hydronephrosis in clinical practice by removing causes for obstruction.

P-172 Metabolite profile signatures associated with accelerated aging and functional deficiency of succinate dehydrogenase in S. cerevisiae

PRESENTING AUTHOR: ***Felice A De Jong, Iroa Technologies LLC, United States***

CO-AUTHORS: ***Marjorie Jones, Haley Albright, Michael Fitch, Felice A de Jong, Tim Garrett, Chris Beecher, John L Hartman IV***

S. cerevisiae is a genetic model for eukaryotic cellular aging. Yeast chronological lifespan (CLS) is measured as stationary phase survival. Genes, nutrients and environmental factors are all known to influence CLS in yeast and animal models. Succinate dehydrogenase (SDH) is an enzyme complex, encoded by four genes (SDH1-4), which functions in the TCA cycle and electron transport chain. We observed loss of SDH activity to be associated with aging, but to date biochemical mechanisms linking SDH energy metabolism and mitochondrial aging are unclear. To assess the functional metabolic effects of SDH on chronological aging, herein we applied isotope ratio outlier analysis (IROA) LC-MS metabolomics to compare the biochemical profiles of individual SDH subunit knockout strains in S. cerevisiae.Metabolite profiles employing complex 13C-labeled internal standards were collected from aging S. cerevisiae cultures to assess the effect of SDHgene knockouts on the temporal changes in metabolite pools in association with reduced CLS. A reference MS-MLS was applied using IROA metabolomics to assess SDH knockouts against parental reference strain controls, 11 time points over 7 days, to identify age-associated signatures. Samples were analyzed using UHPLC-HRMS and data analyzed using ClsterFinder software. Temporal profiles of each metabolite for all strains revealed age- and strain-dependent differential expression. Age-associated differential metabolite expressions were noted comparing strains with normal (wild type and sdh3 knockdown) vs. reduced (sdh1, sdh2, and sdh4 knockouts) CLS, including those upregulated in strains with normal vs. reduced longevity (e.g., glutathione and glutamate), and vice versa (citrulline and inosine).

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MODEL ORGANISMS

P-173 **Metabolic profiling approach to the identification of discrimination markers in high-fat diet-fed dog**

PRESENTING AUTHOR: ***Kim Ye Jin, Incheon National University, Korea, South***

CO-AUTHORS: ***Jae Kwang Kim, Kyoung Min So, Wan-Kyu Lee***

Obesity is known to be a risk factor for many diseases, and a high fat diet is related to obesity. When high fat diet was induced in the long term, metabolic shifts in mice liver tissue, plasma and urine have already been reported. However, few studies have metabolic profiling of dogs. In this study, metabolic profiling in fecal samples of dogs was performed after feeding the regular diet (RD) and high fat diet (HFD). In addition, metabolic differences between the RD and HFD were analyzed at 4 and 8 weeks. Metabolites were detected by using gas chromatography time-of-flight mass spectrometry (GC-TOFMS) and gas chromatography-flame ionization detector (GC-FID). A total of 58 metabolites, 19 amino acids, 14 organic acids, 4 sugars, 3 sugar alcohols, 16 fatty acids, 1 amine, and 1 inorganic acid were identified. Multivariate analyses such as principal component analysis (PCA), partial least-squares discriminant analysis (PLS-DA), Pearson's correlation analysis and hierarchical clustering analysis (HCA) were used to visualize the obtained data. The RD and HFD group were clustered separately in the PLS-DA score plot. Compared with RD group at 8 weeks, the levels of C12:0, C16:0, C18:0 and C20:0 were increased in HFD group. Also, these had significant variable importance in the projection (VIP) value (>1.0) in PLS-DA model and P-value (<0.05) in t-test. These results will be useful to find potential biomarkers for obesity and study the metabolic profiling of dogs in the future.

P-174 **Lipidomics of inflammation-induced optic nerve regeneration**

PRESENTING AUTHOR: ***Anna M. Trzeciecka, University of Miami, United States***

CO-AUTHORS: ***David T. Stark, Jacky M. K. Kwong, Maria C. Piqueras, Sanjoy K. Bhattacharya, Joseph Caprioli***

In adult mammals, retinal ganglion cells (RGCs) fail to regenerate their axons when damaged. As a result, RGCs die after acute injury and in progressive degenerative diseases such as glaucoma leading to permanent visual loss. Little is known about the role of lipidome in the processes of axonal injury and repair. Lipids are one of the end-products of gene expression – metabolites, that together with proteins build cell membranes, myelin sheaths and participate in number of biological processes. Understanding of this missing part of chemistry may provide new clues to the biology of optic nerve (ON) regeneration. Established experimental models of ON regeneration allow exploration of molecular determinants of RGC axon regenerative success and failure. In this study, we analyzed lipidomic profiles of ON and retina after ON crush injury with and without Zymosan treatment using high resolution liquid chromatography-tandem mass spectrometry approach. Our results reveal profound remodeling of retina and ON lipidomes that occur following injury. In retina, Zymosan treatment largely abrogates widespread lipidome alterations. Differently, in ON, Zymosan induces distinct lipid profile rather than restores it to the naïve structure. These results support the concept of lipidome involvement in ON injury and regeneration. We identified number of lipid species, classes and fatty acids that may be involved in the mechanisms of axonal damage and repair. Lipids upregulated during ON regeneration may be interesting candidates for further functional studies.

P-175 **Study of the impact of a commercial formulation of glyphosate on a turtle species, Trachemys scripta elegans by non-targeted metabolic profiling.**

PRESENTING AUTHOR: ***Cédric BERTRAND, PSL Research University, Université de Perpignan Via Domitia, France***

CO-AUTHORS: ***Chandrashekhar Patil, Delphine Raviglione, Olivier Verneau, Cédric Bertrand***

The commercial formulations of glyphosate-based herbicides are suspected to impact human health and biodiversity, mainly in areas with intense agricultural activities such as fruit crops and vineyards. Glyphosate and its degradation product (AMPA), are among the most common pollutants found in the French freshwater ecosystems. In Southern France, especially in the Pyrénées-Orientales department, high concentrations of these products are detected in some watercourses, particularly in the Fosseille River. These pollutants are known to induce an oxidative stress within freshwater organisms, including fishes, amphibians and freshwater turtles. The aim of this study is to test under experimental conditions the non-targeted metabolomic profiling approach to determine metabolic changes in the red-eared slider (Trachemys scripta elegans) and to characterize the discriminating molecules related to oxidative stress, after exposure to commercial herbicide formulation on the one hand and to degraded waters collected in the Fosseille River on the other. The results obtained show that the metabolome of T. s. elegans changes significantly depending on the treatments. Thus metabolomic profiling could be a very sensitive approach to characterize metabolic changes in aquatic organisms following water contamination. On the basis of multivariate statistical analysis (PCA and OPLS-DA) 11 VIPs are selected to be characterized as some potential contamination indicators. Because our experiments in mesocosm validated this method, we are now planning to focus on wild population of Mauremys leprosa, a freshwater turtle species listed in France as 'Vulnerable' in the Red list of the IUCN and which occurs in the Fosseille River.

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MODEL ORGANISMS

P-176 **Metabolic profiling of human blood associated with environmental risk factor in air pollution**

PRESENTING AUTHOR: ***Seoyoung Jang, Korea Basic Science Institute, Korea, South***

CO-AUTHORS: ***Geum-sook Hwang***

Environmental risk factors (ERFs) is the greatest enviromental threat to public health all over the world, and are related to nervous system disorder. We investigated the association between levels of metabolite identified in human blood samples and ERFs concentrations such as heavy metal and fine particulate matter (PM10) in air pollution. In this study, we performed metabolic profiling on blood from 457 Koreans using 1H-NMR. We categorized samples into four groups according to the concentration levels of ERFs. Partial least sqaures - discriminant analysis (PLS-DA) showed separation between the high exposure category and low exposure category in PM10. We found that some metabolites were significantly altered such as fumarate, lactate, leucine, glucose, and glutamine. This approach can be a promising technique to assess the impacts of environmental pollution on human metabolism and to identify potential biomarkers for predicting exposure of pollutants like ERFs.

NEW ADVANCES

P-177 **Nitrogen-Based Metabolomics for functional analysis of urease in Staphylococcus aureus**

PRESENTING AUTHOR: ***Fatema Bhinderwala, Department of Chemistry, University of Nebraska Lincoln, United States***

CO-AUTHORS: ***Samantha Lonergan,Jade Woods, Chunyi Zhou,Paul D. Fey, Robert Powers***

Isotopically labeling a metabolite and tracing its metabolic fate has provided invaluable insights about the role of metabolism in human diseases in addition to a variety of other issues. 13C-labeled metabolite tracers or unlabeled 1H-based NMR experiments are currently the most common application of NMR to metabolomics studies. Unfortunately, the coverage of the metabolome has been consequently limited to the most abundant carbon-containing metabolites. To expand the coverage of the metabolome and enhance the impact of metabolomics studies, we present a protocol for 15N-labeled metabolite tracer experiments that may also be combined with routine 13C-tracer experiments to simultaneously detect both 15N- and 13C-labeled metabolites in metabolic samples. The methodology is demonstrated by labeling Escherichia coli and Staphylococcus aureus metabolomes with 15N1-ammonium chloride, 15N4-arginine, and 13C3-acetate. Efficient 15N and 13C metabolite labeling and identification were achieved utilizing standard cell culture and sample preparation protocols.Nitrogen metabolism in gram-positive bacteria Staphylococcus aureus is highly controlled through conserved mechanisms that respond to changes in the amino acid concentrations and pH environment. We investigate the role of urease in maintaining homeostasis of nitrogen, and subsequently rescuing acid-induced cell death. We utilize our 15N-metabolomics methodology to study the adaptation in nitrogen metabolism in urease deficient strains (JE2 ureB:ΦNΣ) through the 15N-labeled amino acid tracers.

P-178 **Tissue metabolomics in imaging science: which ex-vivo method is best?**

PRESENTING AUTHOR: ***Christoph Trautwein, Eberhard-Karls-University Tuebingen, Werner Siemens Imaging Center, Department of Preclinical Imaging and Radiopharmacy, Germany***

CO-AUTHORS: ***Dr. Marcel A. Krueger, Dr. Andreas M. Schmid, Dr. Janina D'Alvise, Dr. Sascha Dammeier, Prof. Dr. Bernd J. Pichler***

Imaging modalities like PET, MRI or hyperpolarized imaging enable to study metabolism in-vivo. However, currently only a very limited amount of metabolites and pathways can be analyzed. While ex-vivo 1H NMR HR-MAS spectroscopy has the potential to verify in-vivo findings revealed by MRS or PET, deeper analysis however depends on metabolite extraction and advanced bioanalytical methods. Aim of this study was to evaluate technical differences between HR-MAS, liquid NMR, LC-MS (not considering MALDI/DESI) and two extraction methods. For that purpose, we collected three organs with distinct metabolic profiles from three Balb/c nu/nu mice. After cervical dislocation, liver, brain and subcutaneous tumor tissue were directly quenched in liquid nitrogen. Biopsies were punched and analyzed by HR-MAS according established protocols. The remaining tissue was cryogenically pulverized and lyophilized overnight. Homogenous dry powder aliquots (5 mg) were either processed according a commercial LC-MS/MS workflow or with our image-guided 2-phase extraction protocol based on focused ultrasonication. Extracts of both methods were analyzed by 1H NMR (Bruker 600 MHz Avance III) and LC-MS/MS (AB Sciex QTRAP 6500). In HR-MAS, spectra strongly changed during 1h data acquisition at 277 K, which indicates physical destruction and ongoing enzymatic degradation and makes this technique not recommendable. With commercial LC-MS/MS we quantified up to 180 metabolites, however from few compound classes and mainly lipids. By contrast, 1H liquid NMR quantified up to 50 metabolites of most central pathways and yielded spectra directly matchable with in-vivo MRS, making this technique first choice for ex-vivo tissue extract metabolomics in imaging science.

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NEW ADVANCES

P-179 Quantitative Glycolipid Tissue and Plasma Analysis by Broadband and Scanning Quadrupole Data Independent LC-MS Analysis

PRESENTING AUTHOR: ***David Heywood, Waters, United Kingdom***

CO-AUTHORS: ***Mina Mirzaian, Lee A. Gethings, Ningombam Sanjib Meitei, Maria J Ferraz, Kassiani Kytidou, Robert S. Plumb, Johannes P.C. Vissers, Johannes MFG Aerts***

Gaucher disease (GD) is a relatively common recessively inherited lysosomal storage disorder that is caused by a deficiency in the lysosomal beta-glucosidase. A hallmark of GD is the massive accumulation of glucosylceramide (GlcCer) in lysosomes of tissue macrophages, eventually transforming into viable Gaucher cells. Plasma and spleen tissue lipids were extracted using total extract chloroform/methanol and modified Bligh/Dyer based methods. Lipids were separated using reversed phase based chromatographic methods and MS data collected using a broadband DIA technique, where no precursor isolation is applied prior to the collection of MS or MS/MS data, and a scanning quadrupole based DIA method, where a resolving quadrupole mass filter is scanned repetitively with MS and MS/MS data collected at rapid spectral acquisition rates. Comparing the two tissue extraction techniques, both methods yielded a similar number of lipid identifications. However, the total extraction method provided a greater number of glucosylceramides. The majority of these glucosylceramides are shown to be significantly over expressed, typically >10 fold, in GD patients and associated with high significance and %CV values of typically <10%. The analysis of the pre and post treatment plasma samples of GD patients, targeting specific biochemical pathways and interactors, provided complementary information. The results suggest that untargeted quantitative profiling of the lipid complement of spleen tissue and plasma by DIA LC-MS delivers novel insights in Gaucher disease metabolism by revealing glycolipid abundance abnormalities, which are currently undergoing validation.

P-180 Non-targeted Metabolomic and Lipidomic Profiling of FFPE Tissue Samples: Method Development and Proof of Concept Investigation

PRESENTING AUTHOR: ***Sylvia Karin Neef, Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Germany and University of Tübingen, Germany***

CO-AUTHORS: ***Heike Horn, Stefan Winter, Ute Hofmann, Thomas E. Muerdter, Elke Schaeffeler, German Ott, Matthias Schwab, Mathias Haag***

Metabolomic profiling of fresh-frozen tissue is a promising approach in cancer research to examine metabolic alterations and to identify potential biomarkers. Unfortunately, well annotated frozen specimens are a limited resource and complex in terms of storage and handling. Due to this bottleneck, there is an increasing interest in FFPE tissue – the standard preservation format in diagnostic pathology – as valuable and widely available material for retrospective metabolomic investigations. Former studies showed the feasibility of acquiring reproducible metabolomic data from methanolic FFPE tissue extracts using targeted LC-MS/MS. These results indicate that FFPE tissue is a suitable resource to acquire not only metabolomic but also lipidomic data. Therefore, we developed a non-targeted method for the combined metabolomic and lipidomic profiling of FFPE tissue. This optimized approach comprises a two-step extraction procedure followed by LC-QTOF-MS analysis with HILIC and RP separation. The method allows the analysis of a broad range of molecules from FFPE tissue including polar, medium-polar and nonpolar metabolites with high technical precision (averaged CV ≤7%). For reproducibility assessment we used targeted feature extraction on the basis of an inhouse established library containing 271 metabolites as representative subset of the whole metabolome. On average 227 metabolites could be annotated. More than 80 % of these metabolites exhibited CVs ≤25% which demonstrates good overall reproducibility. Finally we performed a proof-of-concept experiment by analyzing a set of five human FFPE kidney cancer samples as well as the corresponding non-tumorous tissue and achieved phenotypic distinction, thereby verifying the applicability of the proposed method.

P-181 Addressing Metabolomic Challenges with a Universal Internal Standard Mixture

PRESENTING AUTHOR: ***Andrew Percy, Cambridge Isotope Laboratories, Inc., United States***

CO-AUTHORS: ***Gerrit Hermann, Michaela Schwaiger, Gunda Koellensperger, Gary Siuzdak, Krista Backiel***

Mass spectrometry (MS)-based assays continue to evolve in metabolomics to help address queries in disease biomarker evaluation and systems biology investigation. While assays can be constructed using label-free approaches, a powerful strategy to reduce analytical variability utilizes isotopically labeled standards. To expand the breadth of labeled metabolites for use as internal standards in various applications, we prepared a uniform 13C-labeled yeast extract comprising 100s of 13C-enriched small molecules. This mix was produced by batch cultivating Pichia pastoris in a culture medium containing 13C6 D-glucose, with boiling ethanolic extraction used after fermentation to isolate the metabolome and lyophilization to stabilize the metabolites. The composition, as revealed by LC-MS/MS, comprises a broad set of reproducibly identified metabolites (e.g., amino and organic acids, sugar phosphates, cofactors), with linkage to a variety of biochemical pathways (e.g., citrate and glyoxylate cycle) and cellular/molecular processes (e.g., immune system, DNA metabolism). The utility of the extract has been demonstrated in a variety of applications with excellent figures of merit. To be presented here is an overview of the developed extract, the methodological advances (e.g., anion-exchange chromatography with high resolution MS), and recent applications (e.g., absolute metabolite quantitation in human plasma and cancer cells). Additionally to be included is the use of this extract to train new algorithms in METLIN/isoMETLIN to generate in silico MS/MS data for improved characterization of known and unknown metabolites.

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NEW ADVANCES

P-183 Validation of a methodology for evaluating longitudinal change of VOCs in breath

PRESENTING AUTHOR: ***Aditya Malkar, Owlstone Medical Ltd, United Kingdom***

CO-AUTHORS: ***Jasper Boschmans, Rob Smith, Russell Parris, Billy Boyle, Simon Kitchen, Duncan Apthorp, Marc van der Schee***

Background: Evaluating longitudinal changes in breath biomarkers holds relevance for a wide range of applications such as disease monitoring, assessing effects of environmental exposures and studying disease pharmacokinetics. This proof-of-concept study aims to validate the use of the Breath Biopsy platform to observe changes in exhaled Volatile Organic Compound (VOC) concentrations over time. Methods: A change in 5 known exhaled VOCs (α-pinene, β-pinene, limonene, eucalyptol and (±)-menthol) was induced by ingestion of a 200 mg peppermint capsule. 500 ml of breath was sampled twice prior to ingestion and every 30 minutes for up to 6.5 hours afterwards, using the ReCIVA Breath Sampler and subsequently analysed by Thermal Desorption Gas Chromatography Mass Spectrometry. The experiment was repeated four times in a single subject. For each compound fold-changes pre and post ingestion along with repeatability were reported. Results: Ingestion of the peppermint capsule resulted in a clear increase in target VOCs 30 minutes after ingestion; for example a 28-fold increase inα-pinenewas measured compared to baseline pre-ingestion controls. Subsequent measurements showed a clear washout curve for the exogenous compounds. Repeat experiments showed an excellent reproducibility, for example the mean average% relative standard deviation(%RSD) of peak area for α-pinene was 8.05. Conclusions: The study demonstrates that the Breath Biopsy platform can be used to study longitudinal changes of exhaled VOCs in a sensitive and repeatable way unlocking potential new usecases for breath analysis.

P-184 SINGLE LIVE CELL AND TISSUE METABOLISM VIA RAMAN IMAGING MICROSCOPY

PRESENTING AUTHOR: ***Adrian Lita, National Institutes of Health, United States***

CO-AUTHORS: ***Mark R. Gilbert, Mioara Larion***

Raman has been used for decades to determine vibrational models linked with structural changes of small molecules. Due to recent progress in the diode lasers and notch filters, study of biological systems became possible in the form of Raman Imaging Microscopy. Focusing the laser through the microscope allows the collection of Raman spectra for each pixel and subsequent movement of the stage permits the acquisition of spectra in a new position. This approach provides a chemical and biochemical mapping of the entire cell in the culture media. Therefore, the technique is non-destructive, non-invasive and can accept a wide range of samples from bulk to microscopic, from solids to liquids or gasses. Since Raman has not been primarily used for biological samples before, there is limiting spectral assignments for these samples. Our goal is to use Raman Imaging microscope in order to monitor metabolic changes in live cells and tissue. We will present some to the advances using this technique such as: cytochrome C release from mitochondria upon TG02 drug treatment; quantification of lipid content in GBM cell lines upon treatment with AGI-5198 drug, as well as mapping of regions containing infiltrating cells in GBM tissues. In the future, we plan to extend the use of Raman technique to other samples and other classes of biochemical compounds.

P-185 Metabolic fate and subchronic biological effects of core-shell structured Fe3O4@SiO2-NH2 nanoparticles

PRESENTING AUTHOR: ***Jianghua Feng, Xiamen University, China***

CO-AUTHORS: ***Yueli Chen, Jianghua Feng***

Core-shell structured Fe3O4@SiO2-NH2nanoparticles (Fe@Si-NPs)demonstrated outstanding potentials in drug targeting and delivery and medical imaging. However,they have limited clinical applications due to unknown chronic bio-effects and potential bio-related risks.In this study, the subchronic biological effects and metabolic fate of 20 nm Fe@Si-NPs in Sprague-Dawley rats in 12 weeks were investigated by combination of traditional serumbiochemical assay, the measurements of ratios of tissue to body weights and iron biodistribution in tissuesand NMR-based metabonomic analysis using an intravenous model. Biofluids (plasma and urine) analysis provided the transportation, absorption and excretion information of Fe@Si-NPs. Urine metabonome displayed a metabolic recovery while self-regulation of plasma metabonome leaded to the parallel metabolic trends between dosed and control groups in 12 weeks. And biological tissues (spleen, liver, kidney and lung) analysis indicated liver and spleen are the targeted-organs of Fe@Si-NPs. The obvious metabolic variations responding to the biodistribution were induced by Fe@Si-NPs although no visible toxic effects were observed in these tissues. Besides the common energy metabolism response to the xenobiotics, Fe@Si-NPs also disturbed the metabolic pathways in glycerophospholipid and sphingolipid metabolism, metabolisms of purine, pyrimidine and nicotinate. Our results provide preliminary validation for the potential use of Fe@Si-NPs in clinical medicine and give identifiable ground for the dose selection and bio-nanoagent optimization.

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NEW ADVANCES

P-186 A spectroscopic and computational insight into the molecular interaction of Schiff base organometallic complexes with α-acid glycoprotein (AGP)

PRESENTING AUTHOR: ***Mohamed AlAjmi, College of Pharmacy, King Saud University, Saudi Arabia***

CO-AUTHORS: ***Multaq Almalki, Tabish Rehman***

Background: AGP is an acute phase plasma protein which acts as immune-modulatory protein by binding and transporting various exogenous and endogenous compounds. Previously, our group has synthesized and characterized novel Schiff base organometallic complexes exhibiting prominent anti-inflammatory properties. In the present study, the mechanism of interaction between Schiff base organometallic complexes with AGP was evaluated. Methods: We examined the interaction between organometallic complexes and AGP by fluorescence spectroscopy, circular dichroism (CD) and molecular docking techniques. Quenching in AGP fluorescence was measured in 285-400 nm range after excitation at 280 nm. Synchronous fluorescence was measured to evaluate the contribution Tyr and Trp residues in quenching. Intermolecular distance between quencher and organometallic complex was measured by FRET. Effect of organometallic complexes binding on the overall AGP structure was measured by far-UV CD. Insight into molecular interaction between AGP and organometallic complexes was gained by molecular docking. Results: Results suggested that organometallic complexes quenched the fluorescence of AGP by binding near to Trp. Binding constants of organometallic compounds were of the order of 105 M-1. FRET demonstrated that the distance between organometallic complexes and Trp residue varied in 2.50 – 3.50 nm range. A conformational change in AGP structure was observed upon organometallic complexes binding. Molecular docking results indicated that hydrophobic interactions and hydrogen bonding played significant role in stabilizing the complex between organometallic complexes and AGP. Conclusions: Knowledge of interaction between AGP and organometallic complexes will helpto evaluate the pharmacokinetics and pharmacodynamics of the organometallic complexes.

P-187 Setting up metabolomics in multicellular tumour spheroids

PRESENTING AUTHOR: ***Mate Rusz, University of Vienna, Hungary***

CO-AUTHORS: ***Luis Galvez, Debora Wernitznig, Michael A. Jakupec, Bernhard K. Keppler, Gunda Koellensperger***

In this work we aimed at the optimisation of metabolomic approaches for the investigation of 3D multicellular tumour spheroids, which emerged as models for testing of anticancer compounds as they better mimic certain features of in vivo tumour tissue. Since their cell number is usually lower than that of two-dimensional cell cultures, it is challenging to set up a reliable tool kit for profiling their metabolome. Therefore, we addressed fundamental questions regarding all steps of analysis. Different sample preparation and normalization procedures were employed for non-targeted and targeted metabolomics by high resolution mass spectrometry. The application of metabolomics in research of acquired drug resistance based on in vitro models is of paramount importance as the mechanisms underlying the development of resistance on the metabolome level remain unclear. Furthermore, hypoxia - a key feature of poorly vascularized solid tumours and tumour spheroids - also triggers cellular adaptations and metabolic shifts. Hence, we addressed metabolomic rearrangements and lead metabolomic alterations in hypoxic and non-hypoxic 3D models in comparison with 2D (monolayer) cultures and upon incubation with oxaliplatin and KP1339, a clinically investigated Ru-based drug. The monolayer and spheroid cultures grown from oxaliplatin-sensitive vs –resistant colorectal cancer cell lines reveal metabolomics patterns correlating with sensitivity and resistance.

P-188 Integrated Data independent analysis LC/MS and MALDI imaging Workflow for Spatial Lipidome Analysis of Liver Tissues

PRESENTING AUTHOR: ***Hernando J. Olivos, Waters Corporation, United States***

CO-AUTHORS: ***Bindesh Shrestha, Hernando Olivos, Qi Liu, Xinmin Yin, Wenke Feng, Xiang Zhang***

Fatty liver diseases are often associated with chronic alcohol consumption. Understanding changes in levels of lipids and their location in the liver can be helpful for studying dysregulated lipid metabolism caused by alcohol consumption. Data independent analysis (DIA) using LC/MS have been used to obtain hypothesis-generating lipid marker candidates, while mass spectrometry imaging (MSI) can show the spatial distribution of such lipids. C57/BL6 mice were pair-fed control diet (PF) or 5% alcohol containing diet (AF) for 10 days followed by oral gavage of a bonus of alcohol. Extracted lipids from mouse liver were analyzed by UPLC/MS using C18 column. Adjacent liver sections were imaged by MALDI MSI. Both UPLC/MS and MALDI data were collected on a SYNAPT HDMS G2-Si instrument. The untargeted UPLC/MS analysis of alcohol-fed mice and their pair-fed counterparts was used to determine differentially expressed lipids as result of alcohol consumption. The identification of those lipids was also obtained simultaneously using DIA. Both analyses was dominated by phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol), fatty acids etc. For example, LC/MS analysis showed PE(38:6) as upregulated for alcohol-fed mice and PI(38:4) as downregulated. The imaging of adjacent tissue sections using MALDI on the same instrument platform showed the spatial distribution of same lipids with minimal instrumental bias. Here we show an integrated DIA-LC/MS and MALDI MS spatio-lipidomic workflow for untargeted lipidomics in liver tissues.

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NEW ADVANCES

P-189 Isomer separation visualization to reveal aldosterone specific distribution

PRESENTING AUTHOR: ***Shuichi Shimma, Osaka University, Japan***

CO-AUTHORS: ***Emi Takao, Yuki Sugiura, Koshiro Nishimoto, Eiichiro Fukusaki***

Visualization of steroid hormones using matrix-assisted laser desorption/ionization (MALDI-IMS) is one of the active subjects for endocrine studies. Unfortunately, it is difficult to ionize steroid hormones due to the polarity and abundance. To overcome the problems, on-tissue chemical derivatization (OTCD) using Girard T reagent were developed, however the problem of structural isomers in steroid hormones is still remain. In this paper, we developed aldosterone specific visualization method using OTCD and tandem mass spectrometry. To confirm the usability of our method, two experiments were performed in rat sodium diet model and human primary aldosteronism (PA), which is one of the causes of secondary hypertension. According to our experimental results, aldosterone and structural isomer of cortisone were clearly visualized separately. In PA patient tissues, we successfully visualized those hormones separately and compared using immunohistochemistry. Our finding in this study was the expression level of aldosterone production enzyme and produced aldosterone were different in distribution.

P-190 Innovations in Ion Mobility/Mass Spectrometry for Metabolomics and Clinical Analysis

PRESENTING AUTHOR: ***Richard A Yost, University of Florida, United States***

CO-AUTHORS: ***Robin H.J. Kemperman, Allison Levy, Russell Lewis, Nicholas R. Oranzi, Michael Wei, Timothy J. Garrett***

Ion mobility/mass spectrometry has tremendous potential for metabolomics, lipidomics, and clinical analysis. Ion mobility can resolve compounds unresolved by LC/MS/MS, provide additional structural information not available from mass spectrometry, and reduce or eliminate the need for chromatographic separation. These features offer significant improvements for quantitative targeted metabolomics and clinical analysis, as well as for untargeted (global) metabolomics studies. This presentation will explore innovations in ion mobility/mass spectrometry for metabolomics, lipidomics, and clinical analysis. Techniques to be covered include both classic drift tube ion mobility (IMS) and high-field asymmetric-waveform ion mobility (FAIMS), in conjunction with HRMS, MS/MS, and LC/MS. Characterization and optimization of instrumental parameters critical for analytical performance will be explored, including ionization techniques, cationization and complexation of analytes for improved mobility separation, and integration with chromatographic separation and MS/MS. Applications will include a range of metabolomics, lipidomics, and targeted clinical analyses. Specific examples will include rapid clinical assays (vitamin D and its epimers), separation of isomeric performance-enhancing steroids, breath analysis for early disease screening, and improvements in mass spec imaging. Recent advances in these areas will be highlighted, along with a perspective on the metabolomics and clinical future of these approaches.

P-191 Wide-targeted LC-MS/MS-based chiral metabolic profiling focusing on amino acids and related metabolites

PRESENTING AUTHOR: ***Yosuke Nakano, Osaka University, Japan***

CO-AUTHORS: ***Moyu Taniguchi, Eiichiro Fukusaki***

Metabolomics has been an evolving science with a wide range of applications in various fields. However, previous studies have rarely focused on metabolite chirality. In this study, to achieve metabolic profiling of chiral amino acids and related metabolites, we developed a high-throughput method using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The combination of two types of chiral columns (with binaphthyl-based crown ether and cinchona alkaloid-derived zwitterionic stationary phases) enabled the analysis of 115 chiral and non-chiral metabolites. By finely optimizing MS/MS parameters, the method allowed the highly sensitive (0.001-50 nmol/mL) and wide dynamic range detection (100-50,000) of target analytes in a standard solution without derivatization. We applied the method to food samples (cheese), and successfully quantified trace levels of metabolites such as D-amino acids in samples. Additionally, we performed principal component analysis (PCA) on the metabolome data and obtained unique profiles that reflected metabolite chirality. These results demonstrated the applicability and feasibility of the LC-MS/MS method as an effective tool for wide-targeted chiral metabolome analysis.

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NEW ADVANCES

P-192 A High Throughput Single Platform For High Throughput Quantitative MultiOmic Studies

PRESENTING AUTHOR: ***Jimmy Yuk, Waters, United States***

CO-AUTHORS: ***Robert S. Plumb, Lee A. Gethings, Billy J. Molloy***

A high throughput targeted UPLC-MS/MS single platform, employing a reversed-phase gradient separation, has been developed for the quantification/monitoring of small molecule metabolites, lipids and peptides. The platform employs a single LC column and mobile phase combination which allows the analysis of multiple analyte classes with either positive or negative ion MRM detection. The use of metabolic profiling (metabonomics/metabolomics) to discover biomarkers of organismal response to environmental and physiological change is now widespread. In biomedical applications metabolic profiling is being deployed as a method for finding novel, mechanistic, biomarkers of disease with obvious potential for improving diagnosis, and patient stratification. Hypothesis driven metabolomics delivers detailed qualitative and quantitative analysis on specific pathways or classes of metabolites, allowing researchers to analyse the effects of disease or treatments in greater detail. These targeted assays usually employ "bespoke" methods which are optimized for each pathway or metabolic class making multiplexing assays difficult. We have developed a single analytical LC-MS/MS platform which is rapid, simple and reliable. The methodology employs a single LC column / mobile-phase combination which facilitate bile acids, biogenic amine, free fatty acids, acyl carnitines, lipids and 100 protein panel. This single platform approach has been employed for the analysis of plasma from a liver cancer study, showing excellent throughput and sensitivity.

P-193 A Rapid Microbore Metabolomics Profiling (RaMMP) Analytical Platform for Discovery Metabolomics and Lipidomics

PRESENTING AUTHOR: ***Robert S. Plumb, Waters, United States***

CO-AUTHORS: ***Lee A. Gethings, Adam M. King, Ian D. Wilson***

A suite of rapid metabolomics LC-MS methodologies, for polar, non-polar small molecule and lipid analysis, has been developed delivering high throughput and high reproducibility biofluid analysis. As global life-styles change we are seeing increasing cases of obesity, diabetes, and mental health issues. Discovery metabolomics, employing unbiased data collection and analysis, offers a valuable and unique insight into the underlying biochemistry of diseases as well as the patients' individual biochemistry "phenotype', diet, health status, age and stress. To deliver this information the analytical data generated is processed via a variety of chemometric modelling and analysis methodologies to deliver the relevant biochemical information. LC-MS based analysis has become the key technology for metabolomics providing data on polar and non-polar metabolites as well as lipids. However these analysis times are typically in the 15-30 minute range which is not compatible with large cohort clinical and epidemiological studies where sample sizes often are in the 1000's range. To address this challenge we have developed a suite of sub 3 minute LC-MS assays based on microbore LC and accurate mass MS. The assays have been employed for the analysis of several rodent toxicology samples and human breast cancer samples. The data generated has been shown to be reproducible, sensitive and reliable delivering similar "biomarkers" to that obtained with more extensive metabolomic methods.

P-194 An Extensive Evaluation of Column Chemistries to Retain Biologically Relevant Metabolites for Targeted Metabolomics Analyses

PRESENTING AUTHOR: ***Baljit K Ubhi, SCIEX, United States***

CO-AUTHORS: ***Si Mou, Lei Xiong***

Metabolomics provides a snapshot of the metabolic system and is an incredibly useful tool for advancing precision/personalized medicine. The challenge with LC-MS/MS metabolomics is the number of different column chemistries which are employed to retain metabolites because of their varying size, polarities, solubility's and charge– therefore no single method can capture all metabolites. We present an extensive evaluation of a variety of column chemistries where the goal was to retain as many biologically relevant metabolites as possible as part of a targeted metabolomics assay. The most commonly used column chemistries were evaluated as well as others available on the market. A total of ten different columns were evaluated, to retain as many biologically relevant metabolites as possible on one assay. All columns were optimized for gradient, temperature, run time, injection volume and chromatographic resolution of isomers. Once optimized the columns were compared for analyte peak shape, sensitivity (s/n) and chromatographic retention of polar metabolites as well as other biologically relevant metabolites. The MRMs were optimized by using authentic standards and verified using plasma extracts. We report on an alternative column which provides superior sensitivity (s/n), peak shape and retains the largest number of metabolites. Interestingly the most commonly employed columns in the metabolomics community today were of the least performing chemistries in terms of sensitivity (s/n), chromatographic resolution and retention. After evaluating biologically relevant metabolite retention on highest performing four columns we recommend a single column for optimal peak shape, sensitivity (s/n), chromatographic retention and resolution for targeted metabolomics analyses.

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NEW ADVANCES

P-195 A NEW 2D LC-MS/MS METHOD FOR QUANTITATION OF CALCITRIOL IN HUMAN SERUM

PRESENTING AUTHOR: ***Laila N Abudulai, The University of Western Australia, Australia***

CO-AUTHORS: ***Lucinda Black, Maike Bollen, Dorothee Hahne, Jahmila Parthenay, Robyn Lucas, Michael W Clarke***

The analysis of the bioactive form of vitamin D, calcitriol (1,25-dihydroxy vitamin D3), is offered in some specialized clinical laboratories. The low concentration of calcitriol in human serum and its short half-life make it a challenging analyte to routinely monitor. We have developed a 2-dimensional (2D) LC-MS/MS method to accurately quantitate calcitriol in adults and children, requiring only 500mL of human serum. The accuracy of the method was validated by comparison of our LC-MS/MS method with other commercial antibody (IDS, EIA and CT) and LC-MS methods from the vitamin D external quality assessment scheme (DEQAS). DEQAS samples (n=19) were extracted using a serum 1,25 kit from ImmunoDiagnostik and assayed using both Agilent C18 and Phenomenex Biphenyl HPLC columns in 2D mode. Analysis was performed on an Agilent 6460 triple quadrupole tandem mass spectrometer in positive ion mode, coupled to a UHPLC system. The results from our new 2D LC-MS/MS method show excellent intra-assay precision (mean concentration calcitriol = 48.5 pg/mL, SD = 1.4, CV% = 3). The inter-assay precision was evaluated using a pooled serum sample (n=12) and the mean concentration of calcitriol detected was 57 pg/mL (CV% = 15.3). Our method showed good inter-assay agreement (p = 0.51) based on results from 54 laboratories (R2 = 0.79). The analysis of calcitriol remains a challenge for clinical diagnostic laboratories. We present for the first time a robust, sensitive and accurate 2D LC-MS/MS method for the quantitation of calcitriol in human serum in both adult and paediatric samples.

P-196 LC-MS/MS ion-pairing method for the analysis of polar compounds: Application to the study of immunometabolic responses of human macrophages to Aspergillus fumigatus.

PRESENTING AUTHOR: ***Jorge Sáiz, Centre of Metabolomics and Bioanalysis (CEMBIO), Spain***

CO-AUTHORS: ***Miguel Fernández-García, Agostinho Carvalho, Coral Barbas.***

Ion-pairing is a useful separation mode for polar compounds by RP-LC in certain applications. We describe a method for the analysis of polar compounds by ion-pairing RP-LC-MS/MS used in our laboratory. The original methoduses tributylamine, with buffer A composed of 97% water and 3% methanol, 10 mM tributylamine, 15 mM glacial acetic acid and buffer B composed of 10 mM tributylamine, 15 mM glacial acetic acid, prepared in methanol. The method uses a quaternary pump (1200 bar) for the gradient chromatography, in a first stage, for the removal of contaminants at the column head for each and every analytical run in back-flush mode with acetonitrile, in a second stage, and for the column regeneration with buffer A, in a third stage. The set-up in our laboratory was different, having a binary pump (1200 bar) and a quaternary pump (400 bar). Therefore, the hardware connection and the chromatography was modifiedin order to keep equivalent stagesto those proposed in the original method. The method was validated and applied to the target analysis of 40 polar metabolites in extracts of human macrophages from patients carrying a specific mutation underlying susceptibility to fungal infection, as well as healthy donors. Samples comprised unstimulated macrophages and macrophages challenged with either wild-type or pksPΔ (melanin-deficient) Aspergillus fumigatus. We found differences in the metabolic composition of the studied batches, indicating the requirement of fungal melanin to induce several metabolic pathways in macrophages, as well as insights into the mechanisms underlying fungal susceptibility in patients.

P-197 Untargeted Metabolomics using Trapped Ion Mobility Spectrometry with Parallel Accumulation SErial Fragmentation (TIMS-PASEF)

PRESENTING AUTHOR: ***Karolina Sulek, University of Copenhagen, Denmark***

CO-AUTHORS: ***Catherine G. Vasilopoulou, Andreas-David Brunner, Florian Meier, Ulrike Schweiger-Hufnagel, Aiko Barsch, Matthias Mann***

The metabolome represents a highly dynamic network of small molecules and ubiquitous isomers exponentiate the complexity. Trapped ion mobility spectrometry (TIMS) has the potential to separate ions based on their shape and thus adds an additional layer of information to conventional liquid chromatography-mass spectrometry. Here, we further integrate “Parallel Accumulation–SErial Fragmentation” (PASEF, Meier et al., PMID: 26538118) to multiply the speed and sensitivity of MS/MS acquisition. We present a data analysis pipeline that makes full use of the four-dimensional feature space for large scale and untargeted metabolite identification. TimsTOF Pro instrument (Bruker Daltonik) allows determining collisional cross sections (CCS). The CCS values for a set of metabolites comprising vitamins, hormones, and lipids were in good agreement with literature values and highly reproducible with CVs deviations below 2%. PASEF exploits the separation in time by rapidly switching the quadrupole isolation window within one TIMS scan. Thereby it increased the total number of MS/MS spectra per 22 min LC-MS run from about 10.000 by a factor of > 10 in metabolite extracts from human plasma. The novel post-processing algorithm in MetaboScape (Bruker Daltonik) assembles 4D-features (‘buckets’) from retention time, m/z, ion mobility and intensity, and connects these features to MS/MS scans. PASEF increased the number of detected buckets with associated MS/MS spectra four times compared to the standard AutoMSMS mode. We anticipate that the accurate measurement of TIMS collisional cross sections values in conjunction with fast PASEF acquisition will enable a deeper characterization of the metabolome, for example, of clinical samples.

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NEW ADVANCES

P-198 Applying Trapped Ion Mobility separation (TIMS) in combination with Parallel Accumulation Serial Fragmentation (PASEF) for analysis of lipidomics samples

PRESENTING AUTHOR: ***Aiko Barsch, Bruker Daltonik, Germany***

CO-AUTHORS: ***Sebastian Götz, Sven W Meyer, Ulrike Schweiger-Hufnagel, Ningombam Sanjib Meitei***

Untargeted lipidomics workflows are aimed at profiling of changes in the lipidome which can lead to the discovery of relevant lipids as potential biomarkers. This approach relies on robustly identifying a large number of lipids and statistically evaluating their relative abundances. We present an improved lipidomics identification workflow based on a combination of chromatographic and TIMS separation combined with a very fast data dependent MS/MS fragmentation (PASEF) showing increased numbers of identifications compared to classical fragmentation methods. Data acquisition was performed with a timsTOF PRO instrument (Bruker). The TIMS technology adds an additional dimension of separation. To evaluate this effect on lipidomics analyses a direct infusion method was applied to a critical pair of isobaric phopsphocholines (PC 18:1) differing only in the position of the unsaturation (9Z vs 6Z), these could be separated with a mobility peak resolution of 175. LC-TIMS-MS measurements in PASEF mode were performed on extracts of commercially available plasma samples from different species (bovine, chicken, pig and human). The resulting data sets were processed in a novel MetaboScape software version using a 4 dimensional feature finder designed to include the mobility separation dimension. The main precursor mass and the most intense fragment spectrum were exported to SimLipid software (Premier Biosoft) for identification. SimLipid compares all submitted fragment spectra with its internal lipid database (> 40,000 entries). First results show 200-400 unique lipid IDs (depending on animal species). Compared to analyses performed without TIMS/PASEF an increase of 30-50% in identifications can be observed.

P-199 Achieving high precision targeted metabolomics: challenges and application to diverse biological samples

PRESENTING AUTHOR: ***Michel Wagner, The Institute of Cancer Research, United Kingdom***

CO-AUTHORS: ***Alan Henley, Akos Pal, Florence Raynaud, Jyoti Choudhary***

Liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS) is a popular platform for targeted metabolomics. The selected reaction monitoring (SRM) mode and the separation performance offered by current LC columns are generally believed to provide sufficient selectivity for metabolite profiling. As such, analytical protocols typically rely on a single SRM transition per metabolite and generic LC gradients. However, several often-overlooked issues can compromise reliable metabolite identification and quantitation, and therefore lead to misleading interpretations. A chemical library comprising 185 polar metabolites has been build up and covers diverse biochemical structures and key metabolic pathways. The analytical system consisted in a SCIEX QTRAP 6500 QqQLIT instrument and a Shimadzu Nexera X2 LC system. MS-infusion experiments were performed to acquire MS and MS/MS spectra and several SRM transitions were optimized for each metabolite. The resulting spectral library was essential for method development and optimisation. A range of HILIC columns comprising different chemistries and mobile phases were investigated to determine optimal LC conditions. A qualitative / quantitative method, relying on multiple SRM transitions per metabolite and data-dependent acquisition (DDA) of MS/MS spectra has been established. The method was then used to systematically evaluate the interferences from complex biological samples including tissues, fluids and cells (mouse kidney, liver, spleen, muscle, heart, lung, brain, plasma and human cells). Our results highlight the importance of 1) carefully controlled chromatography to avoid cross-talk issues (e.g. isobaric species and in-source fragmentation) and 2) using multiple SRM and DDA for accurate quantitation and high-confidence metabolite identification.

P-200 Observation of acetyl phosphate formation in mammalian mitochondria using real-time in-organelle NMR metabolomics

PRESENTING AUTHOR: ***Hoonsik Nam, Seoul National University, Korea, South***

CO-AUTHORS: ***Wen Jun Xu***

Recent studies point out the link between altered mitochondrial metabolism and cancer, and detailed understanding of mitochondrial metabolism requires real-time detection of its metabolites. Metabolomics studies use cell lysates and measure metabolites at a fixed time point, resulting in unavoidable metabolite loss during sample preparation and impossibility of monitoring metabolism in real-time. Employing heteronuclear 2D NMR and 13C3-pyruvate, we propose in-organelle metabolomics that allows for the monitoring of mitochondrial metabolic changes in real-time. The approach identified acetyl phosphate synthesis from pyruvate in human mitochondria for the first time, whose production has been largely neglected in eukaryotic metabolism since its first description about 70 years ago in bacteria. The kinetic profile of acetyl phosphate formation was biphasic, with initial increase and rapid degradation. This transient nature suggested its role as a new metabolic intermediate in mitochondria. The method also allowed for the estimation of pyruvate dehydrogenase (PDH) enzyme activity through monitoring of the acetyl-CoA formation, independent of competing cytosolic metabolism. The results confirmed the positive regulation of mitochondrial PDH activity by p53, a well-known tumor suppressor. This approach enabled us to detect unstable metabolite acetyl phosphate which was hardly known in human, and acetyl-CoA formation in real time, overcoming limitations of previous studies relying on metabolites extraction of whole cell. Our method can be applied to further studies of mitochondrial role in cancer, other organelle-specific metabolism and mitochondria-related disease such as Alzheimer's, Parkinson's and muscular dystrophy.

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NEW ADVANCES

P-201 A Universal Method for Untargeted Metabolomics Applications

PRESENTING AUTHOR: ***Tatjana D. Talamantes, Thermo Fisher Scientific, United States***

CO-AUTHORS: ***Ioanna Ntai, Shannon Eliuk, Amanda L. Souza***

Mass spectrometry has become a significant tool in the field of untargeted metabolomics applications. The necessity of identifying and covering a wide range of endogenous metabolites with a variety of analyte concentrations has become rigorous and time intensive, specifically when determining optimal method settings for these different metabolomics discovery workflows. This investigation focuses on developing a universal method on a Thermo Scientific™ Orbitrap Fusion™ Tribrid™ Mass Spectrometer optimized for metabolomics discovery experiments. The untargeted metabolomics universal method adjusts parameters "on-the-fly" according to spectral complexity/intensity in order to maximize the number of compounds selected for fragmentation in a single run. This improved sampling of the metabolome does not compromise fragmentation quality. On the contrary, it allows for high quality fragmentation spectra to be collected even for precursors of low abundance, which consequently provides an increase in metabolite annotation when searched against spectral libraries, such as mzCloud™. Developing a universal metabolomics method involved optimization of parameters such as maximum injection time and AGC target which impact quality and quantity of spectra per run. The metabolomes of reference samples of Human Plasma (NIST SRM1950), E. coli cell extract (Cambridge Isotopes), and Green Tea Extract (NIST SRM3255) were extracted and then analyzed by data dependent MS/MS in positive mode. Data was processed using Thermo Scientific™ Compound Discoverer™ software searching against mzCloud and Chemspider to obtain metabolome coverage. Employing this universal analytical method for untargeted metabolomics workflows successfully improves metabolome annotation without need for sample-specific method optimization.

P-202 Development of a comprehensive analytical method for dipeptide using LC-MS/MS and CE-MS/MS

PRESENTING AUTHOR: ***Hitoshi Ozawa, Institute for Advanced Biosciences, Keio University, Japan***

CO-AUTHORS: ***Akiyoshi Hirayama, Takamasa Ishikawa, Tomohito Doke, Takuji Ishimoto, Shoichi Maruyama, Tomoyoshi Soga, Masaru Tomita***

Dipeptides are contained in fermented food such as soy sauce, bean paste and Japanese sake, some of which are known to exhibit various physiologically active actions including anxiolytic, hypotensive and analgesic actions. However, comprehensive analysis of these dipeptides is challenging due to structural isomers (e.g. Gln-Gly and Gly-Gln) exists in each dipeptide. In this study, we developed the comprehensive analytical method for the analysis of dipeptides with both liquid chromatography-tandem mass spectrometry and capillary electrophoresis-tandem mass spectrometry. Under optimized analytical conditions, we successfully detected 328 dipeptides separated with its structural isomers and the results of method validation for reproducibility, linearity, sensitivity and recovery were acceptable. The method was applied to the comprehensive analysis of dipeptides in liver tissue obtained from diabetic mouse. As a result, more than 200 dipeptides were detected in mouse liver. Among them, several dipeptides showed significant difference depending on the difference in diet (normal diet and high fat diet). The method provides high accuracy and sensitivity for the quantification of 328 dipeptides and thus could be a powerful tool for the comprehensive analysis of dipeptides.

P-203 Open biphasic microfluidics for on chip metabolite extraction

PRESENTING AUTHOR: ***Ulri Nicole Lee, University of Washington, United States***

CO-AUTHORS: ***Ulri N. Lee, Jean Berthier, Jiaquan Yu, Erwin Berthier, Ashleigh B. Theberge***

Liquid-liquid extraction, a common sample preparation method, partitions small hydrophobic molecules from a complex aqueous matrix into an organic phase for downstream analysis by mass spectrometry. Here, we present an open microfluidic device that enables stable biphasic interfaces for extraction of molecules directly from microscale cell culture. The device consists of a lower aqueous channel for cell culture and an upper organic solvent channel; the aqueous and organic channels are connected through geometrically tuned microscale apertures. Broader applications of this technology include the ability to culture limited numbers of cells from patients while streamlining the sample preparation/analysis workflow for improved personalized medicine and cancer diagnostics. The metabolite profiles observed after analysis are, in part, dependent on the polarity of the extraction solvent. To extend the use of this device to a broader range of solvents, we derived equations to optimize the aperture size based on solvents with varying polarity, density, and interfacial tension. We tested our model with organic solvents (cyclohexane, ethyl acetate, and chloroform) interfaced with cell culture media in 125-800 μm square apertures and identified conditions for stability. Importantly, the open nature of this device enables the user to add and remove microliter quantities of solvent over the aqueous filled apertures with simple pipetting. This microscale extraction method is an advance from traditional liquid-liquid extraction methods because it enables direct coupling to microscale cultures, full recovery of microliter volumes of solvent, and can be arrayed for multiplexed, high throughput studies.

POSTER SESSIONS 1 AND 2 – Monday and Tuesday – all odd number presenters will be at their posters.
POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

NEW ADVANCES

P-204 Vasodilation-on-a-Chip: A novel microfluidic device to study the effect of inflammatory metabolites on blood vessel dilation

PRESENTING AUTHOR: ***Ashley M. Dostie, University of Washington, United States***

CO-AUTHORS: ***Erwin Berthier, Ashleigh B. Theberge***

Effective treatment of chronic inflammation requires an understanding of the complex pathways that generate pro-inflammatory metabolites. Blood vessel dilation (e.g., vasodilation) enables increased blood flow and immune cell recruitment during inflammation. Thousands of small molecule metabolites are produced during inflammation, and the role of the majority of these metabolites in vasodilation is not yet fully identified. Current methods for exploring these factors are inadequate, and do not facilitate the throughput needed to study large metabolite libraries. Here, we present vasodilation-on-a-chip, a novel microfluidic device that can be used to study a variety of metabolic pathways. We encapsulate primary human vascular smooth muscle cells (VSMCs) in type I collagen within a mold designed so that a lumen is generated upon demolding. After gelation, the collagen is removed from the mold so that it is completely detached from any surfaces. This allows the collagen gels to be completely deformable, enabling a dynamic readout of dilation or constriction of the gel upon treatment with molecules that participate in the regulation of blood vessel dilation. This dynamic system enables the treatment of embedded human VSMCs with pro-inflammatory metabolites including cytokines such as prostaglandins and other related fatty acid derivatives. This microscale platform reduces reagent and cell consumption, enabling the study of precious metabolites that are challenging to isolate in large quantities and the use of biologically relevant patient cells.

P-205 Novel Method of Short Chain Fatty Acids in Feces using GC-MS with Automated Sample Preparation

PRESENTING AUTHOR: ***Kuniyo Sugitate, Agilent Technologies, Japan Ltd., Japan***

CO-AUTHORS: ***Takeshi Furuhashi, Takashi Nakai, Yusuke Jikumaru, Genki Ishihara, Sadao Nakamura***

Short chain fatty acids (SCFAs) profiling has been a major topic in gut bacteria studies, while there are some technical difficulties attributed to the highly volatile and hydrophilic characteristics. In this study, an optimized method based on GC-MS with chloroformate derivatization was developed for SCFAs. The advantage of this method is instantaneous reaction in aqueous solution and no heating is required. Although methyl and ethyl esterification are the common, we concluded iso-butyl esterification, the combination of iso-butyl alcohol and iso-butyl chloroformate was the best derivatization. Due to increase in molecular size by butyl esterification, butylated SCFAs can be separated from reagent peak and chromatographic peak resolution can be also improved. As for SCFAs analysis in biological samples, it requires rapidly sample treatment to prevent degradation after sampling. Our protocol is 1: Extract samples with ultrapure water then add iso-butanol and pyridine. Samples are stored at a freezer until sample measurement. 2: Derivatization by iso-butyl chloroformate. 3: n-Hexane extraction for derivatized SCFAs. 4: GC-MS Measurement. Automated sample preparation and GC-MS analysis (2-4) was carried out in a sequential way using GC-MS autosampler which has sample preparation function. Calibration results (R2) in each SCFA from 4-40 pg to 1 ng on-column in scan mode were above 0.995. With regard to biological sample, mammalian feces samples were investigated and acetic acid, propionic acid, butanoic acid and valeric acid were detected as distinct compounds.

P-206 Quantitation of nicotine, nicotine-derived nitrosamine ketone, and their major metabolites in exposed extract using liquid chromatography with high-resolution accurate mass spectrometry.

PRESENTING AUTHOR: ***Quentin Dutertre, Philip Morris International R&D, Switzerland***

CO-AUTHORS: ***Sandra Sendyk, Caroline Mathon, Arno Knorr, Mark Bentley***

Cigarette smoke (CS) contains several thousands of compounds, representing a challenging matrix for analytical investigation. Its highly complex composition, combined with the multiplicity of xenobiotic biotransformation routes, adds complexity for studies concerning the metabolism of compounds derived from CS or aerosol from heat-not-burn tobacco products, like the Tobacco Heating System (THS 2.2). Nicotine can be used as a biomarker of exposure, and its metabolism pathway is well established. Tobacco-specific N-nitrosamines, derived from the nitrosation of nicotine and related compounds, are present in substantial quantities in tobacco, CS, and smokeless tobacco. The procedure was designed for quantitation of nicotine, nicotine-derived nitrosamine ketone, and their major metabolites in extracts of cellular systems exposed to CS and THS aerosol. A liquid chromatography method using two columns (anion exchange and pentafluorophenyl) in series was developed and coupled to high-resolution accurate mass spectrometry in full-scan positive electrospray ionization mode. This method (liquid-chromatography, high-resolution, accurate-mass mass spectrometry (HRAM LC-MS)) provides a sample extraction with multiple stable isotope labelled internal standards and enables quantitation of all compounds of interest within one single run. The method was validated testing selectivity, limit of detection, linearity, precision, and accuracy. The method can be used for simultaneous quantitation of multiple compounds in exposed extracts. The combination of two columns in series enables separation of a broad range of metabolites in terms of structural diversity (e.g., the separation of glucuronic acid conjugates from other less hydrophilic metabolites) and is well suited for xenobiotic metabolism investigation in complex matrices.

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NEW ADVANCES

P-207 Data Independent Acquisition Improves Metabolite Coverage over Traditional Data Dependent Techniques for Untargeted Metabolomics

PRESENTING AUTHOR: ***Zuzana Demianova, SCIEX, Germany***

CO-AUTHORS: ***Cyrus Papan, Joerg Dojahn, Baljit K. Ubhi***

Data independent acquisition (DIA) workflows are well adopted in quantitative discovery proteomics, but still not commonly used in discovery metabolomics. Data dependent acquisition (DDA) techniques are heavily employed in the field of metabolomics. Here, we describe how DIA enables the identification of a higher number of metabolites for untargeted metabolomics workflows compared to traditional DDA techniquesthus enabling a broader profile of the metabolome. At the DDA level, the data demonstrate a significant improvement of metabolite coverage at the MSMS level when comparing the top5 to top25 DDA method. We show over 100% increase of metabolite coverage in plasma extracts by increasing the number of selected precursor ions for DDA acquisition. This result highlights the capability of the QqTOF mass analyzer for fast MSMS acquisition, which allows for the fragmentation of a large number of precursors in a single DDA cycle = a larger number of metabolites identified. We also applied these approaches to common matrices used in metabolomics studies. When comparing the performance in extracted plasma it can be observed that applying a DIA approach with 20 variable windows allows ~55% gains in metabolite coverage versus the top20 DDA acquisition, similar gains as seen in the urine extract. Finally, we shown that quantification on MSMS level provide better selectivity and higher S/N ratio, which allows lower LOQ. More confident MSMS based identifications lead to higher quantifiable metabolites, which at the end allows better understanding of the biology.

P-208 Metabolomic Profiling of Caenorhabditis elegans Using Capillary Electrophoresis Mass Spectrometry (CE-MS)

PRESENTING AUTHOR: ***Brianna M. Garcia, University of Georgia, United States***

CO-AUTHORS: ***Patience Sanderson, Franklin E. Leach III, I. Jonathan Amster, Arthur S. Edison***

Metabolomic studies aim to effectively identify metabolic products related to specific biological problems. This is commonly achieved through the use of analytical separation techniques such as gas and liquid phase chromatography. In capillary electrophoresis (CE), compound separation is dependent on the analyte size and charge, and is therefore well suited for the analysis of polar metabolites. Due to the fundamental differences in the separation mechanism of CE versus chromatographic methods, CE-MS metabolomics has the potential to identify metabolites previously not detected by LC or GC based methods. In this study, metabolites extracted from a mixed population of N2 wild isolate Caenorhabditis elegans were separated on an Agilent HP 3D CE coupled to an EMASS-II CE-MS interface used to introduce the separated sample into a Thermo Fisher Orbitrap Elite mass spectrometer. Consistent with prior literature, extracted ion electropherograms for select signals fell within the expected migration window with times between 4 and 17 minutes. Preliminary assignments have been made for metabolites including arginine and sucrose/trehalose with measured masses within +/- 2ppm of theoretical values. These identifications have also been made by MALDI-FT-ICR MS of the same mixture. Our preliminary results suggest further optimization of the normal polarity separation conditions is required. Upon completion, additional experiments will include reverse polarity negative ion mode CE-MS. In addition, comparative studies of unique and overlapping metabolites found between CE-MS, NMR, and prior in-lab results from MALDI-FT-ICR MS and LC-MS will be performed to determine to robustness of CE-MS based untargeted metabolomics.

P-209 Development of GC/MS based quantitative metabolome analysis methodology by Calibration-Curve-Locking database

PRESENTING AUTHOR: ***Takeshi Bamba, Kyushu University, Japan***

CO-AUTHORS: ***Kosuke Hata, Yuki, Soma, Toshiyuki Yamashita, Masatomo Takahashi, Kuniyo Sugitate, Takeshi Serino, Hiromi Miyagawa, Kenichi Suzuki, Kayoko Yamada, Takatomo Kawamukai, Teruhisa Shiota, Yoshihiro Izumi, Takeshi Bamba***

Calibration-Curve-Locking Databases (CCLD) have been constructed for automatic compound search and semi-quantitative screening by GC/MS in fields of forensic medicine and residual pesticide measuring. CCLDs contain the retention time, calibration curve and electron impact ionization mass spectrum obtained under stable apparatus condition. We constructed a novel CCLD for metabolomics study field. All standard compounds and biological samples were subjected to GC-MS just after the derivatization under stable apparatus conditions using following strategies. 1: DFTPP tuning for reproducible and uniform mass spectrum. 2: Retention-Time Locking technique to fix the retention times. 3: Optimization of derivatization conditions for high sensitivity analysis. 4: Automation of derivatization followed by injection to GC-MS by PAL RTC (CTC Analytics AG). One target (quantifier) ion and one or more qualifier ion were selected for each compound based on the results of standard substances analysis, and a calibration curve was obtained by plotting the peak area ratio of the target compound to the IS versus the amount of target compound. These data were registered as the novel CCLD using MassHunter Quantitative Analysis software (Agilent Technologies), which enables automatic compound search and quantification by target deconvolution and quantification algorithm. We examined the applicability of the constructed database to analyzing SRM1950 Metabolites in Human Plasma samples, resulting time- and labor-saving semi-qualitative screening without the need for standard substances.

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NEW ADVANCES

P-210 Mass spectrometric analysis of purine de novo biosynthesis

PRESENTING AUTHOR: ***Tomas Adam, Palacky University Hospital, Czechia***

CO-AUTHORS: ***Lucie Mádrová, Matyáš Krijt, Veronika Barešová, Jan Václavík, David Friedecký, Dana Dobešová, Olga Součková, Václava Škopová, Marie Zikánová, Tomáš Adam***

Purine nucleotides have vital functions in nucleic acid synthesis, energetic homeostasis, cell signalling and others in both prokaryotes and eukaryotes. Supply of purines is provided by two pathways – salvage pathway and de novo synthesis (PDNS). Although PDNS activity varies during cell cycle, it becomes an important source of purines especially for rapidly dividing cells. Method for detail studying of PDNS is missing due to analytical reasons (sensitivity) and commercial unavailability of the compounds. The aim was to fully describe mass spectrometric fragmentation behaviour of newly synthesized PDNS related metabolites and build up analytical method. With the exception of four initial ribotide PDNS intermediates that preferred losing water, phosphate or cleave forming base of purine ring, all the other metabolites studied cleaved the glycosidic bond in the first fragmentation stage. Fragmentation was possible to 4th-6th stages. Liquid-chromatography-high resolution mass spectrometric method was developed and applied in the analysis of CRISPR-Cas9 genome-edited HeLa cells deficient in individual steps of PDNS and salvage pathway. Identity of newly synthesized intermediates forming under pathological conditions of known and theoretically possible defects of PDNS was confirmed by comparing fragmentation patterns of synthesized metabolites of PDNS with those produced by cells. Use of stable isotope incorporation allowed confirmation of fragmentation mechanisms and provide data for future fluxomic experiments. The method may find its use in diagnosing of PDNS disorders, investigation of purinosome formation, cancer research, enzyme inhibition studies and other applications. Supported by the MEYSCR LO1304, the MHCR (AZV 15-28979A), programmes PRIMUS/17/MED/6 and by IGA_LF_2018_010.

P-211 Improved LC/MS Methods for the Analysis of Anionic Metabolites

PRESENTING AUTHOR: ***Alex Apffel, Agilent Research Laboratories, United States***

CO-AUTHORS: ***Jordy J. Hsiao, Oscar G. Potter, Genevieve C. Van de Bittner, Te-Wei Chu, Hongfeng Yin***

Phosphorylated and carboxylated analytes are difficult to detect or have poor peak shapes in LC/MS experiments due to trace metals within the chromatography systems. Previous methods addressed this through LC system passivation and/or metal chelators such as ethylenediaminetetraacetic acid (EDTA) as mobile phase additives. However, the metal chelators are highly ionizable and cause ion suppression of target analytes. To ameliorate this problem, solvent additives were investigated to reduce metal-analyte interaction with minimal ion suppression effects. Metabolite standards were initially used to assess the performance of several chemicals as solvent additives compared to EDTA. Analytes were separated using Agilent's InfinityLab Poroshell 120 HILIC-Z PEEK-lined columns on a 1290 LC system coupled on-line with a 6545 Q-TOF. The additivewith the best performance wasthen used in experiments to monitor nutrients and metabolic waste in growth media from K562 leukemia cells and to examine intracellular metabolite changes from cells treated with 5 µM methotrexate (MTX) or vehicle (DMSO). Data will be presented showing that addition of chemical additiveat low micromolar concentrations to the mobile phase for LC/MS analysis enhanced the sensitivity of negatively charged molecules with minimal or no signal suppression. The additive improved the peak shape and signal strength for a wide variety of anionic analytes including organic acids, nucleotides, sugar phosphates and phosphopeptides.

P-212 Quantitative Lipidomics in Drosophila melanogaster

PRESENTING AUTHOR: ***Lisa Fan Bettcher, University of Washington, United States***

CO-AUTHORS: ***Cynthia B. Le, Daniel Raftery***

Drosophila melanogasteris a widely used model organism in biomedical research, owing to its short life cycle, ease of culturing and genetic modification, and its ability to produce large quantities of externally laid embryos. Metabolomics offers a powerful approach for the investigation ofDrosophila,including the functions of genes and enzymes under a variety of stresses. Thebroad basedanalysis of lipids offers visualization of energy homeostasis and signaling as well as understanding the membrane structure of the flies. The newLipidyzerplatform, consisting of an ABSciexQTRAP 5500 MS/MS andSelexIONdifferential mobility spectrometry,is capable of measuringup to 1100 unique lipid species from 13 lipid classes with absolute quantitation (in µM) in human serum. However, no method currently exists for the analysis of lipids inDrosophilaby this platform. To overcome this challenge, we have developed a new method for the quantitative analysis of lipids in theDrosophilamodel, which currentlymeasures around 540 lipids in the fly.Thelipid concentrationswere linear insamplesbetween0-20mg(R2> 0.99). In comparison with human serum, thelipid profile ofDrosophilacontainedfatty acids with moderate hydrocarbon chain length, an abundance of phosphatidylethanolamines andtriacylglycerols, and a lack of ceramides.Furthermore,female flies had higher concentrations ofcholesterol esters,lysophosphatidylethanolamines,lysophosphatidylethanolamines, anddiacylglycerolsthan male flies, while males hadhigher concentrations of freefatty acids.In this presentation, we describe our new protocol starting from sample preparation to obtaining the absolute concentration of lipids in flies.

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NEW ADVANCES

P-213 Fabric Phase Sorptive Extraction - A metabolomic pre-processing approach for ionizing radiation injury assessment

PRESENTING AUTHOR: ***Alexandra Taraboletti, Georgetown University, United States***

CO-AUTHORS: ***Alexandra Taraboletti, Maryam Goudarzi, Abuzar Kabir, Bo-Hyun Moon, Jerome Lacombe, Pelagie Ake, David Brenner, Frederic Zenhausern, Albert Fornace Jr.***

The modern application of mass spectrometry (MS)-based metabolomics to the field of radiation-injury and biodosimetry has allowed for high-throughput screenings to identify biomarkers related to ionizing radiation (IR) exposure. Valuable work, done by our team, on radiation biodosimetry and IR injury assessment in easily accessible biofluids (such as urine, blood, saliva) has revealed unique metabolic perturbations in response to radiation quality, dose, and dose rate. Nevertheless, the employment of swift injury assessment in the case of a radiological disaster still remains a challenge as current sample pre-processing can be time-consuming and cause sample deterioration, among other issues. To address these concerns, our team has developed a novel workflow using a MS-compatible fabric phase sorptive extraction (FPSE) technique. The FPSE employs a matrix coated with sol-gel poly(caprolactone-b-dimethylsiloxane-b-caprolactone) (PCL-b-PDMS-b-PCL) that binds both polar and non-polar metabolites in whole blood and serum, minimizing the sample pre-processing steps and improving analyte recovery. Here we confirm that the FPSE technique coupled with UPLC-ToF (Waters G2) can distinguish diverse radiation exposure markers such as taurine, linoleic acid, carnitine, and phosphatidylcholines found 24 hours after 8 Gy irradiation. We also note the effect different fabric materials coated with PCL-b-PDMS-b-PCL have on both metabolite extraction efficiency and temporal stabilization at room temperature. These findings suggest that the FPSE novel approach has the potential to expedite IR injury assessment via biomarker screening by providing a novel method to stabilize biofluids between collection and sample analysis.

P-214 Automated MALDI Magnetic Resonance Mass Spectrometry (MRMS) for biomarker based determination of diabetes during pregnancy

PRESENTING AUTHOR: ***Christopher J Thompson, Bruker Daltonics Inc., United States***

CO-AUTHORS: ***Franklin E. Leach III, Christopher J. Thompson, Jeremy J. Wolff, Jacquelyn Walejko, Anushka Chelliah, Maureen Keller-Wood, Gary Kruppa, Arthur S. Edison***

The occurrence of pregestational diabetes leads to an increased risk of poor fetal and maternal outcomes during the course of pregnancy. In comparison to non-diabetic women, these individuals have a predisposition to variations in glycolysis, glyconeogenesis, and fatty acid metabolism. The data available to determine metabolic changes during this period is limited. Here, we seek to determine the metabolomic alterations in serum and urine of pregnant women with diabetes and compare them to non-diabetic controls during both the intrapartum and post-partum periods by MALDI-MRMS. The selection of a MALDI in conjunction with the high mass accuracy and resolving power of MRMS has enabled increased sample throughput and the direct interrogation of high salt samples (e.g. urine) without the need for any sample prep or purification. In conjunction with advanced acquisition strategies, data has been acquired in a fraction of the time previously required to facilitate high quality data with high sample throughput required for large clinical sample sets. In our preliminary data, samples from three subgoups of women have been examined in positive ion mode. Each acquisition required less than 5 minutes to acquire.We have been able to identify molecular compositions that correspond to over 100 lipid species with a mass error less than 250 ppb. Current efforts focus on the application of multivariate statistics. Future work will extending this MALDI MRMS approach to the full clinical sample set, additional statistical analyses, and the correlation of NMR/MS spectral features to facilitate unknown identification through approaches such as SUMMIT.

P-215 Real-time in-vivo metabolomics of a multicellular eukaryote using HR-MAS NMR

PRESENTING AUTHOR: ***Michael Thomas Judge, University of Georgia, United States***

CO-AUTHORS: ***Michael T. Judge, Yue Yu, John Glushka, James Griffith, Jonathan Arnold, Arthur S. Edison***

Nuclear Magnetic Resonance (NMR) is inherently quantitative and reproducible, and yields a wealth of structural information, making it an ideal platform for untargeted metabolomic approaches where metabolites are either collected in the form of biofluids or extracted before analysis. This approach works well for collecting data on large numbers of independent samples. Metabolomic time-series experiments, on the other hand, tend to be quite labor-intensive and produce data which can be difficult to interpret reliably using this approach. For instance, the trends observed for a given feature are actually statistical composites of static observations on multiple independent samples which must account for both biological and technical variation (e.g. variable extraction efficiency). High-Resolution Magic Angle Spinning (HR-MAS) is a technique which allows high-resolution NMR on mixed-phase samples, thereby permitting untargeted metabolomics without extraction and even in-vivo measurements on microorganisms. We extend HR-MAS to conduct real-time, in-vivo, multi-day metabolomic measurements of single samples of the filamentous fungus, Neurospora crassa. We evaluated the reproducibility of this technique for a classic genetic and biochemical model organism, and developed a first generation of computationaltools for analysis of these novel data. With a temporal resolution on the order of minutes, we find that this technique allows for a rich and unprecedented view of metabolism for a multicellular eukaryote and uncovers properties about NMR metabolomics data that would be difficult or impossible to decipher with traditional approaches.

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NEW ADVANCES

P-216 Separation of anionic metabolite isomers using capillary ion chromatography-differential mobility spectrometry-mass spectrometry

PRESENTING AUTHOR: ***Akiyoshi Hirayama, Keio University, Japan***

CO-AUTHORS: ***Masaru Tomita, Tomoyoshi Soga***

[Introduction] Ion chromatography-mass spectrometry (IC-MS) has potential to be a new platform for the global analysis of charged compounds in metabolomics. We evaluated the use of capillary IC-MS for comprehensive anionic metabolite analysis and obtained good method validation results. However, several isomers which play important roles in central metabolism, such as 2-phosphoglycerate and 3-phosphoglycerate, glyceraldehyde 3-phosphate and dihydroxyacetonephosphate, glucose 1-phosphate and mannose 1-phosphate, could not be separated. In this study, we demonstrate the applicability of ion mobility spectrometry techniques for the separation of these isomers. [Methods] IC-MS analysis was performed using a Dionex ICS-5000+ system (Thermo Fisher Scientific, San Jose, CA) equipped with a Triple TOF 5600 system (SCIEX, San Jose, CA) with a differential mobility spectrometry (DMS) cell (SelexIONTM). An Agilent 1100 series capillary HPLC pump (Agilent Technologies, Waldbronn, Germany) was used to deliver sheath liquid. Anionic metabolites were separated on a Dionex IonPac AS11-HC-4 µm (0.4 × 250 mm, 4 µm; Thermo Fisher Scientific) that was maintained at 35°C. [Preliminary results] When applying DMS, the compensation voltage should be optimized for each isomer. The choice of modifier, which improves the separation efficiency, is also important. Under these optimized parameters all isomers tested were completely separated. Although sensitivity was slightly lower by the use of ion mobility cell, it can be adequately applied to metabolome analysis. [Novel aspects] The developed IC-DMS-MS could be a powerful new tool for anionic metabolome analysis.

P-217 NMRbox; Reproducible computing for computational NMR metabolomics

PRESENTING AUTHOR: ***Hesam Dashti, UW-Madison, United States***

CO-AUTHORS: ***Hesam Dashti, Mark W. Maciejewski, Adam D. Schuyler, Michael R Gryk, Ion Moraru, Michael Wilson, Gerard Weatherby, Jon R Wedell, Kumaran Baskaran, Pedro R Romero, Eldon L Ulrich, Frank Delaglio, Hamid R Eghbalnia, Jeffrey C Hoch***

NMRbox is a free software platform for academic, government, and non-profits that bundles a wide range of NMR software packages into a near zero-configuration virtual machine (VM). The NMRbox VM can be accessed as a cloud-based Platform-as-a-Service (PaaS) or locally via a download. To date the NMRbox VM has well over 100 different NMR software packages installed covering all aspects of NMR data processing and analysis and currently has over 800 registered users.

P-218 Differential Mobility Separation (DMS)-based separation of bile acid isomers

PRESENTING AUTHOR: ***Randy Arnold, SCIEX, United States***

CO-AUTHORS: ***Dietrich Merkel, Cyrus Papan, Joerg Dojahn***

Bile acids are involved in a wide range of biological functions including lipid resorption, immunological functions and metabolic regulation. Through metabolic transformations, isomeric and isobaric variants are generated, which makes the unequivocal identification and quantification of individual chemical species difficult. Differential Mobility Separation (DMS) is an ion mobility technology which separates molecules based on their dipolar moment and used for the separation of bile-acid isomers. While the combination with chromatographic separation may improve selectivity, the separation power of DMS is sufficient at determining isomers through infusion-based quantification without the need for LC development. Using DMS, bile acid standards were infused to determine the compensation voltages (CoV) of the different isomers. The compensation voltage was ramped over a range of 30V to 0V. The results showed almost baseline separation of the different isomers. DMS showed a marked reduction of chemical background resulting in improved signal to noise ratio, enabling better quantitation.

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NEW ADVANCES

P-219 A Wellness Study Using Microflow Targeted Metabolomics to Investigate the Effects of Diet and Exercise on the Metabolome

PRESENTING AUTHOR: ***Khatereh Motamedchaboki, SCIEX, United States***

CO-AUTHORS: ***Khatereh Motamedchaboki, Baljit Ubhi***

Liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis is an essential tool for identification and quantitation of metabolites in complex samples like urine and plasma to investigate the affected metabolic pathways in dietary assessment studies, precision medicine, biomarker discovery and large population-based studies to evaluate the effects of nutritional, pharmaceutical, and environmental exposures. Self-testing is on the rise as more people become interested in monitoring their health with wearable technology and related applications available today to advance into more simplified and meaningful health related data to the everyday consumer. Here, we have analyzed dried blood spots (DBS) with microflow LC coupled to a triple quadrupole mass spectrometry system from a wellness study and monitored over 300 polar metabolites covering all major metabolic pathways. Screening these polar metabolites across the three metabolic states (fasted, fed and active) identified several metabolites that change during these conditions, which are clearly differentiating these three metabolic states. DBS extracts from 3 different spots/ sample which shows no significant variation between different sampling. This method provides accuracy and precision across each of the different DBS extracts obtained from each group. The microflow targeted metabolomics analysis of DBS shows to be a rapid, sensitive, and accurate method for profiling polar metabolites in a variety of biofluids and could be utilized for monitoring these targeted polar metabolites in other wellness-based studies as the method successfully differentiated these three different conditions we monitored during this wellness study.

P-220 Molecular profiling of isoprenoid compounds in blood and liver

PRESENTING AUTHOR: ***Jun Han, University of Victoria, Canada***

CO-AUTHORS: ***Alexandria Doerfler, Ayrea Hurley, William R. Lagor, James Hui, Christoph H. Borchers***

Isoprenoids contain multiple isoprene units in each molecule and play important biochemical functions in eukaryotes, archaea, and some bacteria. Due to high hydrophobicity and the wide molecular weight distributions, many isoprenopids are not readily analyzed by LC-MS using electrospray ionization or atmospheric pressure chemical ionization and are even not analysable by gas chromatography. We developed a new LC-MS method with silver-assisted ionization for molecular profiling of different isoprenoids in human and mouse specimens. The total isoprenoids in blood and liver were quantitatively extracted using single-step liquid-liquid extraction (LLE) and were analyzed by ultrahigh-performance liquid chromatography / high-resolution mass spectrometry on an LTQ-Orbitrap instrument and with 25 μM of silver ion in the mobile phase for coordination-assisted ionization. Identification of menoquinones, ubiquinones, ubiquinols, dolichols, polyprenols, squalene and oxidosqualene was based on accurate mass measurements and MS/MS spectral interpretation, in comparison with some available authentic compounds. After appropriate method optimization and validation, sensitive (middle to high femtomoles) and precise (CVs <12%) quantitation of the isoprenoids in mouse liver was achieved. Combining this method with other two LC-MS methods, which we developed for quantitative analyses of isoprenyl phosphates and sterols respectively, full-profile analyses of the metabolic intermediates and end products in the liver of wild type and gene knockout mice along the mevalonate pathway and its downstream isoprenoid and cholesterol biosynthesis pathways were conducted. The observed up- or down-regulated metabolites at the different branching points of metabolism confirmed the successful depletion of the targeted Fdft1gene in the animals.

P-221 How can ion mobility-mass spectrometry provide deeper structural studies of plant secondary metabolites?

PRESENTING AUTHOR: ***Tim Causon, BOKU Vienna, Austria***

CO-AUTHORS: ***Pieter B. Venter, Stephan Hann, André de Villiers***

Currently of critical importance in the emerging use of ion mobility-mass spectrometry (IM-MS) to support metabolomics studies is establishing the validity of results obtained across different types of commercially available instrumentation. With no established approach for deriving true collision cross section (CCS) distributions for metabolites in the gas phase, only systematic analytical evaluation and inter-laboratory comparisons can be used to establish conditional values for supporting metabolite annotation. Critical factors in such measurements, including the influence of ion heating, instrument duty cycle, and calibration to a traceable set of reference values must all be considered in order for CCS distributions to be reliably reported as a conditional metabolite identification point. Therefore, in this study we have investigated both drift tube (DT) and travelling wave (TW) IMS-MS instrumentation to investigate monomeric and oligomeric phenolic metabolites in plant extracts. In addition to the good agreement observed for the CCS values of identified monomeric phenols, the gas phase behavior of oligomeric species was found to be reproducible, independent of the instrumentation. Multiple drift peaks observed on both instruments for oligomeric tannins indicate the presence of several conformers of these species which could not be separated chromatographically and may correspond to rotational isomers or protomerization. Both IM analysers also provided cleaner mass spectra filtered according to drift time, which significantly improved data quality and formula annotation according to accurate mass and isotope patterns. Consistent data on both instruments were obtained for a range of phenolic compounds, including phenolic acids, procyanidins, ellagitannins, gallotannins, flavanols and flavonols.

POSTER SESSIONS 1 AND 2 – **Monday and Tuesday –** all odd number presenters will be at their posters.
POSTER SESSIONS 3 AND 4 – **Wednesday and Thursday –** all even number presenters will be at their posters.

NEW ADVANCES

P-222 Results of an International Ring Trial for the Biocrates AbsoluteIDQ p400HR Targeted Metabolomics Kit

PRESENTING AUTHOR: ***Barbara Wolf, BIOCRATES Life Sciences AG, Austria***

CO-AUTHORS: ***Lisa St. John-Williams, Tuan Hai Pham, Therese Koal, Anastasia Kalli, Andreas FR Huhmer, John A. Bowden, Stormy Koeniger, Steven Cepa, Florence I Raynaud, Akos Pal, Yasmin Asad, Catherine L. Winder, Andrew Southam, Mark Viant, Warwick Dunn, Cornelia Prehn, Jerzy Adamski, Tong Shen, Gregory Byram, Oliver Fiehn, Luiz Valdiviez, Rupasri Mandal, Jiamin Zheng, Lun Zhang, David Wishart, Facundo M. Fernandez, David A. Gaul, Catherine G. Vasilopoulou, Florian Meier, Matthias Mann, Fuad J Naser, Gary J Patti, Viet D Dang, David J. Borts, M. Arthur Moseley, J. Will Thompson***

Targeted metabolomics has gained popularity due to the ability to rapidly and confidently quantify metabolites. One advantage of using standardized platforms is to enable consistent measurements across experiments and laboratories. We have conducted an international ring trial of the new AbsoluteIDQ p400HR (Biocrates AG), a kit which quantifies up to 408 metabolites across 11 different metabolite classes, using Q Exactive™ Orbitrap mass spectrometers (Thermo Scientific™).Blinded samples (including plasma and serum from male and female donors and plasma from mouse and rat) and kit were provided to each laboratory. Data were collected and submitted to Duke University for aggregation.Of the 408 metabolites, 251 analytes are consistently and reproducibly quantified, including 21 amino acids, 14 biogenic amines, 16 acylcarnitines, 14 lysophosphatidylcholines, 93 phosphatidylcholines, 6 ceramides, 24 sphingomyelins, 12 cholesteryl esters, 14 diacylglycerols, 36 triacylglycerols, and total hexoses. Amino acids were universally and reproducibly quantified (5% intra-lab CV, 12% inter-lab CV). Biogenic amines and total hexoses also perform extremely well (<14% inter-lab CV). Acylcarnitines and lipids showed slightly higher inter-laboratory variance (inter-lab %CV averages between 23% for sphingomyelins and 38% for Lyso-PC). Outlier assessment using PCA shows no significant bias due to geographical location or Q Exactive platform. PCA and hierarchical clustering also shows higher quantitative variance between individual plasma samples than between laboratories.This supports the hypothesis that the p400HR kit will empower translational medicine efforts by enabling accurate metabolite quantitation, an aspect important for comparisons between laboratories and clinical cohorts. Research use only. Not for use in diagnostic purposes.

P-223 An Infusion “Shotgun” Approach for High-Throughput Untargeted Metabolomics.

PRESENTING AUTHOR: ***Devin Keller, SCIEX, United States***

CO-AUTHORS: ***Marialice Maldini, Baljit K Ubhi***

Metabolomics studies the complexity and variety of various chemical compounds. Mass spectrometry-based untargeted metabolomics requires approaches to collect data in an unbiased fashion. LC approaches are biased to the column chemistry and metabolites which can be retained. Infusion or a “shotgun” approach for untargeted metabolomics, allows the collection of the MS/MS of all possible candidates, enabling identification, quantitation and retrospective analysis of the data. Thus, a rapid metabolic fingerprint of each biological sample is captured. By optimizing for mass range, one method was developed to capture all relevant metabolites in a high-throughput mode. Verifying the method through a simple cell line extract case study highlighted the metabolites responsible for differentiating the different sample groups. The “shotgun” approach for Metabolomics results in a powerful and rapid tool to obtain huge and complementary data of numerous samples even if metabolites present only at very low levels in biological samples.

P-224 Development of the high-resolution data-independent acquisition of MS/MS analysis

PRESENTING AUTHOR: ***Yasumune NAKAYAMA, Graduate School of Engineering, Sojo University, Japan***

CO-AUTHORS: ***Takeshi Bamba, Eiichiro Fukusaki***

Data independent acquisition (DIA) is promising technology for biological studies. In metabolomics, comprehensive MS/MS aquisition strongly support non-target analysis. One of the major DIA system, SWATH acquisition is well-established and practical software for metabolomics is developed. Whereas, a wide Q1-window sometimes generate mixed spectra of metabolites in closed m/z and retention time. This problem makes connection of precursor ion and product ion difficult. To solve the problem, we newly developed high-resolution DIA system. In our system, MS/MS spectra are acquired by sequential wide Q1 windows like SWATH. However, the windows are programed to slide at each cycle (here after sliding-SWATH). Because the product ions of a precursor belong to different windows at each cycle, exclusive MS/MS spectra from co-eluted precursor ions could be selected at post-acquisition analysis. To verify the principle, acquisition method that sequential windows are slid at each cycle was constructed on Q-TOF/MS TT5600+ (SCIEX). Acquired data were analyzed by script on the R language with the xcms package. As a result, product ion of co-eluted and closed m/z precursor peaks was distinguished by exclusive MS/MS spectra. This system would contribute high-resolution DIA and make all-MRM of metabolomics possible.

POSTER SESSIONS 1 AND 2 – Monday and Tuesday – all odd number presenters will be at their posters.
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NEW ADVANCES

P-225 A Framework for Ultrafast Metabolic Phenotyping Utilizing Isotopic Fine Structure and Ultra-High Resolution Magnetic Resonance Mass Spectrometry

PRESENTING AUTHOR: ***Matthew R. Lewis, Imperial College London, United Kingdom***

CO-AUTHORS: ***Matthias Witt, Aiko Barsch, Nikolas Kessler, Christopher J. Thompson, Luke Whiley, Elena Chekmeneva, Jake T. M. Pearce, Jeremy K. Nicholson***

As biobanks and clinics open their doors to the promise of metabolic phenotyping for understanding population health, powerful analytical solutions capable of large-scale deployment are needed. The chemical complexity of clinical samples is traditionally measured by mass spectrometry hyphenation with GC or LC. While powerful, the performance of these approaches is dependent on the time invested in chromatographic separation, limiting sample throughput and narrowing the metabolic coverage to only those species amenable to the chromatographic method used. High sample throughput is achieved for complex clinical samples by removing the time-dependent LC step. While this may initially seem counterintuitive, the superior resolving power of the FIA-MRMS can easily resolve 1,000's of compounds from complex human urine samples in a single mass spectrum and is analogous to petroleum based MRMS workflows that can produce over 10,000 compounds in one infusion run. This workflow permits the detection of isotopic fine structure (IFS) which directly provides the molecular formula of the unknown metabolite through resolution of heavy neutrons provided by heteroatoms in a molecule. Matching of extracted features to known metabolites derived from the HMDB database enabled an assignment of approximately 300 metabolites in combined positive and negative ESI data, including highly polar compounds often not readily seen in LCMS workflows. This approach expands the metabolome coverage and increases confidence in molecular assignment while achieving the sample analysis throughput necessary to increase the speed of clinical studies and tackle very large cohorts in population health.

P-226 Extractive Ratio Analysis NMR Spectroscopy for Improved Unknown Metabolite Identification in Crowded Spectra

PRESENTING AUTHOR: ***Liladhar Paudel, University of Washington, United States***

CO-AUTHORS: ***G. A. Nagana Gowda, Daniel Raftery***

Ratio Analysis NMR spectroscopy (RANSY) provides a statistical approach to isolate spectra peaks from individual metabolites and thereby aid in their identification in biological mixtures. However, in some complex biological systems such as urine, signals from different compound classes increase the complexity of NMR spectra, which cause RANSY to be ineffective. As a remedy, selective extraction of a compound class brings a level of simplification to the spectra. Extraction conditions and/or solvents can be manipulated to limit the compounds extracted and give rise to simplified NMR spectra with varying peak intensities that can be modulated for further statistical analysis. Based on this approach, we describe extractive ratio analyisis NMR spectroscopy (E-RANSY) which constructs NMR subspectra of individual metabolites based on a single sample, and demonstrate its application for the identification of human urine metabolites. A set of samples are produced by extraction of a single urine sample using different extraction conditions, such as pH. RANSY is then applied to these extracted samples. This new method works well for the selective identification of carboxylic acid metabolites in urine samples. Further, an improvement to RANSY algorithm, which we refer to as S-RANSY, is provided. S-RANSY provides ratio analysis spectrum with better signal to noise ratio using the mean of peak ratios divided by the square of standard deviations to select metabolite peaks. Both E-RANSY and S-RANSY perform superior to correlation or our original ratio analysis method and exhibit high potential to identify unknown metabolites in complex biological mixtures.

P-227 Development of fully-automated SPE-GC-MS system enabling solid-phase analytical derivatization for the wide targets and the wide concentration range analysis

PRESENTING AUTHOR: ***Ryoichi Sasano, AISTI SCIENCE CO., Ltd., Japan***

CO-AUTHORS: ***Masahiro Furuno, Eiichiro Fukusaki***

In the metabolomics, generally, the analysis of amino acids, organic acids and sugars in the sample was done by using GC-MS in one analysis. However, several problems have emerged when the sugar concentration in the sample is too high. This causes difficulty for measuring low concentrations of amino acids and organic acids, due to the similarity of their retention time. Moreover, high level of sugars can also make the MS becomes dirty and the sensitivity decreases. In this study, we developed an online SPE-GC-MS system using automated solid-phase derivatization method with two-step sample loading for simultaneous analysis of low concentrations of amino acids and organic acids and high concentrations of sugars. In the first sample loading, 40 µL of the sample was loaded on the solid phase cartridge, and amino acids and organic acids were retained on the solid phase while sugars were removed. In the second sample loading, 1 µL of the sample was loaded on the same solid phase cartridge, sugars were retained on the solid phase. Next, the derivatization reagent was added to the solid phase, and derivatized on the solid phase. Subsequently, the derivatives were injected into the GC while eluting from the solid phase, and finally analyzed by GC-MS. As a result, the chromatogram was able to obtain each peak intensity within the same dynamic range. By using this system, simultaneous analysis for the sample containing low concentrations of amino acids and organic acids and high concentrations of sugars was accomplished.

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NEW ADVANCES

P-228 The creation and identification of an IROA sample for any sample type

PRESENTING AUTHOR: ***Chris Beecher, IROA Technologies, United States***

CO-AUTHORS: ***Irwin Kurland, Yunping Qui, Felice de Jong, Chris Beecher***

IROA patterns, in all previous reports, are due to isotopic enrichments in metabolites. These enrichments form distinct mathematical (binomial distribution) patterns from carefully controlled random mixtures of 12C and 13C isotopes. These patterns are unique for any molecular formula and are useful to support Identification and quantitation when used as Internal Standards in Metabolomics experiments. This presentation will highlight the use of a set of derivatization reagents which also create IROA patterns due to a (binomial) randomization design within the derivatization tagging system. While not as strong as the full IROA patterning, this system is still capable of providing a strong level of identification, verification, and quantitation. An IROA patterning derived from the binomial randomization tagging is useful in finding compounds and excluding artifacts, and in the use of Ion Mobility (here DMS) and fragmentation to assure identity. This system is very useful in situations when a pure compound-based IROA pattern is difficult, or expensive, to achieve, and allows the derivatization of an IROA tag to a QC sample for normalization/quantitation of all metabolites that can be so derivatized. Different derivation tags may be required for different chemical classes. Such situations are common for metabolomic profiling in many non-tissue assays, such as urine, CSF, or plasma.

P-229 High-throughput quantification of the levels and labeling abundance of free amino acids by liquid chromatography tandem mass spectrometry

PRESENTING AUTHOR: ***Jean Christophe Cocuron, BioDiscovery Institute, University of North Texas, United States***

CO-AUTHORS: ***Enkhtuul Tsogtbaatar, Ana Paula Alonso***

Amino acids (AAs) are produced from intermediaries of various biochemical pathways, and serve as building blocks for proteins. Protein synthesis is a key process in all living cells, and involves huge flow of carbon through central metabolism for AA production. 13C-based metabolic flux analysis is the most commonly approach to follow and quantify the flow of 13C-carbon throughout biochemical pathways. To date, the determination of AA mass isotopomer distributions (MIDs) relied on the analysis of proteinogenic rather than free AAs. The techniques commonly used are gas chromatography-mass spetrometry or nuclear magnetic resonance, which respectively requires derivatization or substantial amount of material. This work describes the development and validation of a high-throughput liquid chromatography tandem mass spectrometry method allowing the direct quantification of the levels and labeling of free AAs, without derivatization. Sensitivity in the order of the femtomol was achieved using multiple reaction monitoring mode. Finally, this method was successfully applied to determine the MIDs of a total of 18 free AAs extracted from maize embryos cultured with 13C-glutamine or 13C-glucose. Due to the increased application of tandem mass spectrometry for 13C-metabolic flux analysis and dynamic labeling studies, this novel method will enable the assessment of more complete and accurate labeling information of intracellular AAs, and therefore a better definition of the fluxes.

P-230 How to validate comprehensiveness for an LC-ESI method optimization analyzing tiny sample amounts

PRESENTING AUTHOR: ***Carsten Jaeger, Charité - Universitätsmedizin Berlin, Germany***

CO-AUTHORS: ***Jan Lisec***

A recurring challenge in many metabolomics laboratories is to yield a maximum of metabolic information from very limited amounts of sample material. To deal with this in liquid chromatography-mass spectrometry (LC-MS), a major strategy is to optimize the electrospray ionization (ESI) step, e.g. by using miniaturized (nano-ESI) or high-temperature (HT) ion interfaces (JetStream, IonBooster etc.). Potential drawbacks of using the latter, however, include increased analyte decomposition due to elevated nebulization temperature and pressure, leading to reduced chemical coverage achieved in the screening experiment. We here propose a comparative nontargeted evaluation procedure to be carried out in advance of experiments, based on chemical analysis of a defined biological test case followed by a dedicated data analysis procedure: we (a) identify “biological” features based on correlation with sample concentration, (b) inspect linear ranges of the latter over multiple sample concentration levels and (c) analyze the distribution of the features’ parameters over chemical classes. Applying this workflow to an IonBooster versus a standard ESI source indicated highly increased numbers of (true biological) features as well as unaltered relative abundances of organic acids, organic heterocyclic compounds and other major compounds classes. Validation of these results against a large target metabolite library (n>400) confirmed conclusions about the absence of discrimination in terms of ionization and fragmentation behavior. We discuss the transferability of our approach to related use cases and the potential of HT ESI for improving LC-MS metabolomics.

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NEW ADVANCES

P-231 Getting to know unknowns: GC-MS compound investigations with BinVestigate

PRESENTING AUTHOR: ***Matthew Mueller, UC Davis, United States***

CO-AUTHORS: ***Gert Wohlgemuth, Sajjan Singh Mehta, Diego Pedrosa, Oliver Fiehn***

Both GC-TOF MS vendor software and the free MS-DIAL software utilize automatic peak detection and mass spectral deconvolution, yielding approximately 1,000 pure spectra per chromatogram. We have developed the database BinBase that collects, manages, processes and stores all spectra in a consensus repository for over 14 years. Currently, BinBase has accumulated data for over 7,000 spectra in over 1,800 studies of more than 120,000 samples. We now open this database for comparisons and investigations through a public graphic user interface. Users can query any BinBase entry through data published in studies deposited in the NIH MetabolomicsWorkbench, or using own GC-MS data. This interface is called BinVestigate. For testing GC-MS spectra from own investigations, users have to paste spectra along with Kovats retention index information. The tool yields the most similar spectra at a given retention index window, using the most common 5% diphenyldimethylsiloxane columns. Once a bin has been selected, the application provides information about the compound, including if it has been annotated by the WCMC as a known structure, its prevalence of occurrence and its abundance in more than 100 species and more than 70 organs. In addition to the user-friendly front end, BinVestigate exposes a powerful REST API that allows more technical users to query the server directly. This opens the tool up to be used as a component in future workflows, and allows users to process the data directly in whatever way is most useful.

P-232 A Sensitive, High-Throughput LC-MS/MS Method for Measuring Catecholamines in Low Volume Serum

PRESENTING AUTHOR: ***Jiamin Zheng, University of Alberta, Canada***

CO-AUTHORS: ***Rupasri Mandal, David S. Wishart***

A robust, sensitive, high-throughput method for the detection and quantification of catecholamines in serum, including dopamine, 5-methoxytryptamine, tyramine, phenylethylamine, epinephrine, norepinephrine, metanephrine, and normetanephrine is described. It is based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) in a positive scheduled multiple reaction monitoring (MRM) mode. Key to the success of the method is the inclusion of an amine derivatization step, using phenylisothiocyanate (PITC), prior to liquid chromatographic separation of the targeted analytes on a C18 reversed-phase column. Mass spectrometric conditions, e.g., characteristic fragmentations and quantification transitions were also optimized to obtain maximum sensitivity and specificity. The limits of detection for all the target analytes are in the low nanomolar range. The recovery rates of spiked serum samples with three different concentration levels, i.e., low, medium, and high, are in the range of 93.2% to 113% with satisfactory precision values of less than 10.9%. This method was successfully applied to determine the concentrations of catecholamines in multiple human serum samples, with results closely matching those reported in the literature. Comparisons to other reported methods for measuring catecholamines indicate this new approach requires 10-20X less volume, making it ideal for targeted metabolomics studies with volume-limited samples. It is also amenable to analyzing samples in 96-well plate format. Unlike most of other catecholamine measurement methods described to date, our method uses pre-column derivatization instead of a more costly solid phase extraction (SPE) step. We believe this assay holds good promise for its future use or adoption by clinical testing laboratories.

P-233 Mass Spectrometry –based High-throughput Quantitative Assays and Applications to Metabolomics

PRESENTING AUTHOR: ***Rupasri Mandal, University of Alberta, Central African Republic***

CO-AUTHORS: ***Jiamin Zheng, Yichen Xia, Lun Zhang and David S. Wishart***

The Metabolomics Innovation Centre (TMIC) specializes in quantitative metabolomics assays for human, animal, plant and microbial samples. Most recently, TMIC has developed and adapted several quantitative assays to expand its list of detectable metabolites to include catecholamines, oxylipins, uremic toxins, one-carbon metabolites and organic acids. We have also developed several quantitative assays capable of measuring a large number of metabolites (>100) which are biomedically relevant. One such example includes 153 biomedically relevant metabolites (including amino acids, acylcarnitines, biogenic amines, lysolipids, organic acids, vitamins and uremic toxins). Another such example is development of an exposome assay which includes amino acids, vitamins, organic acids, fatty acids, and many metabolite markers related to environmental, toxins, and food exposures. These assays are based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) in multiple reaction monitoring (MRM) mode. Key to the success of the method is the inclusion of derivatization steps, prior to liquid chromatographic separation of the targeted analytes on a C18 reversed-phase column. MS conditions including characteristic fragmentations and quantification transitions were optimized to obtain maximum sensitivity and specificity. The recovery rates of spiked serum samples with three different concentration levels (i.e., low, medium, and high) are in the range of 95% to 110% with satisfactory precision values of less than 10%. These assays were used to successfully analyze human serum and urine samples, with results closely matching those reported in the literature. These new technologies along with a brief description of their applications in human health, agriculture, nutrition and other fields will be presented.

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NEW ADVANCES

P-234 An Ion Chromatography-MS1/DI-MS2 method for Stable Isotope-Resolved Metabolomics (SIRM) mapping of metabolic networks

PRESENTING AUTHOR: ***Qiushi Sun, University of Kentucky, United States***

CO-AUTHORS: ***Andrew N. Lane, Teresa W-M. Fan, Richard M. Higashi***

Introduction: The complexity of metabolic networks makes pathway mapping challenging. The SIRM approach is powerful for tracing the flow of atoms through metabolic pathways where ultra-high resolution (UHR) MS1 is essential for distinguishing isotopes and obtaining their total count. MS2 can help differentiate multiple biosynthetic pathways, but it is difficult to coexist with the time-consuming UHR-MS1 in a single injection. We have now developed a quantitative Ion Chromatography-UHR - MS1/DI-(data independent)-MS2 method that fits this need. We have applied this method to trace the atom fate of [U-13C]-glucose in A549 spheroids and [U-13C, 15N]-glutamine in BEAS-2B cells. Methods: Data-dependent acquisition (DDA-MS2) using a Thermo Fusion was first used to determine and match precursor and product ions to establish a compound-fragment table. Then IC-UHR-MS1/DI-MS2 was set up for target analysis based on this table. Polar metabolites were extracted from A549 spheroids and BEAS-2B cells with cold 70% methanol and acetonitrile/water/chloroform (V/V 2:1.5:1), respectively. Results: This method exhibited excellent reproducibility. The isotope enrichment distributions of the main metabolites from glycolysis, the Krebs cycle, the pentose phosphate pathway, nucleotides, and their 13C, 15N labeled fragments were obtained in both A549 spheroids and BEAS-2B cells. Conclusions: A quantitative IC-UHR-MS1/DI-MS2 method was developed and successfully employed for SIRM studies of both [U-13C]-glucose and [U-13C, 15N]-glutamine traced cancer cells. It provides the overall labeling distribution of metabolites via MS1 and simultaneously yields positional labeling information via MS2. Such information greatly enables the determination of multiple metabolomic pathways. Supported by NCI P01CA163223-01A1, 1U24DK097215-01A1, 1R01CA118434-01A2, 5R21ES025669-02 and P30CA177558)

P-235 Automated NMR and GC-MS Kits for Quantitative Metabolomics

PRESENTING AUTHOR: ***Danuta Chamot, University of Alberta - The Metabolomics Innovation Centre, Canada***

CO-AUTHORS: ***Matthias Lipfert, Manoj Rout, Mark Berjanskii, Yichen Xia, Edison Dong, Debjani Bhattacharyya Chowdhury, Jiamin Zheng, Rupasri Mandal and David S. Wishart***

Automation of metabolomics techniques such as nuclear magnetic spectroscopy (NMR) and gas chromatography mass spectrometry (GC-MS) remains a pressing challenge in the field of metabolomics. Both are powerful techniques, but are limited by the requirement of highly qualified personnel to operate equipment and perform time-consuming spectral profiling and data analysis. The Metabolomics Innovation Centre (TMIC) is developing easy-to-use kits that will streamline metabolomic workflows while increasing throughput and reducing costs. The goal of the kits is to provide everything necessary to run a metabolomics analysis, except the instrument. Our NMR kit contains all components required for 1H analysis, including buffer solution, deuterated internal standard and detailed instructions. The kit also contains an access code for a web-based, automated NMR spectral profiling server, which can automatically determine the concentration of up to 75 NMR-detectable metabolites with ~90% accuracy and ~10% quantification error in less than 5 minutes. This kit is compatible with serum and plasma samples. TMIC's GC-MS kit consists of a derivatization reagent, internal and alkane standards (C8-C20 and C22-C40), and detailed instructions. The kit also contains an access code for GC-AutoFit, an automated, web-based tool, which can identify and quantify up to 120 compounds in urine, serum, plasma, CSF and milk. These kits will enable a wealth of new metabolomics applications in both research and clinical settings.

P-236 Intelligent MSn workflow for maximizing confident metabolite annotations in untargeted metabolomics studies

PRESENTING AUTHOR: ***Ioanna Ntai, Thermo Fisher Scientific, United States***

CO-AUTHORS: ***Iman Mohtashemi, Martin Jones, William Nash, Ralf Tautenhahn, Graeme McAlister, Seema Sharma, Amanda Souza, Vlad Zabrouskov, Mark Viant, Warwick Dunn, Andreas Huhmer***

Metabolite annotation is a current bottleneck in the broad implementation of metabolomics, hindering biological interpretation of results. Here, we utilized an Orbitrap™ Tribrid™ Mass Spectrometer to develop an automated workflow that maximizes the number of metabolites interrogated by MS/MS and MSn, while minimizing the acquisition of uninformative spectra, in the analysis of human plasma (NIST SRM1950) During data-dependent MS/MS, ions are selected based on abundance, without any knowledge of biological relevance or type of ion. Often, irrelevant spectra, resulting from fragmentation of solvent, plasticizer and other background ions dominate the duty cycle, limiting the capacity of the instrument to acquire informative spectra. By enabling the automatic generation and implementation of a background exclusion list based on real-time feature detection in LC-MS data, background ion MS2 spectra were practically eliminated. Highly abundant compounds, in the form of a parent ion or any of its accompanying features, such as isotopes and adducts, may prevent the fragmentation of metabolites of lower abundance. By populating the inclusion list with the preferred ion for each metabolite, more compounds can be sampled by MS/MS in a single run. Additionally, by automatically updating inclusion and exclusion lists during analysis, we can ensure that compounds not selected for MS/MS will be prioritized during a subsequent injection. Implementing this intelligent workflow, in combination with MSn and different ion activation methods (HCD and CID), resulted in fragmentation of more unique metabolites and a greater number of metabolites confidently annotated, in this important reference material.

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NEW ADVANCES

P-237 Expansion of Targeted LC-MS/MS metabolomics to 300 metabolites. How many metabolites are enough?

PRESENTING AUTHOR: ***Robert Pepin, PhD, University of Washington, United States***

CO-AUTHORS: ***Mathew Ellenberger, M. Daniel Raftery, PhD, Tim R. Peterson, PhD***

A targeted LC-MS/MS metabolomics assay consisting of 200+ metabolites in 38 pathways is improved and expanded through the addition of 110 metabolites. 22 additional metabolic pathways or compound classes are represented in the new metabolites. In addition, the coverage of metabolites in 5 pathways was doubled. When combined with the existing pathways in the assay, this gives a fairly thorough coverage of many of the most important metabolic pathways in animal organisms while being broad enough to be of relevance to a broad array of sample types. Adding an additional 55% to the target list gave an improvement of 58% in the different biochemical processes or compound classes probed by this assay. Clearly, there is room for additional coverage within the existing pathways on our target list, but this begs the question: at what point does the addition of more metabolite targets have decreasing benefit? Data examining the feasibility of adding additional metabolites will be presented by looking at the distribution of individual metabolite CV values as a function of increasing target list size. Speculation and prediction about the feasibility and value of substantially increasing the target list will be shown as well. Finally, a metabolomics profile of k562 cancer cells subjected to various treatments will be presented utilizing this expanded target list.

P-238 Emerging Comprehensive Lipidomics Platform for Cancer Research

PRESENTING AUTHOR: ***Gillian Mackay, Cancer Research UK, Beatson Institute, United Kingdom***

CO-AUTHORS: ***Sergey Tumanov, Jurre Kamphorst***

Global changes in lipid metabolism occur when cells undergo transformation and are recognised as a hallmark of many types of cancer. Changes in a lipid profile of a cell can have profound effects on cell metabolism. Investigating the changes in different lipid classes and molecular species in cancer cells is clearly important in understanding their crucial role in supporting tumour growth and for identifying novel therapeutic targets for cancer treatment. Lipidomics is the comprehensive identification and quantification of the thousands of cellular lipid molecular species. Despite recent advances in separation technology and mass spectrometry, detection and measurement of all lipids species remains challenging. The most popular method for lipid extraction is based on chloroform, which does not extract the more polar lipids. We have shown that butanol-based extractions can be used for many of these polar lipids and this method nicely complements our chloroform extractions. Similarly, different liquid chromatography mobile phase solvents are required to measure lipids of very different hydrophobicity. Most phospholipids, glycerides and cholesterol esters exhibited good analytical performance using acetonitrile-isopropanol liquid chromatography gradient, but lipids containing free phosphate groups displayed limited retention and extensive peak broadening. A water-methanol gradient mitigated these issues and provided optimal separation of these and other polar lipids. We found that a combination of two extraction procedures and two types of gradient on a single UPLC column provides a practical approach towards truly comprehensive lipidomics.

P-239 Automated Iterative LC-MS/MS Data Acquisition Improves Coverage of the Plasma Lipidome

PRESENTING AUTHOR: ***Mark J Sartain, Agilent Technologies, Inc., United States***

CO-AUTHORS: ***Timothy J Garrett, Jeremy P Koelmel***

A major challenge in mass spectrometry-based lipidomics is the comprehensive characterization of a large and diverse set of lipid species, spanning a wide concentration range within a biological sample. Coupling liquid chromatography (LC) to MS helps reduce the complexity of the ionized lipid population and can also help elucidate isomeric lipid species. Confident lipid annotation requires data acquisition at the MS/MS level to enable product-ion spectral matching against in-silico generated databases. However, comprehensive lipid annotation from data-dependent (Auto) LC-MS/MS data is generally limited by the number of precursors that can be selected for fragmentation during chromatographic elution. Due to concentration bias this strategy often misses important lipid species of low abundance. Here we introduce a new, fully-automated mode of Q-TOF data acquisition where precursors previously selected for MS/MS fragmentation are excluded on a rolling basis. By excluding both background ions and previously selected high abundance lipid ions for fragmentation, sequential injections provide fragmentation of lower abundance lipid species. As a proof-of-principle, we applied this new Iterative MS/MS functionality to plasma lipid extracts to explore the potential benefits. Compared to replicates of conventional Auto MS/MS analyses, sequential injections of Iterative MS/MS significantly increased lipid annotations in plasma, and showed specific advantage in spectra-dense regions of the chromatogram. In addition, increased coverage of lipid classes of low abundance, such as DAGs and lipids with odd-chain fatty acids, was obtained with Iterative MS/MS. Together these results demonstrate that lipidome coverage can be improved with Iterative MS/MS, a useful addition to the lipidomics toolbox.

POSTER SESSIONS 1 AND 2 – Monday and Tuesday – all odd number presenters will be at their posters.
POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

NEW ADVANCES

P-240 **Derivatized Globally Optimized Targeted Mass Spectrometry (dGOT-MS)**

PRESENTING AUTHOR: ***Renke Zhang, China Agricultural University, China***

CO-AUTHORS: ***Xinyu Zhang, Haiwei Gu, Ping Zhang, Dan Du, Gowda GA, Liladhar Paudel, Daniel Raftery***

dGOT-MSincorporates the advantages of 15N-cholamine tagging and advanced MS methods to improve carboxylic acid detection. 15N-cholamine tagging improves MS detection by adding a positive charge to carboxylic acids, and thus all the carboxylic acids labeled with 15N-cholamine can be detected in positive mode, irrespective of the pH or solvent conditions of the eluting media used for chromatographic separation.15N-cholamine derivatization significantly enhanced the carboxylic acids detection limit in dGOT-MS. For the 172 carboxylic acids standards, the detection sensitivity improved by 3 orders of magnitude. In addition, dGOT-MS has high specificity for the detection of carboxylic acids, since carboxylic acids labeled with 15N-cholamine have the loss of 59.0 Da in neutral loss scanning. Importantly, dGOT-MS uses a QQQ-MS machine, but in a global manner to detect carboxylic acids, with wide coverage and high relibility. As a result, we obtained 516 precursor ions and 1,468 MRM transitions (~500 identified thus far) in the mass range of 130-730 Da from a urine sample. dGOT-MS has excellent analytical performance for global carboxylic acid measurements. In addition, we obtained 300 dGOT-MS MRM transitions from heart cell samples. dGOT-MS was significantly better than traditional global profiling using a quadrupole-time of flight (Q-TOF) instrument of similar vintage, for differentiating control cells from those with ACC2 inhibition. In summary, our results showed that dGOT-MS is a novel and useful method for the global profiling of carboxylic acids, with high potential to be applied in many metabolomics studies.

SYSTEMS BIOLOGY, NETWORKS

P-241 **PDK3 is essential regulator of PDH flux and could be a novel therapeutic target of POD linked diseases.**

PRESENTING AUTHOR: ***HYE JIN HAM, Gwangju Institute of science and technology (GIST), Korea, South***

CO-AUTHORS: ***Byung-Gyu Kim***

Dysfunctions of pyruvate dehydrogenase (PDH) activity have been well known to be involved in pyruvate oxidation defects (POD) linked diseases such as diabetes, neuropathy or cancer. However, the fine tuning mechanisms of in vivo PDH activity and TCA metabolic flux, perturbed by PDH regulating factors such as pyruvate dehydrogenase kinases (PDKs), are largely remain elusive. We generated all isoform of PDKs knock out chromic myeloid leukemia (CML) HAP1 cell lines, respectively. First, we observed that phosphorylation dependent inhibition activity to PDH was almost abolished in PDK3 KO HAP1 cells (K3KO), and proliferation rate of which cells was also significantly slower than other isoform of PDK KO or wild type HAP1 cells with down-regulated SIRT1 and cyclin D1 levels. Second, survival related PI3K/AKT/beta-catenin axis also elevated in K3KO and which level was more boosted by insulin or insulin growth factor 1 (IGF1) stimulation. Third, by using liquid chromatography tandem mass spectrometry-multiple reaction monitoring (LCMS-MRM) based 13C-metabolic flux analysis (MFA) of TCA cycle metabolite, we discovered that in vivo PDH flux was significantly increased in PDK3 KO hap1 cells (89%) even if all isoform PDK KO HAP1 cells showed a higher PDH activity (average 76.25%) than wild type (55%). Hyperactivity of PDH may induce both cell cycle arrest and survival but co-suppression of PDK activity and PI3K/AKT pathway could efficiently led to an apoptosis of CML like HAP1 cells. PDK3 is essential regulator of PDH flux and could be a novel therapeutic target of POD linked diseases.

P-242 **Unravelling complex diseases by precision-medicine: application of system's biology and medicine**

PRESENTING AUTHOR: ***Mariona Jové, University of Lleida, Spain***

CO-AUTHORS: ***Joaquim Sol, Rosanna Cabré, Irene Pradas, Omar Ramírez, Rebeca Berdún, Manuel Portero-Otín, Reinald Pamplona, Aurora Pujol***

The current knowledge on hereditary spastic paraplegia (HSP) and related motor disorders suggests that axonal degeneration is a final consequence of disturbances in diverse processes such as neuron myelin composition or mitochondrial dysfunction, among others. X-adrenoleukodytrophy is amotor neuron disease caused by the alteration of a single gene, ABCD1, but this alteration results in very diverse clinical phenotypes. To date, there is very few knowledge on the cellular and biological basis for explaining these differences in clinical phenotypes and there is not an effectivetreatment. In order to elucidate novel biomarkers of prognosis and to define potential therapeutic pathways of X-ALD, QTOF-based untargeted metabolomic and lipidomic analyses were performed. The results show a modulation on the biosynthetic pathway of specific neurotransmitters in X-ALD patients opening a new window forfurther application of approved drugs to treat this pathogenesis. Further, we offer several lipid species (involved in signalling, structural and bioenergetic functions) as a good candidates as a motor neuron diseases biomarkers. All in all, we conclude that the application of system's biology deriveddisciplines such as metabolomics and lipidomics can pave the way for development of personalized medicines.

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SYSTEMS BIOLOGY, NETWORKS

P-243 Effects of traditional Chinese medicine prescription, Er-Zhi-Wan, on Yin deficiency syndrome of liver and kidney based on metabolomics and network approach

PRESENTING AUTHOR: ***Weifeng Yao, Nanjing University of Chinese Medicine, China***

CO-AUTHORS: ***Weifeng Yao, Yuanyuan Zhai, Jia Xu, Li Zhang***

The therapeutic principles and goals of traditional Chinese medicine (TCM) are different from those in Western medicine. Aging is a process closely linked with Yin deficiency syndrome of liver and kidney, therefore, the term of aging may be employed to explain the syndrome. A novel approach using metabolomics coupled with network approach provides the feasibility for the basic research associated with the study of biological mechanism. In this paper, the metabolites in serum and urine were clustered in metabolic pathways to build compound networks, respectively. According to two well-established node centrality measures, five hub metabolites, namely, arachidonate, L-Arginine, testosterone, taurine and 2-Oxoglutarae were screened. To explore the data further, a gene-protein-metabolite network was constructed through the integration of network analysis and metabolomics, which was used as an effective approach to explain the molecular mechanism of TCM syndrome, yin deficiency of liver and kidney, and the intervention of Er-Zhi-Wan. The results indicated that the syndrome was related to the metabolic pathways of Sphingolipid metabolism, Primary bile acid biosynthesis, Arginine and proline metabolism, Taurine and hypotaurine metabolism, Steroid hormone biosynthesis et al. The shortest pathways of the gene-protein-metabolite network involved aging genes such as ACO1, PTGS1 and CAV1. Our results showed that combining metabolomics and network analysis can provide new strategies and ideas for the interpretation of pathogenesis of disease with full consideration of “gene-protein-metabolite”.

P-244 Acetylation of malate dehydrogenase 1 promotes adipogenic differentiation via activating its enzymatic activity

PRESENTING AUTHOR: ***Eun Young Kim, Daegu Gyeongbuk Institute of Science & Technology/Core Protein Resources Center, Korea, South***

CO-AUTHORS: ***Hyo Eun Kim***

Acetylation is one of the most crucial post-translational modifications that affect protein function. Protein lysine acetylation is catalyzed by acetyltransferases, and acetyl-CoA functions as the source of the acetyl group. Additionally, acetyl-CoA plays critical roles in maintaining the balance between carbohydrate metabolism and fatty acid synthesis. Here, we sought to determine whether lysine acetylation is an important process for adipocyte differentiation. Based on an analysis of the acetylome during adipogenesis, various proteins displaying significant quantitative changes were identified by LC-MS/MS. Of these identified proteins, we focused on malate dehydrogenase 1 (MDH1). The acetylation level of MDH1 was increased up to 6-fold at the late stage of adipogenesis. Moreover, overexpression of MDH1 in 3T3-L1 preadipocytes induced a significant increase in the number of cells undergoing adipogenesis. The introduction of mutations to putative lysine acetylation sites showed a significant loss of the ability of cells to undergo adipogenic differentiation. Furthermore, the acetylation of MDH1 dramatically enhanced its enzymatic activity and subsequently increased the intracellular levels of NADPH. These results clearly suggest that adipogenic differentiation may be regulated by the acetylation of MDH1 and that the acetylation of MDH1 is one of the cross-talk mechanisms between adipogenesis and the intracellular energy level.

P-245 Multi-Omic Characterisation of Bladder and Lung Carcinomas using a Novel Scanning Quadrupole DIA Acquisition Method

PRESENTING AUTHOR: ***Lee A. Gethings, Waters, United Kingdom***

CO-AUTHORS: ***Adam King, Robert S. Plumb***

Cancer is one of the most complex, life threatening diseases, existing in many forms which have unknown pathogenesis. A combination of genetic and lifestyle factors are known to contribute towards increasing the probability of encountering cancer. Lifestyle factors such as smoking are known to contribute towards both lung and bladder cancer, with lung cancer providing over 230,000 diagnosed cases in the United States annually. Here, we describe a multi-omic approach to reveal molecular factors that may be involved in these biomolecular processes. Combining lipidomic, metabolomic and proteomic approaches have helped to identify multi-factorial disease associated components and their related pathways. Plasma samples from three biological states of varying phenotype (control, bladder and lung carcinoma) were used with each group consisting of plasma from six individuals. Protein, metabolite and lipid extracts were LC separated and data acquired using a DIA method, whereby the quadrupole (MS1) was continuously scanned between m/z 400-1000 (lipids/metabolites) and 400-900 (peptides). A quadrupole transmission width of approximately 10 Da and 20 Da were employed for lipidomic/metabolomic and proteomic analyses respectively. The data were processed using a variety of informatic tools and searched with respective databases, providing normalized quantitation results. Biological significance of the results was established by merging data from all three omic experiments and performing pathway analysis. A number of significant pathways including complement activation, B cell mediated immunity and receptor signalling were identified as key pathways.

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SYSTEMS BIOLOGY, NETWORKS

P-246 Cell-type resolved human lung lipidome reveals cellular cooperation in lung function

PRESENTING AUTHOR: ***Charles Ansong, Pacific Northwest National Laboratory, United States***

CO-AUTHORS: ***Jennifer E. Kyle, Geremy C. Clair, Erika M. Zink, Kent J. Bloodworth, Anil K. Shukla, Erin S. Baker, Jeffrey A. Whitsett, Charles Ansong***

The lung is a complex organ comprised of multiple cell types each playing overlapping and niche roles in facilitating normal lung development and function. Cell type-resolved organ maps hold significant promise in facilitating/providing a deeper understanding of human organ functions. Here we applied liquid chromatography-tandem mass spectrometry to characterize the lipidome of major lung cell types (epithelial, endothelial, mesenchymal and mixed immune) isolated from three 20 month old human donors. This represents the first lipidome map of any organ. We coupled this with cell type-resolved proteomics of the same samples which provided complementary proteomics analyses substantiating the functional identity of the isolated cells. Lipidomics analyses identified over 300 unique lipid species across 5 lipid subclasses. The lipidome showed significant variations across major human lung cell types, with differences most evident at the subclass and intra-subclass (i.e. total carbon length of the fatty acid chains) level. Specifically, we noted (i) an enrichment of bis(monoacylglycerol)phosphate (BMP) in immune cells, (ii) support for of lipofibroblasts in mesenchyme cells, (iii) precursor lipids for signaling functions in immune cells, and (iv) and enrichment of lipid classes (e.g., ceramides and diacylglycerophosphoserines (PS) with strong roles in apoptosis and blood coagulation in the endothelial. Further, lipidomic signatures revealed an overarching posture of high cellular cooperation within the human lung to support critical functions. Our complementary cell type-resolved lipid and protein datasets serve as a rich resource for analyses of human lung function.

P-247 Quantitative proteomic and metabolic characterization of primary human enterocytes as an in vitro model for first-pass drug metabolism studies

PRESENTING AUTHOR: ***Abdul Basit, Department of Pharmaceutics, University of Washington, United States***

CO-AUTHORS: ***Mathew Karasu, Albert Li, Bhagwat Prasad***

Cryopreserved primary human enterocytes are considered ideal in vitro system for evaluation new chemical entities for their first-pass metabolism liabilities. However, unlike primary hepatocytes, the enterocytes are not well characterized. In this study, we quantified drug metabolizing enzymes (DMEs) and subcellular marker proteins in human enterocytes (n=10 donors) and compared the quantitative DME abundance with human hepatocytes (n=5 donors). Pooled human enterocytes (IVAL) and human hepatocytes (Lonza) were subjected to membrane or cytosol isolation followed by trypsin digestion followed by surrogate peptide LC-MS/MS analysis per established protocols (Prasad et al., CPT, 2016). A testosterone metabolomics study was also performed. CES2 and UGT2B17 abundance was similar (within 2-fold) in human enterocytes and human hepatocytes confirming important role of these enzymes in the intestinal first-pass metabolism. However, the relative abundance of CYP3A4, CYP3A5, CYP2C19, CYP1A2, CYP reductase, cytochrome b5, UGT1A1, and UGT1A4, UGT2B7, UGT2B15, and ADH1C, was 8-180-fold lower in the enterocytes vs. hepatocytes. CYP2B6, CYP2C8, CYP2C9, CYP2D6, CYP2E1, FMO3, UGT1A6, UGT1A9, ADH1A, AOX and CES1 were only detected in human hepatocytes. Following subcellular marker proteins were detected both in human enterocytes and enterocytes in high abundance: nuclear (lamin B1), mitochondrial (Hsp60), cytoskeleton (vimentin and desmin), ER membrane (CYP reductase), ER lumen (calreticulin), peroxisomes (catalase), and cytosol (Hsp-90 beta and LDH-H). Testosterone metabolic activity was observed in the human enterocyte model, where testosterone glucuronide, 6-beta-hydroxy-testosterone and androstenedione were detected as the major metabolites. Therefore, quantitative proteomics and metabolomics was successfully utilized to characterize the novel enterocyte model for drug metabolism prediction.

P-248 Metabolomics and transcriptomics approach in COPD

PRESENTING AUTHOR: ***Charmion Cruickshank-Quinn, University of Colorado, Anschutz Medical Campus, United States***

CO-AUTHORS: ***Sean Jacobson, Grant Hughes, Roger L. Powell, Irina Petrache, Katerina Kechris, Russell Bowler, Nichole Reisdorph***

Background: Chronic obstructive pulmonary disease (COPD) is a chronic lung disease comprising multiple phenotypes including airflow obstruction, emphysema, and frequent exacerbations. The goal of this pilot study was to delineate pathways associated with these various COPD outcomes. Methods: Blood was collected from 149 current or former smokers with or without COPD. Peripheral blood mononuclear cells were analyzed using Affymetrix Human Genome U133 plus 2.0 Gene Array. Plasma was analyzed using untargeted liquid chromatography mass spectrometry-based metabolomics. Statistically significant transcripts ($p \leq 0.015$, FDR≤0.1) and compounds ($p \leq 0.05$, FDR≤0.15) were mapped to biological pathways using IMPaLA. Gene transcript interactions and metabolite pathway interactions were examined using ConsensusPathDB. Results: There were 175 and 60 unique compounds associated with exacerbation severity and exacerbation frequency, respectively; these compounds were mainly amino acids and carbohydrates. There were 367 and 82 unique transcripts associated with emphysema and exacerbation frequency, respectively. For the interactions analysis, exacerbation severity had more pathway interactions than exacerbation frequency. The omics pathway analysis showed that glycerophospholipid metabolism was associated with worse airflow obstruction and more COPD exacerbations while sphingolipid metabolism was associated with lung function outcomes and exacerbation severity involving hospitalizations. Fat digestion and absorption was associated with impaired FEV1/FVC, arginine and proline metabolism with exacerbation severity, and oxidative phosphorylation with emphysema. Conclusion: The combination of two omics strategies allowed us to gain additional pathway coverage. Overlaying transcriptomics and metabolomics data across pathways discerned outcome and phenotypic differences and identified potential mechanisms, pathways, and molecular targets for intervention studies in animal models.

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SYSTEMS BIOLOGY, NETWORKS

P-249 Integrating metabolomics and transcriptomics data highlights metabolic pathway dysregulation in breast cancer

PRESENTING AUTHOR: ***Jalal Khalid Siddiqui, The Ohio State University, United States***

CO-AUTHORS: ***Andrew Patt, Bofei Zhang, Senyang Hu, Elizabeth Baskin, Mingrui Liu, Joseph McElroy, Kevin Coombes, Ewy Mathé***

Metabolomics data are increasingly integrated with other omics data, such as transcriptomic. To facilitate this integration, we have developed IntLIM (Integration through LInear Modeling) to uncover gene-metabolite pairs that are significantly correlated in one phenotype and oppositely or not correlated in another. While this approach does not model the complexities of biochemical reactions (reaction rates and mechanisms), co-regulated genes and metabolites tend to be associated with functional roles and our approach can thus help identify putative gene-metabolite associations to be tested experimentally. To this point, we applied IntLIM to metabolomics and transcriptomics data from tumor (N=61) and adjacent non-tumor (N=47) breast tissue from a previously published breast cancer study. Our goal is to identify gene-metabolite associations that are specific to tumors, their types (e.g. ER+), and other clinical factors (e.g. race). Further, we extend IntLIM to identify clusters of gene-gene and metabolite-metabolite pairs that are correlated in subsets of our samples. To better interpret the biological significance of phenotype-specific pairs, we performed pathway enrichment analysis, which includes clustering of significant pathways by their content overlap, using our comprehensive database RaMP (Relational database of Metabolomics Pathways). RaMP integrates KEGG, HMDB, Reactome, and WikiPathways, and thus contains up-to-date and comprehensive annotations for genes and metabolites. IntLIM (https://github.com/Mathelab/IntLIM) and RaMP (https://github.com/Mathelab/RaMP-DB) are publicly available and contain user-friendly web interfaces. Application of these approaches to breast cancer data provides mechanistic insight into tumor growth/development especially with regard to cancer sub-types and other clinical factors. This insight will assist in the development of therapeutic strategies.

P-250 Qualitative Flux Analysis of LCLs to Study Metabolome Changes and Cellular Energy Flux Among Passages

PRESENTING AUTHOR: ***Songjie Chen, Stanford University, United States***

CO-AUTHORS: ***Yuqin Dai, Lihua Jiang, Michael Snyder***

Lymphoblastoid cell lines (LCLs) have been widely used for genetic and functional research, however, the consistence and dynamics of the cellular metabolome among different clones and passages are mostly unknown. We present a novel solution that applied LC/Q-TOF MS metabolomics profiling and qualitative flux analysis to study the consistence of LCL metabolome among passages and the variation of the cellular energy flux. A robust IP-RP based Q-TOF LC/MS method was developed for metabolomics profiling of broad classes of endogenous metabolites and qualitative flux analysis of pathways of interest. The retention time reproducibility and compound separation of the IP-RP chromatography combined with the high resolution and mass accuracy of the Q-TOF resulted in improved selectivity, sensitivity, and reproducibility for metabolites in metabolomics profiling and flux studies. The metabolomics analysis of the standard reference LCLs indicated a diverse covariation of metabolites among different passages and clones. The qualitative flux analysis is further studied to demonstrate the consistence of flux changes. The metabolites with relatively high consistence or high variation are characterized as the quality control for further metabolomics analysis of more LCLs genotyped in the ENCODE project, which will demonstrate the variation of gene expression among individuals and populations at the metabolic level.

P-251 Integrated Metabolomic and Transcriptomic Analysis of Human Macrophages Co-Cultured with Pseudomonas aeruginosa Biofilms

PRESENTING AUTHOR: ***Amanda L. Fuchs, Montana State University - Bozeman, United States***

CO-AUTHORS: ***Sage Schiller, Valerie Copie, Mary Cloud B. Ammons***

Pseudomonas aeruginosa is a Gram-negative, facultative anaerobic bacterium that has been associated with acute and chronic wound infections. Dissimilar to acute wounds, chronic wounds fail to progress through the reparative stages of wound healing in a timely, orderly manner and feature a prolonged inflammatory response. Over 50% of chronic wounds in the U.S. demonstrate colonization by P. aeruginosa biofilms, immobile microbial communities encased in an extracellular polymeric substance. Bacteria growing within a biofilm have several advantages over planktonic microbes, including greater antibiotic resistance and an enhanced ability to evade the endogenous immune system. Acute wound healing is achieved through the coordinated efforts of multiple host cells, including but not limited to neutrophils, macrophages, fibroblasts, and keratinocytes. Macrophages, a type of phagocyte, are of notable interest in the context of chronic wounds because they are heavily involved in the transition from inflammation to proliferation during wound healing. Since this transition is delayed in chronic wounds, we are investigating the impact of P. aeruginosa biofilms on human macrophages using a simplified in vitro co-culture model. Using 1D 1H NMR-based metabolomics, our studies indicate that macrophage metabolism is differentially modulated in the presence of P. aeruginosa biofilms relative to control macrophages. In addition, we are utilizing qPCR arrays to determine alterations in macrophage gene expression upon co-culture with P. aeruginosa biofilms. By correlating our metabolomics and transcriptomics data sets we will gain a better understanding of how P. aeruginosa biofilms impact macrophage metabolism and function to interfere with the host innate immune response.

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SYSTEMS BIOLOGY, NETWORKS

P-252 SIMPLEX: from extraction to underlying molecular mechanisms in heart

PRESENTING AUTHOR: *Cristina Coman, Leibniz-Institut für Analytische Wissenschaften - ISAS - e.V., Germany*

CO-AUTHORS: *Canan Has, Andreas Roos, Kristina Lorenz, Robert Ahrends*

To gain deeper insights into the underlying molecular mechanisms of a living system, a comprehensive and representative analysis demands a deep and parallel coverage of a broad spectrum of molecular species. Therefore, we developed SIMPLEX (SImultaneous Metabolite, Protein, Lipid EXtraction procedure), a mass spectrometry based strategy for the quantitative investigation of lipids, metabolites and proteins. Despite significant therapeutic advances, various cardiovascular diseases, including myocardial ischemia or infarction and inherited or acquired cardiomyopathies merge into the final common pathway of heart failure. Hence, with the aim to better understand these mechanisms, the cardiac muscle of P104L mutant caveolin-3 transgenic mice, presenting cardiomyopathy and of their wild-type littermates was subjected to the SIMPLEX workflow. In brief, the tissue was incubated with cold MeOH, MTBE was added and water was utilized to induce phase separation. The individual fractions containing lipids (top phase), metabolites (lower phase) and proteins (pellet) were then subjected to the individual mass spectrometry-based workflows. Here we demonstrate (i) how SIMPLEX is suited to analyze complex tissues (e.g. heart), (ii) how to investigate the interlinked proteome, lipidome and metabolome at the systems scale, and (iii) how a missense mutation influences the heart metabolism of mice.

P-253 Genome-scale metabolic models to identify early metabolite changes as markers for toxicant-induced organ injuries

PRESENTING AUTHOR: *Venkat R Pannala, Biotechnology HPC Software Applications Institute, United States*

CO-AUTHORS: *Martha L. Wall, Shanea K. Estes, Irina Trenary, Tracy P. O'Brien, Richard L. Printz, Kalyan C. Vinnakota, Jaques Reifman, Masakazu Shiota, Jamey D. Young, Anders Wallqvist*

In order to provide timely treatment for organ damage initiated by therapeutic drugs or exposure to environmental toxicants, we first need to identify markers that provide an early diagnosis of potential adverse effects before permanent damage occurs. Specifically, the liver, as a primary organ prone to toxicant-induced injuries, lacks diagnostic markers that are specific and sensitive to the early onset of injury. Here, to identify plasma metabolites as markers of early toxicant-induced injury, we used a constraint-based modeling approach with a genome-scale network reconstruction of rat liver metabolism to incorporate perturbations of gene expression induced by acetaminophen, a known hepatotoxicant. A comparison of the model results against the metabolomics data revealed that our approach satisfactorily predicted altered plasma metabolite levels as early as 5 h after exposure to 2 g/kg of acetaminophen, and that after 10 h of exposure, the predictions significantly improved when we integrated measured central carbon fluxes into the network model. Overall, the model correctly predicted the direction of change for 68% of the plasma metabolites for the extended APAP exposure (10 h). We found metabolites such as, cytidine, choline, uracil, proline, and chenodeoxycholic acid among others that were significantly altered in plasma and are consistent with our model predictions. Our approach is solely driven by gene expression and physiological boundary conditions, and does not rely on any toxicant-specific model component. As such, it provides a mechanistic model that serves as a first step in identifying putative plasma metabolites as markers of toxicant-induced organ injuries.

P-254 Using Multi-Omics Approach to Reveal the Metabolism Change in Pulmonary Arterial Smooth Muscle Cells in Patient with Loss-of-function of ALDH1

PRESENTING AUTHOR: *Yuqin Dai, Agilent Technologies Inc., United States*

CO-AUTHORS: *Dan Li, Songjie Chen, Michael Snyder, Marlene Rabinovitch*

Pulmonary arterial hypertension (PAH) that leads to heart failure is not effectively cured by current therapies. It is characterized by progressive occlusion of the vascular lumen owing to the abnormal growth of pulmonary artery smooth muscle cells (PASMC) occupying the neointima. RNAseq analyses of PASMC from 12 PAH vs. 9 control subjects identified heightened expression of aldehyde dehydrogenase 1 family (ALDH1). Loss-of-function of ALDH1 reduces the abnormal growth of PAH PASMC. Here, we present an integrated metabolomics and proteomics approach to reveal the mechanism of metabolites responsible for PAH PASMC with loss-of function of ALDH1 (siALDH1). An IP-RP Q-TOF LC/MS method was used for separation and detection of metabolites from cell extracts. We found that reduced ALDH1 by RNAi decreased the proliferation, and the metabolites in TCA cycle, glycolytic, and pentose phosphate pathways. These pathways contribute to the energy metabolism and nucleotide synthesis. We also correlated the enzymes in catalyzing these metabolites at protein level by proteomics. To get insight of the regulation mechanism, we perform a qualitative flux analysis to study the dynamic changes of the downstream metabolites. The metabolomics and fluxomics results were further integrated with the relative quantification of cellular proteome to characterize the dysfunction pathways of PAH.

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SYSTEMS BIOLOGY, NETWORKS

P-255 OnPLS-Based Multi-Block Data Integration: A Multivariate Approach to Interrogating Biological Interactions in Asthma

PRESENTING AUTHOR: ***Stacey Reinke, Edith Cowan University, Australia***

CO-AUTHORS: ***Beatriz Galindo-Prieto, Tomas Skotare, David I. Broadhurst, Akul Singhania, Daniel Horowitz, Ratko Djukanović, Timothy S.C. Hinks, Paul Geladi, Johan Trygg, Craig E. Wheelock***

Multi-omics data integration is a key challenge in systems-based studies. OnPLS is a multi-block method for the modelling of predictive and orthogonal variation that reduces feature space without relying on a priori biological knowledge of variables. However, these models can be mathematically complex and difficult to interpret. We addressed this obstacle by applying intuitive data visualisation methods to ease OnPLS model interpretation. OnPLS was used to interrogate a subset of a previously published asthma cohort (12 healthy controls, 10 severe asthmatics). Six data blocks were simultaneously modelled to discover shared structural information: transcriptomics; metabolomics; targeted sphingolipids, oxylipins, and fatty acids; and clinical data. The OnPLS model identified 7 components, 2 sharing globally joint structure (all blocks) and 5 sharing locally joint structure (from 2-5 blocks). The first two components were the most informative, identifying differences between healthy controls and asthmatics, plus a disease-gender interaction. The variables contributing to each component and their associated interactions were then identified using the multi-block extension of variable influence on projection (MB-VIOP) in combination with chord-plot visualisation. MB-VIOP identified multiple variables associated with each component that described biological interactions. For example, 5 metabolites associated with corticosteroid treatment described asthma-related differences, confirming the utility of the method to identify known biology. These metabolites correlated with 23 transcripts, including ATP6V1G1, which is implicated in osteoporosis, a known side-effect of corticosteroid treatment. Together, these findings highlight the utility of multi-block models for omics-based data integration and the ability to identify novel biological interactions, such as off-target drug effects.

P-256 A Six-Month Multi-Omics Systems Toxicology Inhalation/Cessation Study in ApoE−/− Mice to Investigate Respiratory and Cardiovascular Exposure Effects of Potential and Candidate Modified Risk Tobacco Products Compared with Conventional Cigarettes

PRESENTING AUTHOR: ***Bjoern Titz, PMI R&D, Philip Morris Products S.A., Switzerland***

CO-AUTHORS: ***Blaine Phillips, Justyna Szostak, Alain Sewer, Catherine Nury, Thomas Schneider, Oksana Lavrynenko, Ashraf Elamin, Emmanuel Guedj, Ee Tsin Wong, Marja Talikka, Stefan Lebrun, Grégory Vuillaume, Athanasios Kondylis, Patrice Leroy, Brian Keppler, Ansgar Buettner, Nikolai V. Ivanov, Patrick Vanscheeuwijck, Florian Martin, Manuel C. Peitsch, Julia Hoeng***

Cigarette smoke (CS) causes adverse health effects that may occur shortly after smoking initiation and lead to the development of respiratory disease (chronic obstructive pulmonary disease), cardiovascular disease, and cancer. To reduce the risk of smokers developing smoking-related diseases, Philip Morris International is developing modified risk tobacco products (MRTP). Within a systems toxicology study, we integrated multi-omics measurements (metabolomics, proteomics, and mRNA/miRNA transcriptomics) with classical endpoints to assess the effects in ApoE-/- mice of six months of exposure to CS (at 29.9 µg nicotine/L) or to aerosols from one potential and one candidate MRTP (CHTP 1.2 and THS 2.2, respectively) at matched nicotine concentrations. The impact of cessation or switching to CHTP 1.2 after three months of CS exposure was also evaluated. Multi-omics data analysis included multivariate dimension reduction and biological network analysis. Exposure to CS caused adverse effects on the lungs, including lung inflammation, and on the cardiovascular system, with aortic plaque formation. In contrast, exposure to MRTP aerosols did not induce lung inflammation or enhance plaque development. Cessation or switching to CHTP 1.2 reversed lung inflammation and halted progression of aortic plaques. Metabolomics, together with the other omics endpoints, further supported apical endpoints and provided insights into the underlying molecular mechanisms. In this study, exposure to MRTP aerosols had minimal adverse respiratory and cardiovascular effects. Cessation or switching to CHTP 1.2 delayed the progression of CS-induced atherosclerotic and lung emphysematous changes. This work exemplifies how multi-omics approaches, including metabolomics, can be leveraged effectively within a systems toxicology study.

P-257 Data analysis for multiomics (metabolomics, proteomics, fluxomics and transcriptomics) on Garuda, a connectivity platform for biological analytics.

PRESENTING AUTHOR: ***Hiroyuki Yasuda, Shimadzu Corporation, Japan***

CO-AUTHORS: ***Yohei Yamada, Shinji Kanazawa, Fumio Matsuda, Samik Ghosh, Takeshi Hase, Nikolaos Tsorman, Yukiko Matsuoka, Shigeki Kajihara, Hiroaki Kitano, Eiichiro Fukusaki, Junko Iida***

In order to understand biological systems, it has become common to analyze over 100 metabolites. In particular, multiomics analysis which attempts to understand biological systems from multiomics data, has been utilized. With the increase in the number of metabolites and the number of proteins to be analyzed, there is now a big need for a tool to quickly look the many measurement results obtained and to create new knowledge and hypotheses. We previously reported that we developed a pipeline for automated visualization of the multiomics data combining protein, metabolite and metabolic flux on the Garuda platform that provides the framework to connect, discover, and navigate through different software called “gadgets”. This study has made it possible to handle transcriptome data. In this study, we attempted a visualization of four omics layers using four kinds of data. In addition to the three sets of data (proteome, metabolome and metabolic flux) that could already be visualized, this study has made it possible to handle transcriptome data. Specifically, we transformed transcriptome data analyzed by microarray into text format and succeeded in reading with the “Load” function on the “Shimadzu MSI Data Import” gadget on the Garuda platform. Then, the transcriptome, proteome, metabolome and metabolic flux data were read by the “Shimadzu MS Data Import” gadget, respectively, and the integrated data was transferred to the “Multiomics Data Mapper” gadget using the “Discovery” function. Here we will present its applications to cyanobacteria.

POSTER SESSIONS 1 AND 2 – Monday and Tuesday – all odd number presenters will be at their posters.
POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

SYSTEMS BIOLOGY, NETWORKS

P-258 Network-based approaches for metabolomics data interpretation and multi-omics integration

PRESENTING AUTHOR: *Jianguo Xia, McGill University, Canada*

CO-AUTHORS: *Jasmine Chong, Guangyan Zhou, Othman Soufan*

The growing application of metabolomics requires improved support for comprehensivedata interpretation and integration with other omics data. Network-based approaches are particularly appealing in that networks can intuitively capture our current knowledge to enable a more holistic view of the experimental results. When coupled with appropriate analytics and visualization support, such presentations can significantly promote more informed hypothesis generation and decision making. Three network-based tools have been recently developed in our group to support metabolomics data interpretation and integration with other omics data. The Network Explorer allows users to explore their resultsfrom targeted metabolomics within multiple types of networks curated based on known connections of metabolite-gene/protein, metabolite-disease, metabolite-metabolite, and metabolite-gene-disease relationships; The MS peaks to Pathways allows users to explore their results from LC-MS based untargeted metabolomics within the context of global metabolic networks based on the mummichog algorithm; Finally, the OmicsNet supports integration of metabolomics with several other layers of omics data. Users can upload one or multiple lists of molecules of interest to create and merge different types of biological networks and visually explore the results in a 3Dvisual analytics systembased on the powerful WebGL technology. OmicsNet supports standard 3D force layout as well asmulti-layered 2D perspective layout to help navigate complex networks. Thesethree tools are freely available as two new modules in MetaboAnalyst 4.0 (metaboanalyst.ca) and a web-based application - OmicsNet (omicsnet.ca) to supportcomprehensive analysis and understanding of metabolomics data.

P-259 Multiscale, multifactorial networks to integrate metabolomics with high-dimensional immunology

PRESENTING AUTHOR: *Yating Wang, Emory University, United States*

CO-AUTHORS: *Luiz Gardinassi, Lu Xiong, Shuzhao Li*

Powerful as multi-omics integration can be for biomedical research, it is still challenging to combine metabolomics, especially untargeted metabolomics, with other high-dimensional data. Through our recent studies, we have developed a computational framework to combine meaningful dimension reduction, hierarchical networks and partial least square regression, to successfully interpret metabolomics in the context of big data immunology (Li et al, 2017. Cell 169:862). We report here our effort to further develop software and server tools based on this framework of multiscale, multifactorial networks. This incorporates into multi-omics integration mummichog version 2, our pathway software for metabolomics, and will be available as free web tools and as docker images. We will demonstrate the software on a study of controlled malaria infection in humans. The integration of transcriptomics and metabolomics revealed concerted molecular events triggered by the infection, notably on platelet activation, innate immunity and T cell signaling. Additional experiment confirmed that the metabolites associated with platelet activation genes were indeed enriched in platelet metabolome. We will also report the application of these tools on large-scale mining of public data.

P-260 Metabolic phenotyping in the population based cohort studies of Tohoku Medical Megabank Project

PRESENTING AUTHOR: *Seizo Koshiba, Tohoku University, Japan*

CO-AUTHORS: *Daisuke Saigusa, Ikuko Motoike, Jin Inoue, Matsuyuki Shirota, Yuichi Aoki, Shu Tadaka, Kengo Kinoshita, Masayuki Yamamoto*

Information of molecular phenotypes of individuals, such as genome and omics, is important for modern medical sciences. Especially, metabolic phenotypes are critically important, as metabolome is a good indicator of the effects of genetic and environmental factors to individual phenotypes. However, omics research is challenging in the case of population-based prospective cohort studies, because these cohorts collect a large number of samples from participants for a long time. Hence, high throughput metabolome analyses are indispensable for cohort studies. Here, we report recent progress in large-scale metabolome analyses in cohort studies of the Tohoku Medical Megabank (TMM) Project, which have been conducted in Japan. We have determined concentration distributions of metabolites in more than 5,000 Japanese plasma samples and released the results as a database, Japanese Multi Omics Reference Panel (jMorp). In this database, we also provide information of age-dependency of plasma metabolite concentrations and correlation between metabolites in plasma. Moreover, we have obtained metabolome data from more than 10,000 participants and are planning to update our jMorp database this year. We are also conducting metabolome genome-wide association study (MGWAS) and other association studies with blood test values and items of questionnaires, which will be included into our database. Thus, we have been working on the construction of strong basis for the metabolic phenotyping in TMM and we believe that this endeavor will contribute to the establishment of our future medicine.

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SYSTEMS BIOLOGY, NETWORKS

P-261 Translating Metabolomics into Therapeutics Insights using Artificial Intelligence

PRESENTING AUTHOR: ***Leila Pirhaji, ReviveMed Inc, United States***

CO-AUTHORS: ***Demarcus Briers, Jike Cui, Pamela Milani, Mathias Leidl, Timothy Curran, Julian Avila-Pacheco, Clary B Clish, Forest M White, Alan Saghatelian, Ernest Fraenkel***

Metabolomics is an essential tool for discovering the right therapeutics for the right patients. However, it has been underutilized in precision-medicine because of the challenges in identifying a large-set of metabolites for each patient. We have developed a pioneering artificial intelligence (AI) platform to overcome the challenges of identifying a large set of metabolites in biofluids and tissues and translate metabolomics into therapeutic insights. Large-scale metabolomic data, which is the global measurements of the masses of metabolites, has been largely neglected due to the ambiguous identity of each metabolite mass. By leveraging our proprietary database and AI algorithm, we overcome the ambiguities of metabolite masses and discover their biological context. First, we find all the possible metabolites corresponding to a metabolite mass by mass matching. We then use network-based machine-learning optimization to predict the putative identity of each mass, while simultaneously discover associated molecular pathways. We further showed the ability of our platform to connect metabolomics to other omics, including proteomics. We further validated this multi-omic data integration is essential to obtain a comprehensive view of biological processes. We experimentally validated our technology by studying Huntington's disease, a genetic neurodegenerative disorder. We demonstrated our technology can discover novel disease-associated pathways, proteins, and metabolites. We also identified existing drugs and novel therapeutic targets associated with the disease and their underlying molecular mechanisms.

P-263 Taiwan Biobank: A large cohort with genomics and metabolomics study

PRESENTING AUTHOR: ***YU, TSUNG FU, Taiwan Biobank, Academia Sinica, Taiwan***

Taiwan Biobank is a longitudinal project aiming to collect the plasma and urine of 200,000 Taiwanese on a population-based design and track their health and lifestyle for at least 10 years. The "Bio-Bank plans to use prospective cohort studies based on ethnicity (population-based) that will help determine the effects of the environment or the gene alone and of gene–gene interactions and gene–environmental factor interactions in chronicdiseases". Taiwan biobank now have conducted following both genomics and metbolomics study, such as genotyping(SNP chip: 653,291 SNPs) (26,000cases) ; whole genomic sequencing(1600cases);DNA methylation (1600cases), HLA typing (1200cases), Blood metabolome (400 cases), Urine metabolome (400 cases). Taiwan Biobank is also starting to collect the plasma, urine and tissue of 5000-10,000 specific disease cases (for each Breast cancer, Lung cancer, Colon cancer, Liver cancer, Head/Neck cancer, CVD, Stroke, DM, Alzheimer's disease, Chronic Kidney disease, Asthma and Endometreosis) and track their health and lifestyle from November 2016. (1000of 100,000 participants) In this disease cohort will also conducted genomics and metabolomics study as above. Thislarge cohort with genomics and metabolomics study will shed light on howto improve the health promotion, chronic disease prevention and the prognosis and treatment of diseases.

P-264 Bioanalytical studies related to Cri du Chat Syndrome (CdCS)

PRESENTING AUTHOR: ***Nilson Antonio Assunção, UNIFESP, Brazil***

CO-AUTHORS: ***Danielle Zildeana Sousa Furtado, Vinicius Guimarães Ferreirab, Fernando Brunale Vilela de Moura Leite, Emanuel Carrilho***

The Cri-du-chat syndrome (CdCS) is a rare genetic disease in which there is a deletion of a part of the short arm of chromosome 5 (5p-). The estimate is that this syndrome affects from 1 / 15,000 to 1 /50,000 children born in the world. One of the main characteristics is that all births present microcephaly and at birth produces a cry similar to the cry of a cat due to reduction of the larynx. Therefore, an untargeted evaluation was proposed in patients with Cri Du Chat Syndrome to help uncover the biochemical changes resulting this disease. A total of 24 samples of urine samples were collected and divided into two groups with the same age range and analyzed in the study untargeted by Capillary Electrophoresis coupled to the Mass Spectrometer. Volcano Plot statistical test was applied to the data and metabolomics pathway analysis was used. A total of 141 metabolites were identified and were found 48 metabolites significance in patients with SCdC. These alterations metabolites are in the majority amino-acids, sugars, vitamin, pentose and lipids. Untargeted analysis revealed changes in the following biochemical pathways: alanine, aspartate and asparagine metabolism, serine, glycine and threonine metabolism, histidine metabolism, arginine and proline metabolism. In general the catabolism of some biochemical pathways in patients with SCdC are affected, mainly the cycle of TCA, the metabolism of glycine, serine and threonine. These changes are related to the process of energy recovery and glycolysis.

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SYSTEMS BIOLOGY, NETWORKS

P-265 Integrated multi-Omic analysis Identifies Altered Metabolic Process in Sporadic Amyotrophic Lateral Sclerosis

PRESENTING AUTHOR: ***Dipa Roy Choudhury, Agilent Technologies, United States***

CO-AUTHORS: ***Qiuying Chen, Shweta Shukradas, Davinder Sandhu, Csaba Konrad, Benjamin Schwartz, Steven. S. Gross***

Integrating biology is challenging, with -omic technologies potentially generating millions of data points that must be interpreted for coherent insights. Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive motor neuron death. In this study, we used a multi-omic approach to uncover molecular mechanisms that underlie cellular dysfunctions in sporadic ALS (sALS) cases. Metabolite profiling detected >1,000 distinct metabolite features from sALS and control patient-derived fibroblasts, of which 41 were differentially-abundant in sALS. Additionally, microarray analysis queried >53,000 transcripts (mRNA and miRNA) from matched samples. Due to the comprehensive nature of gene annotation databases, we manually curated the searchable database to include candidate gene sets based on previously published sALS studies. Using this curated database, Gene Set Enrichment (GSE) analysis identified significantly different expression of genes involved in the regulation of primary metabolic processes and apoptosis in sALS. Mapping integrated transcriptome, microRNAome and metabolome data onto metabolic pathways (e.g. KEGG) indicated that cysteine, methionine, purine, and carbohydrate metabolic pathways were significantly enriched in sALS cases ($P < 0.00023$). Interestingly, upregulation of the trans-sulfuration pathway was identified as a top hit for metabolic pathway enrichment, suggesting an important bridge in sALS between the methionine cycleand cysteine production for glutathione biosynthesis. Together, multiomic data from sALS cases implicate metabolic adaptations to survive limited cysteine/GSH availability, potentially triggered by hypermetabolism and accelerated GSH oxidation. Overall, integrated multi-omics provided insights into sALS-associated disease processes, offering a potentially generic strategy for probing metabolic processes and gene networks in diverse disease states.

P-266 Systems Biology Approach Unveiled that Oral PBDE Exposure Modulates Metabolic Syndrome-related Aqueous Metabolites in a Gut Microbiome-dependent Manner

PRESENTING AUTHOR: ***David K Scoville, University of Washington, United States***

CO-AUTHORS: ***Cindy Yanfei Li, Dongfang Wang, Daniel Raftery, Haiwei Gu, Julia Yue Cui***

Background: Gut microbiome is an important regulator of host metabolism. Polybrominated diphenyl ethers (PBDEs) are persistent environmental toxicants associated with increased risk for metabolic syndrome. We hypothesized that PBDEs reduce beneficial intermediary metabolites in a gut microbiome-dependent manner. Methods: Nine-week old male conventional and germ-free C57BL/6 mice were orally gavaged once daily with vehicle, BDE-47, or BDE-99 (100 µmol/kg) for 4-days (n=3/group). Gut microbiome (16S rDNA sequencing), liver transcriptome (RNA-Seq), and intermediary metabolites in serum, liver, and small and large intestinal contents (SIC and LIC) (LC-MS) were examined. Gene-metabolite networks were generated using Metaboanalyst (http://www.metaboanalyst.ca). Statistical significance was determined using two-way ANOVA and Tukey's post hoc test (adjusted p-value <0.05). Results: LIC had the highest number of differentially regulated metabolites; most were regulated by PBDEs but not enterotype. In contrast, in serum, liver, and SIC, both enterotype and PBDEs altered intermediary metabolites. Importantly, gut microbiome was necessary for PBDE-mediated decreases in aromatic and branched chain amino acid metabolites including 3-indole-propionic acid, recently shown to be involved in inflammation and diabetes. Gene-metabolite networks revealed a positive association between hepatic Alg12 expression and mannose, which are important for protein glycosylation, a dysregulated process observed in metabolic syndrome patients. 23 bacterial taxa were regulated by PBDEs, and correlations of certain taxa with distinct serum metabolites further highlight a modulatory role of the microbiome for PBDE-mediated toxicity. Conclusion: This study showed that PBDEs impact intermediary metabolism in a gut microbiome-dependent manner, suggesting that dysbiosis may contribute to PBDE-mediated toxicities including metabolic syndrome.

P-267 Integrated metabolomic-proteomic analyses of atherosclerotic lesions from a single mouse arterial segment

PRESENTING AUTHOR: ***Jihan Talib, Victor Chang Cardiac Research Institute, Australia***

CO-AUTHORS: ***Peter G Hains, Philip J Robinson, Mark Hodson, Roland Stocker***

The apolipoprotein E gene-deficient (Apoe-/-) mouse serves as an essential tool in elucidating the pathophysiology of atherosclerosis due to its propensity to spontaneously develop arterial lesions. To date, however, an integrated omics assessment of atherosclerotic lesions in individual Apoe-/- mice has been challenging due to the small amount of diseased and non-diseased tissue available. To address this current limitation, we are aiming to develop an integrated metabolomic and proteomic method that utilizes the Barocycler 2320EXT for tissue homogenization and digestion, coupled with high sensitivity mass spectrometry to assess arterial tissue from Apoe-/- mice fed a Western Diet for 6 months. Untargeted LC/MS based metabolomics, lipid profiling and proteomics will be conducted using the Sciex TripleTOF® 6600 mass spectrometer. Targeted metabolomics analyses will utilize the Agilent Metabolomics dMRM method for 219 metabolites. Our preliminary analyses show that from aqueous extracts of a single segment of mouse arterial lesion (~1 mg tissue) we can detect up to 80 metabolites and 1,000 features from the Agilent Metabolomics dMRM method and untargeted metabolomics respectively. The proteomics analyses indicate that we can quantify around 1,000 proteins. We will apply this method to compare metabolomics and proteomic profiles of lesion tissue from Apoe-/- mice to non-lesion tissue from age- and sex-matched C57BL/6J mice. If successful, our method of simultaneous profiling of metabolites and proteins may also be applicable to other situations where only small and valuable tissue samples are available, such as biopsy materials.

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SYSTEMS BIOLOGY, NETWORKS

P-268 **Multi-Omics analyses reveal new cyclic peptides associated with the virulence of F. graminearum in wheat.**

PRESENTING AUTHOR: ***David P. Overy, Agriculture AgriFood Canada, Canada***

CO-AUTHORS: ***Kristina Shostak, Amanda Sproule, John Vierula, Gopal Subramaniam***

Fusarium head blight (FHB) and Gibberella ear rot are diseases of cereal crops (wheat, barley, oat) and corn that are caused by several related fungal species of the genus Fusarium. In particular F. graminearum is the most notorious, due to the accumulation of regulated trichothecene mycotoxins (notably DON – deoxynivalenol, and derivatives) in infected kernels that result in major economic losses worldwide and impose a serious trade burden upon farmers. To develop effective disease management strategies, a comprehensive understanding of the organism's pathogenicity is required. F. graminearum is predicted to possess 67 secondary metabolic gene clusters encoding small secondary metabolites, which could contribute to pathogenicity under specific conditions. Although transcription factor Tri6 positively regulates trichothecene production, evidence suggests that Tri6 is a global regulator affecting multiple pathways involved in virulence. Combined metabolomics and transcriptomic analyses of Tri6 disruption mutants were carried out to establish the role of Tri6 in the production of unique secondary metabolites that contribute to the virulence of F. graminearum inwheat (Triticum aestivum). Analyses results will be presented outlining the identification of a non-ribosomal peptide synthase (NRPS) gene cluster encoding for two new cyclic peptides that are regulated by Tri6; where deletion of the biosynthetic gene results in a loss of product accumulation and pathogenicity in wheat coleoptiles.

P-269 **IMTAP: A computational analysis tool for integrative modeling of metabolites, genes, and proteins**

PRESENTING AUTHOR: ***Ling Huang, Salk Institute for Biological Studies, United States***

CO-AUTHORS: ***Max Shokhirev***

Metabolites provide a functional readout of cell responses and can be used to understand phenotypic differences and disease progression. With the recent advancement in high-throughput untargeted liquid chromatography/mass spectrometry, it is possible to measure thousands of metabolites from a single experiment in an unbiased and system-wide way. However, it is still challenging to interpret the data in the biological context due to the complexity in the network structure of biochemical reactions that produce and consume metabolites. Integrating steady-state metabolomics data with transcriptomics and proteomics data has been shown to improve the understanding of the biological processes. Here, we present IMTAP, a novel computational multi-omics integrative analysis tool. IMTAP uses the reaction rates estimated from measured abundances of enzymes, reactants, and products to establish a bridge between metabolites and genes/proteins. Metabolic network information is pre-constructed from the KEGG database and is customizable by users. The tool uses the statistical package limma for differential expression analysis and presents the result in an interactive portable network graph that allows users to explore specific sub-network modules of interest. IMTAP also makes predictions about the directionality of the change in the metabolites that are not measured in the experiment, providing suggestions for follow-up studies. In addition, the tool will be hosted on a web server that gives easy and free access to researchers interested in integrative multi-omics analysis around the world.

BIG DATA, STATISTICS, INFORMATICS

P-271 **MetaboAnalystR: An R package for reproducible and flexible analysis of metabolomics data**

PRESENTING AUTHOR: ***Jasmine Chong, McGill University, Canada***

CO-AUTHORS: ***Jeff Xia***

MetaboAnalyst (metaboanalyst.ca) has been widely used forcomprehensive metabolomic data analysis, visualization, and functional interpretation. As metabolomics is widely used across different fields and biological systems, data analysis is certainly not"one size fits all". Theweb-based interface, being user-friendly and easily accessible, presents inherent constrains in terms of flexibility of data analysis, batch processing and handling ofbig data.In addition, MetaboAnalyst is continuously updated to keep the pace with technological advancements and users feedback, which can makereproducible data analysischallenging.To address these issues, we have recently developed MetaboAnalystR - a MetaboAnalyst companion R package that is already used by the MetaboAnalyst web application,and canbe run locally on a user's computer.The R package has been thoroughly tested to ensure the same commands will produce identical results from both interfaces. The utility of MetaboAnalystR will be demonstrated on a large-scale dataset.MetaboAnalystRtherein provides a flexible solution for more advanced users to tailor the R code to their data, as well as extend package capabilities by facilitating the construction of custom metabolomics workflows. Further, it complements the corresponding web server to enable transparent, flexible and reproducible analysis of big metabolomics data. MetaboAnalystR is freely available fromhttps://github.com/xia-lab/MetaboAnalystR.

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BIG DATA, STATISTICS, INFORMATICS

P-272 Ensemble Feature Selection for Metabolite Biomarker Discovery

PRESENTING AUTHOR: ***Xiang Zhang, University Louisville, United States***

CO-AUTHORS: ***Aliasghar Shahrjooihaghighi, Biyun Shi, Xinmin Yin, Ameni Trabelsi, Xiaoli Wei, Hichem Frigui, Xiang Zhang***

Biomarker discovery is one of the major applications of metabolomics. The goal of biomarker discovery is to select the few most discriminative features among a large number of irreverent ones. The high dimensionality and small sample size of metabolomics data make feature selection a challenging task in metabolite biomarker discovery. Feature selection and its applications have become an important topic for machine learning researchers. To improve the reliability of metabolite biomarker discovery, we developed an ensemble-based approach by combining the results of five filter-based feature selection methods, including rank product, fold change ratio, area between the curve and the rising diagonal (ABCR), t-test, and partial least squares discriminant analysis (PLS-DA). Permutation test was used in each feature selection method to find potential biomarkers that were ranked by their permutation p-values. We then developed a rank-based method, a variation of Borda count, to combine the individual ranks. Two sets of LC-MS data were used to evaluate the accuracy of the proposed approach. Both were metabolite extract from biological samples containing metabolite standards with different spike-in concentrations. Our results indicated that, for all concentration levels, fusion outperforms the best individual algorithm. We further applied the developed method for metabolite biomarker discovery to study alcohol liver disease. More metabolites in the same pathways were detected as biomarkers, demonstrating that the ensemble learning improves the accuracy of feature selection by combining multiple algorithms that have complementary information.

P-273 Visualizing abundance changes in metabolic networks data with MetL

PRESENTING AUTHOR: ***Hannah Manning, Oregon Health and Science University, United States***

CO-AUTHORS: ***Ozgun Babur, Emek Demir***

We are developing Metabolome Linker (MetL): a free web-based resource that contextualizes a researcher'smetabolic findings by mapping them onto known pathways curated by Pathway Commons. MetL filters dataaccording to user-specifications (i.e. Pearson correlations, significance, etc.) to elucidate notable differences ineach analyte's quantity in conjunction with the changes occurring in its metabolic neighborhood. Given thatindirect interactions undermine Pearson correlations between pairs of molecules (notoriously so inmetabolomics), our approach causally links metabolic changes by leveraging only well documentedintermolecular relationships. Moreover, we will likely include the option of replacing Pearson correlations withGaussian graphical models which, for a given metabolite, condition each of its correlation coefficients againstall others. Additional functionalities of this tool include normalization, mapping user data to ChEBI IDs (vialibChEBI), highlighting compounds found in LIPID MAPS, and the option to include pathway relationshipsamong isomers and ions of the user's analytes. Once MetL has reached production quality, it will be madeavailable to the public alongside other Pathway Commons tools such as CyPath2 and ChiBE.

P-274 tindeResting: using shiny to harvest expert knowledge in an active learning setting.

PRESENTING AUTHOR: ***Charlie Beirnaert, Adrem Data Lab, University of Antwerp, Belgium***

CO-AUTHORS: ***Laura Peeters, Kenne Foubert, Deborah Custers, Anastasia Van der Auwera, Sebastiaan Bijtebier, Wout Bittremieux, Luc Pieters, Kris Laukens***

Dynamic metabolomics consists of collecting metabolomics data over time. The merit of this approach is that it takes into account the highly variable nature of metabolomic processes. This is important in cases like prodrugs being metabolized into pharmacologically active compounds. Few techniques exist to analyze these time-resolved metabolomics data. Most do not take into account the temporal aspect, and the techniques which are suited for this type of data are often not specifically for metabolomics. We provide a case study whereby data-analysis techniques from genomics are combined with the knowledge of experienced metabolomics data-analysts and machine learning. The advantage of genomic tools is that they are highly flexible with respect to the dynamic profile (metabolites can exhibit very different behavior over time). The results contain a large number of significant peaks but a substantial part are false positives. These are easily recognized by the human eye, but reviewing them all is tedious work. To remedy this problem we built an active learning random forest model to rate the results. To avoid the cold start problem a shiny app was built to let users quickly rate many features. A performance analysis revealed that the model was capable of correctly predicting the reviewers responses in the majority of cases (AUC: 0.902 with 10 fold cross-validation). The app and accompanying code are freely available on GitHub. This is, to our knowledge, the first application of active learning to identify interesting features for dynamic metabolomics, this will lead to a decrease in false positives.

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BIG DATA, STATISTICS, INFORMATICS

P-275 Automated Identification of glycerolipid, diacyl-, monacyl- glycerophospholipids with detailed analyses of the fatty acyl composition using tandem mass spectrometry

PRESENTING AUTHOR: ***Ningombam Sanjib Meitei, PREMIER Biosoft, India***

CO-AUTHORS: ***Rajesh Pujari, Himani Gupta, Ulrike Schweiger Hufnagel, Sebastian Goetz, Sven Meyer, Aiko Barsch***

Tandem MS lipidomic data facilitates not only the identification of glycerophospho/glycero-lipids based on their head groups but also the differentiation between saturated, moderately unsaturated (18:1 or 18:2) and highly unsaturated (20:4 or 22:6) acyl residues [1-2]. However, interpretation of MS/MS lipidomic data requires efficient software workflows. We present software workflows to facilitate identification of the fatty acyl composition in glycerolipid, diacyl-, monacyl- glycerophospholipids. Lipids were extracted from the NIST SRM 1950 Metabolites in Human Plasma by liquid-liquid extraction methods. LC separation was performed using a Thermo RSLC UHPLC system with Waters Acquity UPLC CSH C18 column. MS data were acquired using a Bruker impact II QTOF-MS instrument in data dependent acquisition mode (ESI +/- modes). SimLipid MS/MS database search was performed for both the precursor ions, and the product ions. A total of 1036 unique lipid species were identified: FA(n=60), GL(n=534), GP(n=375), SP(n=66), and ST(n=1). The lipid IDs with annotated MS/MS spectra –m/z peaks, fragment names– were exported into MS excel files. Spreadsheet formulas were used to identify the fatty acyl compositions: MG(n= 3; M=0; 1f=3), DG(n= 20; M=1; 1f=5,2f=14), TG(n= 307; M=1; 1f=34,2f=102,3f=170), PC(n= 224; M=89; 1f=88,2f=47), and LysoPC(n= 34; M=14; 1f=20); where M is the number of lipids identified with only the headgroup or parent molecular ions, 1f, 2f and 3f represents the number of lipids identified with characteristic ions corresponding to one, two and three fatty acid chains, respectively. References: 1. Schiller J et al. Front Biosci. 2007; 12:2568–2579. 2. Palusinska-Szysz et al. PloS one. 2014; 9(7):e101243.

P-276 Identifying Metabolite Signals in Mass Spectrometric Analysis of Complex Mixtures

PRESENTING AUTHOR: ***Bety Rostandy, University of North Carolina, Greensboro, United States***

CO-AUTHORS: ***Nadja B. Cech, Xiaoli Gao***

The chemical complexity of the biological sample as well as the multiplex parameters of the analytical process make it challenging for analytical chemists to identify metabolite signals in natural complex mixtures via electrospray-ionization mass spectrometry. The ESI-MS linear dynamic range of a natural complex mixtures has not been thoroughly studied to this day. However, the metabolites in complex mixtures had been shown to follow a log-normal distribution function in their responses. As a test case for this study, a medicinal plant extract of goldenseal (Hydrastis canadensis L.) was employed. The extracts were analyzed using Acquity Ultra Performance Liquid Chromatograph® (Waters Corporation, USA) coupled to a Q-ExactiveTM Plus Hybrid Quadrupole-OrbitrapTM Mass Spectrometer (ThermoFisher Scientifics, USA). The hypothesis for this study is that the parameters of the distribution at any given concentration of a same extract should independently and identically follow the known distribution with same parameters. Therefore, relying on the observed distribution of ions, we expect to be able to determine which ions belong to a given sample at a high level of confidence. The determination of which ions are of metabolites signals often relied on subtraction from the blank (i.e. the negative control of biological sample). This procedure is usually under discretion of the analysts to set up an arbitrary p-value as a cut-off point. Advanced statistical methodologies were utilized to perform simultaneous multiple testing efficiently to enable optimization of signal-to-noise cutoff. Monte-Carlo simulation studies was performed to test the validity of assumed distribution function for the metabolite signals.

P-277 The Undiscovered Complexity of Electrospray Ionisation and its Impact on High Quality Metabolite Annotation

PRESENTING AUTHOR: ***William Nash, University of Birmingham, United Kingdom***

CO-AUTHORS: ***Ralf Weber, Warwick Dunn***

Electrospray-ionisation (ESI) is routinely applied in metabolomics studies. ESI sources give rise to a variety of ion types. Previous research highlighted this complexity for a single instrument type, showing that adducts, fragments and multiply charged species along with isotopic peaks can be detected with multiple ‘metabolite features’ detected for a single metabolite [1]. For accurate annotation of metabolites, the different metabolite features must be grouped together and the ‘ion type’ annotated to allow accurate empirical formula determination. If derivative features are not grouped then false positive annotations will occur. Deep knowledge of the types of metabolite features and their frequency is highly important. 104 untargeted LC-ESI-MS datasets were obtained from Metabolights, Metabolomics Workbench and Phenome Centre Birmingham. Common m/z distances relating to derivative features were identified across all datasets applying two rules (1) the feature pair has similar retention times, all pairs within 5 second overlapping RT windows were grouped and the m/z distances between them were calculated; (2) their intensities across the samples should be highly correlated, Pearson correlation coefficient and associated p-values were calculated allowing results filtering. The most frequently observed distances were annotated, 1038 unique grouped m/z distances had a frequency >=40. 211 groups are currently annotated including 48 of the top 50 most frequently detected. The 12C-13C isotope difference is the most common difference observed, interestingly multiply charged species were more frequently observed than expected including quintuply charged species. Results have been integrated within new software called BEAMS which is publicly available.

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BIG DATA, STATISTICS, INFORMATICS

P-278 Trial for Food Quality Evaluation with Machine Learning Schemes

PRESENTING AUTHOR: ***Takero Sakai, Shimadzu Corporation, Japan***

CO-AUTHORS: ***Tairo Ogura, Yoshihiro Aoyama, Kiyomi Arakawa***

Metabolomics is one of the mightytools for creating models for regression and classification of the sample because it deals with multivariate data. Quality evaluating method of food using multivariategathers more and more attention as Metabolomics getting popular. One of the issues againstpromoting multivariatequality evaluationcould bethe schemefor data processing. Conventionally in Metabolomics, some regular data processing schemes such as Principal Component Analysis (PCA) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) are preferably utilized. Although they havebeen playinga notably important role for development of Metabolomics, we cannot ignore the other strategiesthat deal with the multivariate data that Metabolomics can produce.Machine learning (ML) related data processing under the existence of big data is getting more and more attention recently, and we think they may have great possibility for Metabolomics. In this poster, we preparedthe various parts of beef as food sample and stored it in 4°Cand 40 °C for 3 hours asgood and bad quality, respectively. We grilled it in SPMEvial and the generated gas was analyzed. We collected the data and tried to create the model that discriminates good and bad beef using not only conventional schemes suchas PCA and OPLS-DA but ML related schemes suchas Support Vector Machine (SVM), k-Nearest Neighbors (k-NN) and Artificial Neural Network (ANN). We demonstrated a glimpse of the possibility and usefulness of ML schemes in Metabolomics.

P-279 Accounting for thermodynamic feasibility when inferring tissue-specific genome-wide metabolic models

PRESENTING AUTHOR: ***Oliver Bodeit, University of Amsterdam, Germany***

CO-AUTHORS: ***Johannes van Beek***

Genome-wide models of metabolism containing reactions found in the human body are available. Not all reactions are active in all cell types. At least twenty algorithms exist to predict tissue-specific metabolism from gene or protein expression. The iMAT algorithm (Shlomi et al., Nature Biotechnology, 2008) is one of the most used. iMAT maximizes the correspondence between utilized reactions and global gene or protein expression of the respective enzymes. Resulting flux distributions may contain infeasible loops of reactions operating in closed cycles, which violates thermodynamic principles. We found that the result of iMAT can be affected by such infeasible loops and incorporated a method to identify and remove them (Desouki et al., Bioinformatics, 2015). We compared this cycle-free version of iMAT with the conventional variant, using data on protein expression in quiescent and proliferating endothelial cells (Patella et al., Mol Cell Proteomics, 2015). Differences were found in the number of reactions which were considered upregulated in 10 metabolic subsystems (e.g. inositol phosphate and heme metabolism) or downregulated in 14 subsystems (e.g. glycolysis, vitamin A metabolism). For instance, four reactions in glutamate metabolism were called downregulated in proliferating cells by conventional iMAT while zero were called downregulated by cycle-free iMAT. Five reactions were considered upregulated in proliferating cells in taurine metabolism by conventional iMAT while zero were considered upregulated by cycle-free iMAT. We conclude that the conventional iMAT algorithm is vulnerable to thermodynamically infeasible results and have complemented iMAT to repair this.

P-280 Combining ASCA and mixed models to analyse high dimensional designed data.

PRESENTING AUTHOR: ***Manon Martin, ISBA – IMMAQ – UCL, Belgium***

CO-AUTHORS: ***Pascal De Tullio, Bernadette Govaerts***

Metabolomics data produce high-dimensional multivariate data matrices, here defined as the response matrices, usually with a larger number of variables than samples and a high biological/instrumental variability. Moreover, they often involve an advanced experimental design that must be considered during their analysis (e.g. presence of random effects). To be able to extract meaningful information from these high dimensional designed data matrices, two popular approaches, namely ASCA(+) and APCA(+) combine the strengths of statistical modelling and PCA. They have two main steps: (1) the response matrix decomposition using ANOVA or GLM estimators leading to the fixed effect matrices and (2) the multivariate analysis (PCA) of those (residual-augmented) effect matrices. The scope of this research is to further extend the ASCA/APCA methodology using mixed models with random effects to analyse advanced designs and models, such as repeatability/reproducibility or repeated measures designs. The main modifications are the following: the parallel ANOVA or GLM modelling is replaced by a parallel mixed modelling; a global measure of factor importance for both fixed and random effects is quantified and their statistical significance is tested based on a likelihood ratio statistic and on a parametric bootstrap procedure. This extended methodology has been successfully applied to human serum and urine 1H NMR metabolomics datasets to study the spectral repeatability/reproducibility with one fixed and two random factors. It enabled to quantify the fixed and random effects importance as well as their statistical significance.

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POSTER SESSIONS 3 AND 4 – **Wednesday and Thursday –** all even number presenters will be at their posters.

BIG DATA, STATISTICS, INFORMATICS

P-281 Expansion of metabolite reference libraries through deep learning

PRESENTING AUTHOR: ***Sean Colby, Pacific Northwest National Laboratory, United States***

CO-AUTHORS: ***Sean Colby, Jamie Nunez, Nathan Hodas, Courtney Corley, Ryan Renslow***

Robust and comprehensive identification of small metabolites in complex samples will revolutionize our understanding of metabolic interactions in biological systems. Existing and emerging technologies have enabled measurement of chemical properties of molecules in complex mixtures and, in concert, are sensitive enough to resolve even stereoisomers. Despite these experimental advances, small molecule identification is inhibited by (i) a deficiency in reference properties (e.g. mass spectra, collisional cross section, and other measurable properties), limiting the number of possible identifications, and (ii) the lack of a method to generate candidate matches from experimental features. To this end, we developed a variational autoencoder to learn a continuous numerical, or latent, representation of molecular structure to simultaneously characterize and expand reference libraries for small molecule identification. We extended the VAE to include a chemical property decoder, trained as a multi-task network, in order to shape the latent representation such that it self-assembles according to said properties. We found correlations embedded in the latent representation, enabling molecule generation along manifolds defined by chemical property analogues. Thus, the network can be used to predict chemical properties from structure, as well as generate candidate structures with desired chemical properties. This configuration enables novel molecule discovery from previously unidentifiable metabolomics features in complex biological mixtures. We applied our approach to the Universal Natural Products Databaseand the Human Metabolomics Databaseto generate plausible candidate molecules that corresponded to statistically significant features in Type II Diabetes urine samples and in soil samples taken from several locations around the US.

P-282 Skyline and Panorama for Targeted Metabolomics and Automated Quality Control

PRESENTING AUTHOR: ***Brian Pratt, University of Washington Genome Sciences, MacCoss Lab, United States***

CO-AUTHORS: ***J. Will Thompson, Josh Eckels, Erin Baker, Vagisha Sharma, Michael J. MacCoss, Brendan MacLean***

The Skyline Targeted Mass Spectrometry Environment is a well-known tool originally developed for chromatography-based quantitative proteomics. Originally for selected reaction monitoring (SRM), this popular and freely available software has grown to support full-spectrum methods including MS1 filtering, parallel reaction monitoring (PRM) and data independent acquisition (DIA – including the approach popularized as SWATH). Skyline's ability to read native MS files also makes it ideal for automating quality control, especially in concert with Panorama and AutoQC. Researchers increasingly find themselves working in more than one "-omics" area, and many prefer to use the same tools in different regimes when possible. User demand has led to Skyline being increasingly adapted for use with generalized molecules and ionization modes. Ease of integration with existing small molecule workflows is an ongoing conversation with Skyline's users, leading to support for molecular identifiers such as InChI, CAS, HMDB etc, and import of popular small molecule spectral library formats such as NIST. Skyline also supports building custom experiment or lab specific spectral libraries for small molecule research, as has become increasingly popular in proteomics research. Support for direct infusion experiments is in progress. Ion mobility separation is of particular benefit in metabolomics where isobaric precursors are common, and we see increasing interest in collisional cross section (CCS) as a library property. Recent work in Skyline includes deriving CCS values from experimental data for inclusion in spectral libraries, using the capabilities provided by an ever increasing list of instrument vendors with IMS capability, including Agilent, Bruker and Waters.

P-283 Referencing LC-MS, GC-MS, and NMR workflows on the W4M Galaxy infrastructure for reproducible metabolomics data analysis

PRESENTING AUTHOR: ***Dr Yann GUITTON, LABERCA, Oniris, INRA, Université Bretagne-Loire, France***

CO-AUTHORS: ***Marie Tremblay-Franco Gildas Le Corguillé Jean-François Martin Mélanie Pétéra Pierrick Roger-Mele Alexis Delabrière Sophie Goulitquer Misharl Monsoor Bruno Le Bizec Christophe Duperier Cécile Canlet Rémi Servien Patrick Tardivel Christophe Caron Franck Giacomoni Etienne A. Thévenot***

Metabolomics data analysis is a complex, multistep process, which is constantly evolving with the development of new analytical technologies, mathematical methods, and bioinformatics tools and databases. The Workflow4Metabolomics Galaxy online infrastructure (W4M, http://workflow4metabolomics.org) provides a unique centralized, user-friendly, and high-performance environment to build, run, and share metabolomics workflows for LC-MS, GC-MS, and NMR technologies. W4M now supports the publication of workflows online: for the first time, users have the opportunity to get a reference ID for their study. The whole workflow (i.e. the modules and the parameter values) and the input/output data can thus be publicly accessed and cited with a simple Digital Object Identifier (DOI). By referencing your history, you get a permanent DOI which you can cite in your publications. Making your workflows and associated data available to the community is essential to demonstrate the value and the reproducibility of your analysis (e.g., to reviewers). This initiative follow the four FAIR principles "Findability, Accessibility, Interoperability, and Reusability" of data, algorithms and tools. As for raw data, journal editors will increasingly require that the process of generating the results (code, parameter values, output data) is made available on reference repositories. Funding agencies such as European Programmes also require that the generated data are made public. Finally, by sharing your analysis, you get the opportunity to receive feedback on your results, be cited, initiate new collaborations. Workflow4Metabolomics thus not only offers a unified, evolving environment for metabolomics data analysis, but also should become the reference repository for shared workflows.

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POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

BIG DATA, STATISTICS, INFORMATICS

P-284 Tackling the Challenges in Untargeted NMR Metabolomics Data Preprocessing for Multi-cohort Epidemiological Studies

PRESENTING AUTHOR: *Ibrahim Karaman, Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, United Kingdom*

CO-AUTHORS: *Claire Boulangé, Rui Climaco Pinto, Gonçalo Graça, Abbas Dehghan, Paul Elliott, Ioanna Tzoulaki, David Herrington, Timothy Ebbels*

Untargeted NMR metabolomics has been widely used in large-scale epidemiological studies. Metabolic profiles of human biofluids contain many metabolites and an NMR spectrum of a biofluid (e.g. blood serum) is extremely complex. The complexity of the data grows when thousands of samples are analysed in different experimental runs. Changes in peak positions across the samples can become more difficult to align and normalisation of the samples becomes more important. Further, in epidemiological studies the presence of different cohorts results in additional complexity as comparability of independent cohorts needs to be established. Therefore NMR data must undergo intensive preprocessing to make the data useful for studies of disease.In this work, we used CPMG and standard 1D NMR data from blood serum samples acquired from three cohorts totalling >10,000 individuals. Each dataset consisted of more than 9,000 spectra including the quality control (QC) sample spectra. The proposed workflow involves the following steps: Removing outlying spectral regions Spectral peak alignment using QC samples to generate a reference Sample normalisation Identification of outlying samples Spectral binning and annotation Experimental batch / cohort adjustment using log transformation and auto-scaling The validity of the preprocessing pipeline was examined using PCA, exploiting the QC samples for quality assessment. This workflow offers insights about sources of error and variance in NMR metabolomics from large-scale epidemiological studies and illustrates a practical solution for metabolomics scientists who intend to analyse multi-cohort NMR data.

P-285 Bayesian Estimation of the Number of Protonation Sites for Urinary Metabolites from NMR Spectroscopic Data

PRESENTING AUTHOR: *Timothy Ebbels, Imperial College London, United Kingdom*

CO-AUTHORS: *Lifeng Yi, Maria De Iorio*

Background: To aid the development of better algorithms for NMR data analysis, such as alignment or peak-fitting, it is important to characterise and model chemical shift changes caused by variation in pH. The number of protonation sites, a key parameter in the theoretical relationship between pH and chemical shift, is traditionally estimated from the molecular structure, which is often unknown in untargeted metabolomics applications. Objective: To use observed NMR chemical shift titration data to estimate the number of protonation sites for a range of urinary metabolites. Methods: A pool of urine from healthy subjects was titrated in the range pH 2-12, standard 1H NMR spectra were acquired and positions of 51 peaks (corresponding to 32 identified metabolites) were recorded. A theoretical model of chemical shift was fit to the data using a Bayesian statistical framework, using model selection procedures in a Markov Chain Monte Carlo algorithm to estimate the number of protonation sites for each molecule. Results: The estimated number of protonation sites was found to be correct for 41 out of 51 peaks. In some cases, the number of sites was incorrectly estimated, due to very close pKa values or a limited amount of data in the required pH range. Conclusions: Given appropriate data, it is possible to estimate the number of protonation sites for many metabolites typically observed in 1H NMR metabolomics without knowledge of the molecular structure. This approach may be a valuable resource for the development of future automated metabolite alignment, annotation and peak fitting algorithms.

P-286 Developing a core database for steroid data of the human KORA cohort

PRESENTING AUTHOR: *Alexander Cecil, Helmholtz Zentrum München, Germany*

CO-AUTHORS: *Cornelia Prehn, Florian Schederecker, Annette Peters, Barbara Thorand, Jerzy Adamski*

Developing a core database for large cohort studies is always challenging. Especially in a metabolomics setting as the correct data normalization for a reliable statistical evaluation of the dataset is of utmost importance. The limited sample number on 96 well plates as well as the effects of the measurement batch have to be taken into account. First, a 96-well plate normalization has to be run to bring all plates of one study on the same comparable level of metabolite quantities. This is normally done by using quality control samples or reference samples, which run in parallel to the normal samples on each plate. Second, data transformation to log scale is commonly used to achieve normal data distribution in order to be able to run parametric statistic tests. However, to analyze data from large human cohort studies, such simple normalization processes may not be enough to account for day to day variations in measurements, let alone for measured steroid quantities of a heterogeneous cohort. In this work we compare log transformed data to cube root transformed data and also have a look at possible additional benefits of various data scaling methods to produce the best possible core database for the KORA cohort.

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BIG DATA, STATISTICS, INFORMATICS

P-287 NS-kNN: A modified k-nearest neighbors approach for imputing metabolomics data

PRESENTING AUTHOR: ***Justin Y. Lee, Georgia Institute of Technology, United States***

CO-AUTHORS: ***Mark P. Styczynski***

A frequent problem in large metabolomics datasets is the substantial amount of missing data that can occur, which can cause significant bias in downstream analysis or even prevent the use of certain data analysis methods. Values may be missing due to instrument error and can be referred to as missing completely at random (MCAR), while values missing because they are below or near the instrument's limit of detection have a different pattern of missingness and can be referred to as missing not at random (MNAR). K-nearest neighbors (kNN) imputation is a commonly-used method that estimates a missing value using the abundances of metabolites with similar profiles across all samples in the dataset. Assumptions in typical kNN implementations make it more effective for imputing MCAR values than the MNAR values that are quite prevalent in metabolomics. We have developed an approach called No Skip-kNN (NS-kNN) – a modified version of the original kNN algorithm – that acknowledges MNAR types. When imputing with NS-kNN, missing values across the dataset are used as indicators of increased likelihood of low abundance. We compared both methods on several realistic metabolomics datasets with varying amounts and types of missingness. We show that NS-kNN yields lower imputation errors than kNN if at least 40% of missing values in a dataset are MNAR, which is likely typical. NS-kNN is thus a viable and improved method for metabolomics data imputation that is also generalizable to other data types with many MNAR values.

P-288 Enhancing Compound Identification for Untargeted Ion Mobility-MS Workflows

PRESENTING AUTHOR: ***Aivett Bilbao, Pacific Northwest National Laboratory, United States***

CO-AUTHORS: ***Joon Y. Lee, Hania Khouri, Thomas O. Metz, Richard D. Smith, Erin S. Baker, John Fjeldsted, Samuel H. Payne***

Advanced identification algorithms can enhance current methods using collision cross section (CCS) libraries in untargeted ion mobility-mass spectrometry (IM-MS) workflows. Here we investigate multidimensional scoring strategies for improved metabolite identification within a complete informatics pipeline to study the alpha-proteobacterium Rhodopseudomonas palustris. Samples were obtained under three different growth conditions: aerobic, anaerobic nitrogen fixing and anaerobic photosynthetic. Polar and non-polar metabolite fractions were collected (i.e., metabolite and lipid rich phases) and analyzed in triplicate on an Agilent IM-Q-TOF MS. The PNNL PreProcessor software was used to improve raw data quality. Mass Profiler was used for feature extraction and alignment. Scoring algorithms for metabolite identification were implemented in R. Downstream analyses were performed in Mass Profiler Professional. An in-house CCS database from 500+ standards was used to annotate features. Our preliminary results show that by combining accurate mass and CCS in a simple tolerance-based search the specificity is increased and more features can uniquely be identified. For instance, a feature with 118.0863 m/z and CCS of 120.68 Å was uniquely matched to Betaine within 1% CCS tolerance and distinguished from L-Valine, which has the same formula but 10% CCS difference. Similarly, this feature would have been matched to at least two entries having the same formula in the METLIN database. The feature was found to be downregulated when comparing the aerobic samples versus both anaerobic conditions. Different scoring strategies are being investigated. Among those, nearest neighbor classification taking into account the 3 replicate measurements in our CCS database and different distance metrics.

P-289 Discovery based determination of metabolomic biomarkers using GC x GC - TOFMS and Fisher-ratio (F-Ratio) analysis

PRESENTING AUTHOR: ***Sarah Prebihalo, University of Washington - Seattle, United States***

CO-AUTHORS: ***Sarah E. Prebihalo, Nathanial E. Watson, Robert E. Synovec***

Non-targeted discovery-based analysis of metabolomics has been performed using comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC × GC – TOFMS). Due to the complex matrices, large, information-rich data cube are produced, which often require the use of chemometric data analysis methods to elucidate information. Among these techniques are classification and feature selection tools which allow the analyst to quickly identify analytes of importance. Tile-based Fisher-ratio (F-Ratio) has been developed for rapid discovery of analytes which distinguish between sample classes. Important chromatographic features identified in the F-ratio "hit list" are further investigated using complementary deconvolution methods for deconvolution, identification, and quantification. This analytical workflow combines experimental design, instrumental and computational methods for high-quality extraction of relevant sample features from complex discovery chromatographic studies in a brief period. The use of F-ratio has been successfully validated for use in metabolomic studies via reducing 70 GC × GC – TOFMS chromatograms to 96 metabolites that distinguish yeast metabolomic states. Of importance is the decrease in analysis time from 1 year using traditional analysis methods verses 1 week via F-ratio analysis. Most recently, the use of F-Ratio has been applied to the identification of disease state biomarkers in human plasma.

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BIG DATA, STATISTICS, INFORMATICS

P-290 Data-driven strain engineering: Our quest to produce hundreds of novel molecules

PRESENTING AUTHOR: ***Yang Zhang, Amyris, United States***

Amyris was founded over a decade ago on the premise that synthetic biology could help address some of the world's most pressing challenges. Focusing on supply-limited molecules of societal and economic value, such as medicines, nutrients, and specialty chemicals, Amyris has enabled their cost-effective and sustainable production by building a high-throughput genetic engineering platform coupled to industrial scale fermentation. To enable automated bioengineering, we implemented a suite of computational algorithms to design metabolic pathways, direct their synthesis and phenotyping, learn from the data, and iterate on the design for improved target molecule production. In this presentation I will describe our progress in creating discrete strains capable of making 450 novel molecules. In the process of strain optimization, we routinely collect >400,000 data points each week, largely dominated by metabolomics, and are now learning how to best feed these measurements back into proprietary machine learning and optimization algorithms to drive strain re-design in an automated and iterative fashion. Our intent is to create the first fully automated organism engineering platform.

P-291 Toward complete standards-free metabolomics and exposomics

PRESENTING AUTHOR: ***Ryan S. Renslow, Pacific Northwest National Laboratory, United States***

CO-AUTHORS: ***Sean Colby, Dennis Thomas, Jamie Nuñez, Yasemin Yesiltepe, Niranjan Govind, John Cort, Justin Teeguarden, Thomas O. Metz***

Conventional metabolomics and small molecule identification approaches have demonstrated immense value for disease diagnosis, evaluation of environmental exposures, and discovery of novel molecules. In contrast to genetic and proteomic information available from rapid genome and proteome sequencing, far less is understood about the total 'sequence' of the human metabolome and exposome. However, there are no authentic reference materials for the preponderance of these molecules, and without chemical standards, unambiguous chemical identification is currently limited to slow de novo structure elucidation approaches. We are advancing the use of in silico methods to identify molecules without the use of authentic reference materials. Leveraging innovations in computational quantum chemistry, we have developed a platform to overcome a significant obstacle in metabolomics: the absence of methods to accurately and comprehensively identify small molecules without relying on authentic chemical standards. Currently our approach uses accurate mass, isotopic signature, collision cross section (CCS), and NMR chemical shifts, which can be accurately predicted and consistently measured, enabling confident identification. Our pipeline uses a large-scale quantum chemistry platform for calculating chemical properties, which exploits PNNL's high-performance software, NWChem. Compared to experimental data, initial results indicate <1-2% CCS and <1 ppm NMR chemical shift errors. The values, with accurate mass information, can enable identification of metabolites with high accuracy. Here we discuss several applications of our "standards-free" metabolomics approach, including identification of environmental degradation products, molecular isomer separation, decoding complex blinded mixtures, and correcting mislabeled isobaric isosteres. We also discuss the challenges and future plans of this approach.

P-292 Metabolomics in the Cloud: Scaling Computational Tools to Big Data

PRESENTING AUTHOR: ***Noureddin Mahdi Sadawi, Imperial College London, United Kingdom***

CO-AUTHORS: ***Jianliang Gao, Ibrahim Karaman, Jake T M Pearce, Marco Capuccini, Anders Larsson, Ola Spjuth, Pablo Moreno, Robert C Glen, Timothy M D Ebbels***

Metabolomic datasets are becoming increasingly large and complex, with multiple types of algorithms and workflows needed to process and analyse them. This makes it difficult for researchers to interpret their data without extensive computational and bioinformatics support. The PhenoMeNal project has developed a cloud infrastructure with portable software tools, that enables the processing of larger datasets than typically possible at small labs, thus resolving bottlenecks and facilitating new discoveries. To demonstrate the benefits of up-scaling analyses to a cloud solution, we took two tools, BATMAN (for automated NMR processing) and PAPY (for statistical power and sample size determination) and examined their performance on increasing availability of compute resource. We performed tests at three different levels: a high-end stand-alone desktop machine (8 cores), a medium-scale cluster (80 cores), and a large-scale cluster (>1000 cores). In each case we used BATMAN to quantify 9 metabolites in 2000 1H NMR spectra of blood serum and used PAPY to estimate statistical power across a grid of sample and effect sizes, using 84 of the same spectra as pilot data. Results show that considerable speedup is achievable for both tools using all three systems. For example, BATMAN achieved near-linear scaling up to ~⅓ of the available resource, meaning that an analysis taking more than 3 days to process on one core could be processed in just 10 minutes on the large scale cluster. Overall, this investigation demonstrates the benefits, but also the limitations, of large scale compute infrastructures in processing large metabolomic datasets.

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BIG DATA, STATISTICS, INFORMATICS

P-293 A computational workflow for detecting unknown compounds from untargeted GC/MS and LC/MS metabolomics data and an online library of unknowns detected in plasma and urine

PRESENTING AUTHOR: *Xiuxia Du, University of North Carolina at Charlotte, United States*

CO-AUTHORS: *Aleksandr Smirnov, Yuanyuan Li, Wimal Pathmasiri, Susan Sumner*

Compound identification is considered a top grand challenge that the metabolomics field faces. Generally, more than 50% of the peaks detected by GC/MS or LC/MS from a data file cannot be assigned to any compound. Successful identification of even a small portion of these unknown peaks can significantly increase the information yield from untargeted metabolomics. In order to facilitate the eventual identification of these unknown peaks, we aim to create a publicly available library that collects and shares information of unknown peaks across different labs and studies. As the first step towards this end, we have developed a computational workflow for more accurately detecting unknowns, applied it for detecting unknowns from plasma and urine samples, and provided the resulting unknowns as an online library. For LC-MS, computational steps of the workflow include construction of extracted ion chromatograms (EICs), detection of peaks from EICs, grouping of peaks, determination of isotopic clusters, and determination of the most likely adduct form for each isotopic cluster. For GC-MS data, the most critical step is spectral deconvolution. The online library will be constantly updated when more data files are analyzed. Users of the online library will be able to search the library for individual masses, GC-MS fragmentation spectra, and pseudo mass spectra (consisting of peaks in LC-MS data that originate from the same compound) to find out how frequent the same information has been collected in other labs and look for additional information that can help with the subsequent challenging task of compound identification.

P-294 MetFamily – A Novel Software Tool to Identify Regulated Metabolite Families

PRESENTING AUTHOR: *Gerd Ulrich Balcke, Leibniz Institute of Plant Biochemistry, Germany*

CO-AUTHORS: *Hendrik Treutler, René Meier, Christoph Ruttkies, Alain Tissier, Steffen Neumann*

Untargeted metabolomics with GC or LC coupled to high resolution mass spectrometry allows the detection of several thousand metabolite signals per chromatographic run. But, due to the high complexity of the data, the lack of pure standards, and ambiguous conditions for the assessment of CID-MS/MS spectra, most of those mass features cannot be annotated and assigned to known chemical structures. However, enzyme promiscuity and the grid-like structure of metabolic pathways often result in the production of families of metabolites that have common structural features. Since this similarity is often conserved in the MS fragmentation pattern it is possible to identify regulated metabolite families from comparative metabolomics measurements. This rationale was the basis for the development of the software MetFamily, which is freely accessible under https://msbi.ipb-halle.de/MetFamily/1. Metabolite families can be systematically classified by chemical ontologies such as ChemOnt2. We used Classyfire2 to systematically annotate mass spectra of thousands of pure standards from public sources with the corresponding metabolite family terms from ChemOnt and extracted characteristic fragment motifs of hundreds of metabolite families from their consensus spectra. Characteristic motifs were used for the de novo prediction of metabolite families from mass spectra of unknown compounds and individual families were compared for their regulation in light stress experiments using Arabidopsis thaliana. In analogy to Pfam3 for the annotation of protein classes we here introduce Mfam which allows to automatically assign MS2 fragmentation patterns to certain metabolite families. 1Anal Chem. 2016,88(16):8082-90, 2J Cheminform, 2016, 8:61, 3Nucleic Acids Res. 2016, 44(D1):D279-85.

P-295 Data-adaptive pipeline for filtering and normalizing metabolomics data.

PRESENTING AUTHOR: *Courtney Schiffman, University of California, Berkeley, United States*

CO-AUTHORS: *Lauren Petrick, Kelsi Perttula, Sandrine Dudoit, Stephen Rappaport*

Untargeted metabolomics datasets are affected by a variety of nuisance technical effects and large proportions of uninformative features that can bias subsequent statistical analyses in ways that obscure the underlying biological phenomena of interest. Thus, there is a need for versatile and data-adaptive methods for filtering and normalizing data prior to investigating the underlying biology. Here we present a data-adaptive pipeline for filtering and normalizing metabolomics data that are generated by liquid chromatography-mass spectrometry platforms. Our pipeline includes novel methods for filtering features based on blank samples, proportions of missing values, and estimated intra-class correlation coefficients, andincorporates a variant of the k-nearest-neighbor technique for imputation of missing values. Importantly, we also adapted an RNA-Seq R package, scone, to select an appropriate normalization scheme for removing unwanted variation from metabolomics datasets. Using two untargeted metabolomics datasets that were generated in our laboratory from human samples, we compared our data-adaptive pipeline with a traditional filtering and normalization scheme. The data-adaptive approach outperformed the traditional pipeline in almost all metrics related to the removal of unwanted variation and maintenance of biological variation. Our proposed data-adaptive pipeline should be considered for interrogation of biospecimens to investigate biological phenomena of interest.The R code for running the data-adaptive pipeline is freely available online.

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BIG DATA, STATISTICS, INFORMATICS

P-296 Uniting metabolomics data processing and highly confident annotation across four MS instrumental set ups: MetaboScape 4.0

PRESENTING AUTHOR: ***Nikolas Kessler, Bruker, Germany***

CO-AUTHORS: ***Nikolas Kessler, Wiebke Timm, Sascha Winter, Ulrike Schweiger-Hufnagel, Sven Meyer, Aiko Barsch, Heiko Neuweger***

Metabolomics approaches may be motivated in a variety of ways, pushing different criteria into foreground: speed (throughput), separation, and/or accuracy. Tailored to these prioritized criteria different instrumental set ups will fall into favor. Combining different platforms, complementing their respective strengths, ultimately closes the gap between high-throughput and in-depth analysis methods. MetaboScape 4.0, including the feature extraction and ion deconvolution algorithms T-ReX 2D, 3D and 4D, integrates the processing-, dereplication- and unknown annotation-workflows for FIA-MRMS, LC-MRMS, LC-ESI-TOF, and LC-ESI-TIMS-TOF in a single software. Both automated and manual tools for metabolite annotation seamlessly adapt to the annotation quality criteria each instrumental platform provides. The chromatographic dimension from LCs is a proven indicator for annotation of knowns. MRMS instruments unlock high-resolution mass accuracy and the power to resolve isotopic fine structure. TIMS-TOF data immediately enables all MS/MS workflows to profit from Parallel Accumulation Serial Fragmentation (PASEF) by comprehensive MS/MS fragmentation coverage. Ion mobility can serve as an additional indicator for annotations. To assess the confidence in any annotation all five criteria are reported in a concise but detailed summary, called the annotation quality. Within data types, processing results from positive and negative mode acquisitions can be merged. Additionally, MetaboScape allows generating and extending analyte lists and spectral libraries including all five criteria, making it easy to combine annotation results even across instrumental setups. This finally allows uniting high throughput workflows with the maximum of in-depth exploitation of all the mass spectral information for specific metabolites.

P-297 Propagating annotations on molecular networks using in silico fragmentation

PRESENTING AUTHOR: ***Ricardo R. da Silva, University of California, San Diego, United States***

CO-AUTHORS: ***Mingxun Wang, Louis-Félix Nothias, Justin J. J. van der Hooft, Andrés Mauricio Caraballo-Rodríguez, Evan Fox, Marcy J. Balunas, Jonathan L. Klassen, Norberto Peporine Lopes, Pieter C. Dorrestein***

The annotation/identification of small molecules is one of the most challenging and important steps in untargeted mass spectrometry analysis. Molecular networking has emerged as a structured way to organize and mine data from untargeted tandem mass spectrometry (MS/MS) experiments and has been widely applied to propagateannotations. However, propagation is done through manual inspection of MS/MS spectra connected in the spectral networks and is only possible when a reference library spectrum is available. Here we show how molecular networking can be used to improve the accuracy ofin silicopredictions through propagation of structuralannotations, even when there is no match to an MS/MS spectrum in spectral libraries. NAP is built on top ofin silicofragmentation performed with MetFrag, searching biologically relevant databases GNPS, HMDB, SUPER NATURAL II, ChEBI and DNP (367,204 unique small molecules) and as a general database PubChem. There are two scoring approaches utilized by NAP to re-rank candidates. When there is a spectral library match within a molecular family of the molecular network NAP utilizes the MetFragin silicoprediction with the MetFusion score to re-rank candidates (Fusionscoring). When there are none or very few spectral matches, a network consensus scoring will obtain the structural similarity from the candidate structures instead, exploiting the structural similarity of thein silicocandidates (Consensusscoring). For NIST benchmark, networkFusionandConsensusscores had 29.0% and 19.8%, increase in the first position, respectively, compared to MetFrag alone.NAP is accessible at GNPS web-platformhttps://gnps.ucsd.edu/ProteoSAFe/static/gnps-theoretical.jsp.

P-298 Metabolomics studies to predict potency of mesenchymal stem cell using NMR

PRESENTING AUTHOR: ***Xunan Shen, CCRC, China***

We are part of a new NSF Engineering Research Center for Cell Manufacturing Technologies (CMaT). The overall goal of CMaT is to improve the production and distribution of cells as therapies. Mesenchymal stem cells (MSCs) have potential in stem cell-based therapies for tissue repair, organ transplantation and the treatment of autoimmune disease [1]. However, unintended differentiation and unpredictable performance of transplanted MSCs in human present challenges in the clinical application of MSC-based therapies [2]. We are using metabolomics, other phenotypic measurements, and modeling to discover critical quality attributes (CQAs), which could be used to predict the efficacy and potency of MSCs for transplantation. In this poster, we will show our results using NMR to attempt to discover CQAs of human umbilical MSCs. MSC media were periodically sampled for 48 hours during culturing with and without fetal bovine serum. Media and cell endometabolites were characterized by 1H proton NMR experiments after culturing. We will be correlating changes in the media and cellular metabolites with various phenotypic cell surface and other markers. The goal is to determine CQAs that can predict the function of these MSCs.

POSTER SESSIONS 1 AND 2 – **Monday and Tuesday –** all odd number presenters will be at their posters.
POSTER SESSIONS 3 AND 4 – **Wednesday and Thursday –** all even number presenters will be at their posters.

BIG DATA, STATISTICS, INFORMATICS

P-299 PlaSMA: decoding plant specialized metabolome by the suite of mass spectrometry cheminformatics

PRESENTING AUTHOR: ***Hiroshi Tsugawa, RIKEN CSRS, Japan***

CO-AUTHORS: ***Hiroshi Tsugawa, Ryo Nakabayashi, Amit Rai, Tetsuya Mori, Yutaka Yamada, Hiroyuki Yamamoto, Makoto Arita, Masanori Arita, Kazuki Saito***

Mass spectrometry (MS) cheminformatics aims at the identification of known/unknown metabolites illuminating the dark matter of biological mechanisms. We address the comprehensive annotation for plant specialized metabolites, termed as PlaSMA (Plant Specialized Metabolome Annotation) project, by using LC-MS/MS with the development of MS cheminformatics technologies in addition to human curation effort: the aerial and underground parts of 12 plant species are currently investigated. The annotation is first executed by publicly- and commercially available spectral databases in addition to our newly recorded MS/MS spectra of 420 plant metabolites. For remaining unknowns, the carbon element count is determined by the fully 13C labeled plant tissues with MS-DIAL isotope tracking technology, followed by the molecular formula determination tested as more than 99% accuracy. The structure candidates from a total of 21 metabolome structure databases are ranked by MS-FINDER in silico fragmenter with hydrogen rearrangement rules and newly developed molecular fingerprint. Furthermore, the structure candidate is further curated by confirming positive/negative ion features, unique product ions and neutral losses, and metabolite literatures in addition to molecular networking based on in silico biotransformation, metabolite ontology similarity, and MS/MS spectral similarity. For unknown-unknowns, the metabolite classes are proposed by newly developed fragment set enrichment analysis (FSEA) method which suggests the metabolite ontology via Fisher's test by the set of metabolite class and its fragment ontologies. Consequently, more than 1,000 plant specialized metabolites have already been annotated from the plant extracts, where new structures such as aminesulfoxides, hexosylphospholipids, amino acid-, phenylpropanoid-, and saponin derivatives are also showcased.

P-300 PhenoMeNal: uncomplicated metabolomics data analysis in the cloud

PRESENTING AUTHOR: ***Pablo Moreno, EMBL-EBI European Bioinformatics Institute, United Kingdom***

CO-AUTHORS: ***Namrata Kale, Luca Pireddu, Pierrick Roger, David Johnson, Ralf Weber, Michael Van Vliet Noureddin Sadawi, Kenneth Haug, Claire O'Donovan, Etienne Thevenot, Steffen Neumann, Tim Ebbels, Ola Spjuth, Christoph Steinbeck, PhenoMeNal Consortium***

Metabolomics data analysis is hampered by the lack of bioinformatics resources in many institutions and is complicated by the maintenance requirements and scalability of scientific software. PhenoMeNal is an e-infrastructure for metabolomics data analysis which aims to bridge these gaps, reducing scientific software and pipelines setup complications and allowing to scale up easily and reproducibly. A PhenoMeNal Cloud Research Environment (CRE), which the user can easily create on different clouds through the PhenoMeNal Portal, provides access to more than 180 metabolomics data analysis modules which rely on state of the art software packages such as XCMS, OpenMS, MetFrag and NMRProcFlow, among others. PhenoMeNal's setup creates an scalable and fault-tolerant computer cluster on a cloud provider of choice, both public and private, where the user does not compete for computational resources with other users. Within their own PhenoMeNal CRE, users can interact with the data analysis modules via user friendly workflows (Galaxy, among others) or through programmatic environments. Additionally, PhenoMeNal provides a public version of the CRE, where users can test the tools and Galaxy workflow environment before deciding to create their own CRE. We will describe the infrastructure and give an overview of the available analysis workflows.

P-301 Large-scale kinetic modeling of Neurospora crassa driven by real-time, in-vivo metabolomics data

PRESENTING AUTHOR: ***Yue Wu, University of Georgia, United States***

CO-AUTHORS: ***Michael Judge, Heinz-Bernd Schüttler, Jonathan Arnold, Arthur S. Edison***

Large-scale Flux Balance Analysis (FBA) and small-scale kinetic models have been developed for the classic biochemical model, Neurospora crassa. However, Neurospora still lacks a large-scale kinetic model (LSKM), which would deepen our understanding of global metabolism and its regulation, including the roles of central metabolism in circadian regulation. LSKMs are difficult to construct due to scale of models and limited metabolic data. Time-series metabolomic measurements can offer crucial data on the parameters for an LSKM, but these experiments are labor-intensive, while high temporal resolution is difficult (if possible) to obtain. Furthermore, biological and extraction variance complicate quantification of metabolites and modeling. Using High-Resolution-Magic Angle Spinning (HR-MAS) NMR, we obtain real-time, in-vivo, untargeted metabolomics data on single Neurospora cultures (see poster by Michael Judge). This eliminates the problem of extraction variance, while yielding a true time series for each biological replicate. We map kinetic parameters mined from BRENDA and SABIO-RK onto existing network of Neurospora central metabolism from a previous FBA model. The network was also reformatted by graph partition to promote parallel computation. We then will fit and optimize the parameters of this model using HR-MAS time-series data. This novel approach may open the door to finer-resolution kinetic models, where the restrictions posed by these models might inform identifications in untargeted metabolomics data, while allowing estimation of dynamics that are difficult to measure directly using NMR.

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POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

BIG DATA, STATISTICS, INFORMATICS

P-302 MASTR-Quant: An Open-Source Web Based Software Tool for the Quantitation of Mass Spectrometry-based Data

PRESENTING AUTHOR: ***Saravanan Dayalan, Metabolomics Australia, The University of Melbourne, Australia***

CO-AUTHORS: ***Ruobing Leng, Komal Kanojia, Vinod K. Narayana, Konstantinos Kouremenos, Thusitha Rupasinghe, Ute Roessner, Malcolm McConville, Richard Sinnott***

We present an open-source, web-based software tool that allows users to efficiently calculate the concentration values of features in mass spectrometry data through visualising and defining calibration curves based on several adjustable parameters. MASTR-Quant presents the user options of inputting data in a .csv format, including and excluding data points in order to define the linear or quadratic calibration range. The tool also includes options to assign individual internal standards to specific analytes, to apply weighting factors, background subtraction and dilution ratios. In addition, the tool allows normalization of the data based on internal standards and other external measurements. MASTR-Quant also includes the option of assessing quality of the data through the calculation of coefficient of variation values of each sample group. The final output is downloadable as an excel file with multiple sheets containing detailed results of the individual steps involved in the calculation process. MASTR-Quant allows the user to import data from GC-MS or LC-MS platforms with either full scan or multiple reaction monitoring (MRM) data. Advantages of MASTR-Quant include that it is freely available, easy to use through a web interface and can import data from different instrument platforms. It is also transparent in the underlying calculations and provides users complete control over the setting of parameters. MASTR-Quant also overcomes the disadvantage of manual calculations in spreadsheets, which are known to be time consuming and error-prone. MASTR-Quant therefore offers a fast, efficient and reproducible method of calculating concentrations of analytes.

P-303 An automated framework for on-line monitoring, evaluation and annotation of untargeted mass spectrometry-based metabolomics experiments.

PRESENTING AUTHOR: ***Ralf J. M. Weber, University of Birmingham, United Kingdom***

CO-AUTHORS: ***Jordi Capellades, Gavin R. Lloyd, Thomas N. Lawson, Andris Jankevics, Martin R. Jones, Oscar Yanes, Ralf J. M. Weber***

Mass spectrometry (MS) based metabolomics approaches are establishing themselves as affordable, high-content analysis assays in biomedical and clinical contexts. Constant optimal performance of MS instruments is important to ensure high quality data collection. Instrument performance and data quality can be assessed through the analysis of quality control (QC) samples. This is typically done after the data has been collected, which is inefficient and might result in unnecessary data collection of biological samples when the data quality is found to be poor. Currently, the number of robust and open-source solutions that automatically and systematically monitor instrument performance, conduct statistical analysis to assess data quality, and rank m/z features for subsequent fragmentation experiments is limited. Here we present a computational framework, compatible with Python, R and the web-based platform Galaxy, to enable on-line automated data-processing, statistical analysis and annotation of LC-MS, LC-MS/MS and DIMS data. Experimental metadata and processed data are stored, and can be accessed for subsequent statistical analysis and "real-time" visualisation of instrument performance using process control charts. MS2 QC files, when available, are processed and features are annotated using compound databases/libraries, spectral matching and "in silico" based methods. The results are then used to create ranked target lists for further directed fragmentation experiments. The proposed solution will 1) help to facilitate important decision making for on-going and/or subsequent data collection and analysis; 2) enhance high-throughput capabilities by allowing rapid automated assessment of instrument performance in "real time" and the automated generation of fragmentation targets for improved putative metabolite annotation.

P-304 A prototypic small molecule database for bronchoalveolar lavage-based metabolomics

PRESENTING AUTHOR: ***Scott J Walmsley, University of Colorado Denver-Anschutz, United States***

CO-AUTHORS: ***Charmion Cruickshank-Quinn, Kevin Quinn, Xing Zhang, Irina Petrache, Russell P Bowle, Richard Reisdorph, Nichole Reisdorph***

The analysis of bronchoalveolar lavage fluid (BALF) using mass spectrometry-based metabolomics can provide insight into lung diseases, such as asthma. However, the important step of compound identification is hindered by the lack of a small molecule database that is specific for BALF. Here we describe prototypic, small molecule databases derived from human BALF samples (n=117) assembled using a custom informatics workflow written in R and PHP/SQL. This workflowcomputesion statistics, removescontaminants, aidsvisualization of tandem mass spectrometry results, and filterspotential artifacts. It includesmethods to evaluate realistic isotope ratios and to remove ions identified by in-source fragmentation.The end result is a automatically curated and comprehensive BALF database.To populate the database, human BALF was extracted into lipid and aqueous fractions and analyzed usingLC/MSn.The resulting BALF databases (BALF-DBs) contain 11,736 lipid and 658 aqueous compounds. Over 10% of these where found in 100% of the samples, which by nature are highly variable. TreeMaps of identified compounds from Lipid Maps and the Human Metabolome Database indicated enrichment of specific classes of molecules from both extractions; these include Glycerolipids, Sphingolipids, and Polyketides. Testing the BALF-DBs using nested test sets produced a 99% match rate for lipids and 47% match rate for aqueous molecules. Searching an independent dataset resulted in 45% matching to the lipid BALF-DB compared to < 25% when general databases are searched. Overall, the BALF-DBs can increase confidence in compound identification by saving, cleaning, and searching the reliably detected compounds detected across many samples.

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POSTER SESSIONS 3 AND 4 – **Wednesday and Thursday –** all even number presenters will be at their posters.

BIG DATA, STATISTICS, INFORMATICS

P-305 The nPYc toolbox, a python module for the pre-processing, quality-control, and analysis of metabolic profiling datasets

PRESENTING AUTHOR: ***Jake TM Pearce, Imperial College London, United Kingdom***

CO-AUTHORS: ***Caroline J. Sands, Arnaud M. Wolfer, Noureddin Sadawi, Gonçalo S. Correia, Matthew R. Lewis, Elaine C. Holmes, Robert C. Glen, Jeremy K. Nicholson***

Ensuring cross-platform, -study, and -centre reproducibility is critical to the wider application of metabolic profiling technologies.The nPYc-toolbox provides an open-source python module for the import, quality control and visualization of metabolic profiling datasets. The toolbox may be used interactively, or to build automated pipelines, and embodies our current understanding of the state-of-the-art in analyical QC for untargeted UHPLC-MS and NMR, and targeted analyses, as well as enabling multivaraite visulisationsto examine data-quality in the context of the entire dataset. UHPLC-MS QC is agnostic to peak-picking software and design to characterise and filter features in terms of RSDs, linearity of response, and run-order and batch effects. NMR QC is based oncharacterisingbaselinedeviations, quality of water suppression, and line-width, in addition toassessing chemical shift deviations. In order to support transfer of QC'd data out of the pipeline it supports the export of datasets using the ISATAB study description format in addition tobasic tabular outputs, alongside generation of HTML reports of data-quality.

P-306 Matching Liquid Chromatography - Mass Spectrometry Metabolomic Features Across Datasets: Finding the Same Needles in Two Haystacks

PRESENTING AUTHOR: ***Rui Climaco Pinto, Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, United Kingdom***

CO-AUTHORS: ***Ibrahim Karaman, Matt R. Lewis, Jenny Hällqvist, Manuja Kaluarachchi, Gonçalo Graça, Elena Chekmeneva, Abbas Dehghan, Paul Elliott, Ioanna Tzoulaki, David Herrington, Timothy Ebbels***

This work addresses the important and unsolved problem of matching LC-MS features between different datasets. In untargeted metabolomics, statistical analysis is typically performed on metabolomic features for which the median retention time, m/z, and intensity are known, but not the metabolite identity. Matching features within a dataset is relatively straightforward, as changes in peak positions of successive (run-order) samples are minor. Nevertheless, it is often desirable to combine different datasets (e.g. analytical batches or different cohorts) acquired with a similar setup, but assayed and peak-picked separately. However, matching un-annotated features across different sets is challenging due to their large numbers, within-dataset retention time and m/z similarities, between-dataset retention time and m/z shifts, and different total numbers of features detected using automated peak detection algorithms in each set. We propose a method for matching features between two datasets acquired in similar experimental conditions, to be used after peak-picking. As input, the method uses only the median retention time and median m/z value of each feature (optionally also the features' median intensity). It begins by finding all matches within manually-defined retention time and m/z thresholds, then selects a single match from multiple possible ones, and finally detects false positive matches using discriminant analysis. We illustrate the new method's performance by matching thousands of features from two large studies of serum samples. For validation, we compare intensities of matched features, manually identified compounds in both cohorts, and strength of association of matched features with age and gender.

P-307 Cheminformatics Tools For Enabling Metabolomics

PRESENTING AUTHOR: ***Yannick Djoumbou Feunang, University of Alberta, Canada***

CO-AUTHORS: ***David S Wishart***

The constitution of the human chemical exposome is function of the exposure to exogenous chemicals (e.g. foods, contaminants), and endogenous chemicals (e.g. lipids) that are produced or modified during genetically programmed events, or in response to various stimuli. Understanding the effects of such exposures on humans and the environment is very crucial, yet hampered by several factors. These include, among others, the significantly high ratio of unknown chemicals from the human exposome (>95% of ~ 3,000,000 compounds), and the desperately low ratio (~2%) of detectable mass spectrometry (MS) peaks that are identifiable in large-scale Metabolomics experiments. These factors highlight the need for computational tools to enhance metabolomics. It is believed that the dark matter of the human chemical exposome is largely explained by biotransformations of known chemicals. Moreover, structurally similar molecules tend to undergo similar metabolic or degradation pathways, and even fragment similarly in MS experiments. Therefore, a structural categorization of compounds could help detect common biotransformation and fragmentation patterns, which would assist the prediction of metabolites and MS spectra, using Cheminformatics approaches. Based on these principles, we have developed: 1) BioTransformer, a freely available software tool for small molecule metabolism prediction, and metabolite identification, in mammalians and the environment, and; 2) CFM-ID 3.0, an extension of CFM-ID, a freely available software tool and web server designed to accurately predict mass spectra for rapid compound identification. In this presentation, I will describe both tools, and provide some applications that demonstrate how they could be used to enhance Metabolomics.

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BIG DATA, STATISTICS, INFORMATICS

P-308 Software Utilizing Positive and Negative Ion MS2 / MS3 HCD and CID Spectra for Improved LC-MSn Lipid Annotation

PRESENTING AUTHOR: ***David A Peake, Thermo Fisher Scientific, United States***

CO-AUTHORS: ***Reiko Kiyonami, Gavin E Reid, Yasuto Yokoi, Andreas Hühmer***

We present improvements to the latest version of Thermo Scientific LipidSearch software. New algorithms are introduced to reduce false positives, improve quantitation and to automate searching of LC-MSn data obtained by higher collisional energy (HCD) and linear ion trap collisional induced dissociation (CID) fragmentation methods. Bovine heart and liver total lipid extracts were analyzed by data dependent LC/MS2 and MS3 and these spectra were searched against the predicted product ions and neutral losses for all of lipid species present in the LipidSearch database. Each lipid adduct ion is ranked by mass tolerance, match to the predicted fragmentation and fraction of total MS-MS intensity for predicted ions. The number of lipid species annotated in each experiment were assessed at the sum composition (MS) and isomer (MSn) levels. LC-dd-MS2/MS3 spectra for potential lipid species were annotated separately from positive and negative ion adducts. Each lipid annotation was correlated within a chromatographic time window by merging the positive and negative ion MSn data into a single lipid result. This approach provides lipid annotation that reflects the appropriate level of MS2/MS3 product ions and neutral losses from the entire dataset giving higher confidence in lipid annotations. The merged results were filtered by minimum number of data points, signal-to-noise ratio, adduct ion, match score, ID quality, and coefficient of variation from replicate sample injections. Compared to the results generated only from dd-MS2 HCD results, a combination of HCD and CID LC-MSn gave significantly higher quality lipid identifications.

P-309 The Human Fecal Metabolome Database

PRESENTING AUTHOR: ***Jennifer Reid, The Metabolomics Innovation Centre, University of Alberta, Canada***

CO-AUTHORS: ***Naama Karu, Lu Deng, Mordechai Slae, An Chi Guo, Tanvir Sajed , Hien Huynh, Eytan Wine, David S. Wishart***

Metabolomic analysis of human biospecimens had progressed quickly over the past decade. Technological and methodological advances have led to the comprehensive characterization of human serum, urine, cerebrospinal fluid and saliva metabolomes and the creation of freely available metabolome reference databases. Fecal metabolite analysis is an emerging field of metabolomics that provides wide metabolic coverage of an easily accessible and biologically-valuable biospecimen. However, characterization of the human fecal metabolome lags behind the other metabolomes in terms of the availability of standardized methods and freely available resources. Here we will provide a comprehensive overview of fecal metabolomics based on manual review of over 200 PubMed and Google Scholar abstracts, as well as the human fecal metabolome database (HFMDB, http://www.fecalmetabolome.ca), a freely available, manually curated resource that currently contains over 6000 identified human fecal metabolites. Each entry in the HFMDB includes extensive chemical and biological information about the metabolites as well as the genes, proteins, pathways and diseases with which they are associated. The HFMDB allows convenient browsing and searching options, and provides vital information that can be used by analytical chemists, biologists and clinicians.

P-310 Metabolomics and Galaxy: Tools and workflows for increased reproducibility and transparency of metabolomics data processing and analysis.

PRESENTING AUTHOR: ***James Bradbury, University of Birmingham, United Kingdom***

CO-AUTHORS: ***Thomas N. Lawson, Martin R. Jones, Andris Jankevics, Gavin R. Lloyd, Mark R. Viant, Ralf J. M. Weber***

Metabolomics data processing and analysis workflows are often complex and include many open source tools, each with their own dependencies, resource requirements and scripting languages. Configuring these tools is often complicated, especially for those untrained in informatics. Furthermore, many tools require users to input parameters that can significantly affect outputs and performance, though reporting of these parameters is not always clear. These challenges, faced when using metabolomics data processing workflows, hinder the reproducibility of metabolomics research, which must strive towards making data processing workflows more accessible and better reported. The Galaxy Project [galaxyproject.org], originally developed for the genomics community, offers an open web-based platform enabling developers to make data processing tools and workflows available via an intuitive graphical user interface. Over the past few years this platform has been expanded, through projects including PhenoMeNal [phenomenal-h2020.eu], Workflow4Metabolomics [workflow4metabolomics.org] and MetaboFlow [metaboflow.org], to include many useful and powerful metabolomics tools. Tools included in this platform can be incorporated into defined and shareable workflows, helping to improve reproducibility and to extend their accessibility and utility by the wider scientific community. Our team at the University of Birmingham, UK, is actively involved in the initiatives reported above. Through these efforts a range of well-tested tools have been developed that can be integrated into standardised and automated workflows, within the Galaxy platform, for LC-MS(/MS) and direct-infusion MS based metabolomics data processing. Here, we provide an overview of the Galaxy tools and workflows arising through these initiatives.

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BIG DATA, STATISTICS, INFORMATICS

P-311 Binner: A tool for annotation of degenerate features in untargeted LC-MS metabolomics data

PRESENTING AUTHOR: ***Maureen Kachman, University of Michigan, United States***

CO-AUTHORS: ***Hani Habra, William Duren, Janis Wigginton, Peter Sajjakulnukit, George Michailidis, Charles Burant, Alla Karnovsky***

Untargeted metabolomics studies aim to provide a comprehensive view of all the measurable metabolites in biological samples. The advantage of the untargeted approach is that it achieves a broad coverage of the metabolome, but the tradeoff is the presence of a large number of unknown signalscommonly called features. When a metabolite is analyzed by electrospray ionization-mass spectrometry (ESI-MS), it is usually detected as multiple ion species due to the presence of isotopologues, adducts, and in-source fragments that cannot be readily identified. As a result, a large portion of unknown metabolome (sometimes as high as 70%) is highly redundant, which complicates statistical analysis, as well as compound identification. To address this problem we developed a Java based tool Binner (http://metscape.med.umich.edu/). Binner workflow consists of the following steps: data pre-processing, binning, isotope detection, clustering, computing mass differences, and annotation. Users can choose to run Binner in an automated mode and accept the annotations provided, or to perform interactive data exploration. In addition to possible feature annotations, Binner provides users with the wealth of supporting evidence. An important distinguishing feature of Binner is the ability to recursively build annotation libraries for user-specific experimental platform to provide relevant chemical context combined with intuitive data visualization.

P-312 Machine Learning-based Automated Quantification of Plasma Metabolite from NMR Spectra and Its Application to the jMorp Database

PRESENTING AUTHOR: ***Yuichi Aoki, Tohoku University, Japan***

CO-AUTHORS: ***Yuichi Aoki, Ikuko N. Motoike, Daisuke Saigusa, Seizo Koshiba, Matsuyuki Shirota, Shu Tadaka, Kengo Kinoshita, Masayuki Yamamoto***

Plasma concentrations of biomolecules vary among individuals, depending on genetic and environmental factors. Hence, measurement of such biomolecules, called omics study, can provide valuable information for evaluating individual phenotypes, especially diseases.Tohoku Medical Megabank Organization (ToMMo) has completed the multi-omics (metabolome and proteome) analysis of 5093 plasma samples collected from Japanese (male: 2077, female: 3016) residents who participated in population-based adult cohort studies by the Tohoku Medical Megabank Project. This data has been integrated into the database “Japanese Multi Omics Reference Panel (jMorp)” which is publicly available online [https://jmorp.megabank.tohoku.ac.jp].Metabolome data were measured by proton NMR and LC-MS, and proteome data were obtained by nanoLC-MS. In current state, we released distributions of concentrations of 37 metabolites identified by NMR, distributions of peak intensities of 257 characterized metabolites by LC-MS, and observed frequencies of 256 abundant proteins. Additionally, correlation networks between metabolites are also observed using an interactive network viewer.For this database, we newly developed the program for the estimation of metabolite concentrations from the NMR data, using several linear regression and neural network models. In order to achieve the accurate automatic quantification of plasma metabolites, we intoroducedacombination techniqueof unsupervised and supervisedlearning algorithms, which can buffer thenegative effects ofpeakfluctuation due to thedifference of physico-chemical properties among analyzedsamples.We expect that jMorp is an outstanding resource for a wide range of researchers, particularly those in the fields of medical science, applied molecular biology, and biochemistry.

P-313 A New Set of Metrics for Evaluating LC-MS Global Metabolite Profiling Data Quality

PRESENTING AUTHOR: ***Xinyu Zhang, University of Washington, United States***

CO-AUTHORS: ***Jiyang Dong, Haiwei Gu, Qiang Fei, Daniel Raftery***

Data quality in global metabolite profiling, including metabolite coverage, missing values and reproducibility, are of great significance and often a major challenge for data processing and statistical analysis. We propose an expanded set of metrics be reported in global metabolomics studies to provide an improved evaluation of data quality. In this work, we use a combination of new metrics along with well-known measures to characterize the data quality of global metabolite profiling. A pooled human serum sample was run 50 times on an Agilent LC-QTOF-MS (6545) to provide high-resolution profile and centroid MS data. These data were processed by Progenesis Qi, and then evaluated by a set of 5 metrics. In ESI+ mode, 5213 features with two or more ions were extracted from the profile data and 1348 were identified using the HMDB. A novel evaluation approach that plotted the compound number versus frequency extracted from the samples was developed to evaluate the missing values. 5137 compounds were detected from all samples and 5210 were detected from 70%. In the compounds free of missing values, more than 70 % had CVs smaller than 10% and only 7% had CVs larger than 25%. The Pearson and intra-class correlation coefficients were calculated after log transformation of the data and showed improved performance from the profile data over the centroid data. To better understand the data quality quantitatively, the raw data were also collected from an older Agilent 6520 QTOF as a reference.

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BIG DATA, STATISTICS, INFORMATICS

P-314 Systems approaches to understanding altered metabolic landscapes in Alzheimer's disease

PRESENTING AUTHOR: ***Priyanka Baloni, Institute for Systems Biology, United States***

CO-AUTHORS: ***Cory Funk, Vangelis Simeonidis, Arnold Matthias, Gabi Kastenmueller, Rima Kaddurah-Daouk, Nathan Price***

Alzheimer's disease (AD) is the leading cause of dementia and is characterized by a progressive decline in cognition. With disease etiology believed to begin ~20 years prior to symptoms with variable onset and progression reflects the complex and multifactorial nature of AD. Several studies have implicated metabolic dysfunction as contributing or coinciding with neurodegeneration. The genes and metabolites involved in altered metabolic pathways of AD are potential therapeutic candidates or biomarkers. We used high-throughput transcriptome data of post-mortem brain samples from AD patients and controls to generate region-specific metabolic networks of the human brain, using the most recent human metabolic reconstruction (Recon3D) as a template, to understand the changing metabolic landscape in AD. We have generated draft metabolic networks for multiple AD-affected brain regions. Previous work identified a role for circulating bile acids (BA) in AD, along with altered cholesterol metabolism. Increased levels of secondary BAs and ratios to their primary BA educts have been linked to AD and cognitive decline. By integrating metabolomics measurements of BAs from brain tissue of AD patients and controls within these metabolic networks, our models can capture in silico changes in these pathways, highlighting the role of brain BA metabolism in AD pathophysiology.

P-315 Using multiple types of controls to normalize metabolomics data

PRESENTING AUTHOR: ***Gavriel Olshansky, School of Mathematics and Statistics, The University of Melbourne, Australia***

CO-AUTHORS: ***Terry Speed, Peter Meikle, BIS investigator Group, Alysha M De Livera***

Normalization is an integral part of the statistical analysis of metabolomics data. Due to unavoidable exposure of metabolomics studies to external factors during sample collection, processing, storage, extraction and machine run, the use of an appropriate normalization method is required to improve identification of biomarkers, clustering and classification of the data in answering biological questions of interest. There are several types of controls which could be used in metabolomics studies, including pooled biological samples, technical control samples, internal standards and low varying metabolites. Existing metabolomics normalization approaches do not make full use all of these. Here we propose a novel normalization strategy using the RUV-III framework that can use all available control data in the normalization,capturing different components of unwanted variation. We compare the proposed approach with existing methods using targeted UPLC-MS based lipidomics data from plasma samples in the Barwon Infant Study, which includes 2 types of technical replicates and 27 metabolites as internal standards. This case study involves identifying metabolites that are differentially abundant in relation to gestational age. Our preliminary findings using the proposed approach suggest the identification of a number of biologically meaningful metabolites that were not previously detected due to confounding with unwanted variation. An overall improvement in the detected signal and the correlation structure is also achieved. Using simulations based on this data, we further investigate the robustness of the proposed approach for use in a range of scenarios that are encountered in metabolomics, and provide guidance regarding the applicability of the approach.

P-316 Study of correlations between metabolic and neutral genetic diversities based on data integration

PRESENTING AUTHOR: ***Marie Tremblay-Franco, INRA, UMR 1331, PF MetaToul-AXIOM, Toxalim, France***

CO-AUTHORS: ***Florence Souard, Olivier J. Hardy, Anais Gorel, Jérôme Duminil, Cédric Delporte, Jean-François Martin, Jean-Louis Doucet, Pierre Van Antwerpen, Caroline Stévigny, Nausicaa Noret***

To understand how evolutionary forces interact to shape phenotypic variations observed among natural populations, it is essential to elucidate the relationship between the diversity of adaptive phenotypic traits and the diversity of neutral genetic markers. A population with a high diversity of adaptive phenotypic traits is expected to better face a range of selective pressures. Microsatellites are repeated DNA sequences dispersed in the genome. They are a priori neutral with respect to adaptation of individuals to their environment. A nice example of plant potential adaptive traits is highlighted by the metabolome present in a plant organ at a given date, metabolites being the end-product of the omics' cascade. The objective of the present study was to test whether populations (e.g. wild tropical Erythrophleum trees) with high neutral genetic diversity also have a large diversity of metabolites expected to be related to a higher adaptive potential in the natural environment. Neutral genetic markers (microsatellites) and metabolites (LC-MS) were assessed in individuals from 5 genetically distinct populations growing in a common garden in Cameroon. The kernel PCA method was used to integrate metabolomics and microsatellite preprocessed data. Kernel enables to take into account data heterogeneity. Once individuals were discriminated according to the modalities of the biological factor of interest like species, important features within each block were determined using permutations. Good correlations between metabolome and neutral genetic markers have been obtained at various scales.

POSTER SESSIONS 1 AND 2 – Monday and Tuesday – all odd number presenters will be at their posters.
POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

BIG DATA, STATISTICS, INFORMATICS

P-317 Peak picking revisited in untargeted metabolomics

PRESENTING AUTHOR: *Yasin El Abiead, University of Vienna, Austria*

CO-AUTHORS: *Michaela Schwaiger, Gerrit Hermann, Gunda Koellensperger*

A crucial step of untargeted metabolomics workflows is peak picking, a task that is usually performed by software algorithms. In this work, we will thoroughly discuss different data evaluation strategies determining whether a detected peak should be accepted as reliable or not. Decision rules will be postulated for different scenarios. One commonly accepted strategy is to accept features based on isotopologues, others exploit thresholds based on peak intensity and/or peak shape parameters. The influence of different chromatographic separations and decision rules on untargeted data evaluation will be presented. For this purpose a metabolite standard (>80 compounds) was measured at different concentrations. The trueness will be evaluated by comparison of an untargeted analysis output with a targeted data evaluation output which was manually curated. We will show that the requirements for the feature detection settings depend strongly on the question to be answered by the untargeted metabolomics workflow. We will also test different approaches on how to identify reliable features and evaluate them for their ability to get rid of as many features as possible without losing peaks found by the targeted workflow. In order to test the reliability of the optimized procedure the complexity of the sample was increased by spiking serum samples at different concentration levels.

P-318 Annotation, Alignment, Clustering, and Standardization of Nontargeted LC-MS Datasets

PRESENTING AUTHOR: *Daniel Hitchcock, Broad Institute, United States*

CO-AUTHORS: *Julian Avila-Pacheco, Kevin Bullock, Amy Deik, Courtney Dennis, Sarah Jeanfavre, Kerry Pierce, Justin Scott, Clary Clish*

Nontargeted liquid-chromatography tandem mass-spectrometry (LC-MS) based metabolomics methods can yield data on thousands of features. After peak detection and integration, a number of post-processing steps are required before a dataset may be used for analysis. For instance, features must be annotated with metabolite IDs, have their abundances scaled to account for batch effects, and redundant features produced during ionization must be removed. Moreover, if data were acquired and processed in batches, the datasets must be combined. These steps are complicated by variation in a feature's measured mass-to-charge ratio (m/z), retention time (RT), and relative abundance across samples and batches. We have developed three semi-automated software scripts that aid our nontargeted metabolomics workflow. The first is a novel, interactive alignment tool that matches features between two processed datasets. This program can be used to align features in multi-batch studies, as well as to annotate features by aligning a newly processed dataset with an annotated reference dataset. The second is a data cleaning tool that clusters features and annotates them based on potential combinations of ion adducts, neutral losses, source fragments, and so forth. This tool has also revealed, for example, heterodimeric ion species formed during ESI that can be mistakenly identified as a unique metabolites when searched against databases. Finally, the third script is a data standardization tool that uses pooled reference samples to adjust for drift in instrument sensitivity over time and to scale data between batches. These tools have been implemented as web apps for ease of use.

P-319 Open-source software for detecting metabolites in complex mixtures by scanning precursors with predetermined neutral losses from MS/MS

PRESENTING AUTHOR: *Komal Kedia, Pacific Northwest National Laboratory, United States*

CO-AUTHORS: *Aivett Bilbao, Mowei Zhou, Samuel H. Payne, John R. Cort*

We developed a software tool that enables rapid screening of precursor ions showing predetermined neutral losses in MS/MS. The tool takes a list of any predetermined neutral loss masses as input and provides precursor m/z, charge state, scan number, pair of peaks corresponding to the neutral loss found, and other useful information. This tool will be beneficial in a plethora of MS-based study areas such as metabolomics, lipidomics, and proteomics as well as drug metabolism of xenobiotics and natural products. The tool was implemented in R with few dependencies for an easy-to-use application and open-source to facilitate customization and extension. As a proof of concept, we analyzed data collected for the drug pentosan polysulfate sodium (PPS) sold under the brand name Elmiron. PPS, is a heterogeneous mixtures of sulfated polysaccharide and is the only approved drug for the treatment of interstitial cystitis. We treated this drug with a counterion (pentylammonium) to protect labile sulfate groups from undergoing in-source fragmentation and ran it on a reversed phase Waters system interfaced with Velos-Orbitap. This data represents a good test case for our tool owing to the complexity of PPS. We were successful in identifying precursors showing signature neutral losses specific to PPS and were able to compare the PPS molecular profile of Elmiron vs a foreign PPS product. We then applied the tool to data collected on urine from healthy and PPS-treated donors, to identify PPS and likely PPS metabolites in urine.

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BIG DATA, STATISTICS, INFORMATICS

P-320 Structure Aware Multiple Alignment (SAMA): an XCMS Plug-in to Improve Retention Time Alignment

PRESENTING AUTHOR: ***Chiung-Ting Wu, Virginia Tech, Taiwan***

CO-AUTHORS: ***Yizhi Wang, Timothy Ebbels, David Herrington, Yue Wang, Guoqiang Yu***

XCMS is a popular program to analyze Liquid Chromatography Mass Spectrometry (LC-MS) data. One of the major functions of XCMS is retention time (RT) alignment, which aims to achieve the goal that the same molecule has the same retention time across samples. When performing RT alignment, XCMS relies on the assumption that a single warping function can be applied to all m/z bins in the same sample. While this assumption sounds reasonable and empirical evidences show that in many cases this assumption is satisfied, we do observe that quite a few compounds do not follow this assumption. So, analysis results for these compounds are not reliable. Here, we develop Structure Aware Multiple Alignment (SAMA), a novel profile-based alignment algorithm to help XCMS address this problem. SAMA takes advantage of the observation that two samples processed within a short time period have smaller retention time shift than the two samples separated by a large time interval. SAMA incorporates the experimental processing time order of the samples, and utilizes this information to help alignment. SAMA can align each m/z bin separately, without the assumption that all the m/z bins have the same warping function, so that SAMA can fix these warping functions of the misaligned compounds. In addition, we develop an approach to detect the m/z bins that may contain misaligned compounds from XCMS results, to avoid realigning all the m/z bins. In conclusion, we develop a plug-in to help XCMS to detect and fix the RT misalignments.

P-321 Mutiblocks analysis to enhance NMR metabolomics data classification with physiological or biochemical data.

PRESENTING AUTHOR: ***Mohamed Nawal TRIBA, Sorbonne Paris Cité, Laboratoire de Chimie, Structures et Propriétés de Biomateriaux et d'Agents Therapeutiques, University Paris, France***

CO-AUTHORS: ***Florian Messier, Laurence Le Moyec, Nadia Bouchemal, Céline Robert, Fabienne Durand, Eric Barrey, Philippe Savarin***

CCSWA (1) is an un-supervised method used to analyze simultaneously data blocks measured with different techniques on the same subjects. The latent variables computed with CCSWA summarize the common variability of the blocks. On another hand, the paired analysis of data from a same subject reduces the interindividual variability and therefore enhances the variability from other origin. The CCSWA analysis was applied to characterize the effects of altitude on the running performance of non-acclimatized human subjects on the plasma metabolome and the cardio-respiratory variables and for endurance horses, to investigate the relation between biochemistry modulations and plasma metabolome after races of 90, 120 and 160 km. The application of the CCSWA models to these data enhance the discrimination capacity between exercise effects at sea level and at 1200 m altitude and demonstrate the relation between respiratory variables and metabolic pathways modulation for energy supply. In the case of horses involved in endurance races, the CCSWA models demonstrated the successive metabolic pathways involved as the race distance increases. The results obtained show that combining CCSWA with multilevel methods significantly reduced the effect of the interindividual variability and allowed the detection of the common information between different types of data. Moreover, it is possible to determine the variables that are discriminative in each block giving further arguments for the interpretation of the biological mechanism. 1. QANNARI E. M., COURCOUX Ph., VIGNEAU E. (2001). Common Components and specific weights analysis performed on preference data. Food Quality and Preference. 12, 365-368

P-322 Comparison of free software tools MSDIAL, MZmine and XCMSonline for feature detection from the untargeted LC-MS metabolomics dataset

PRESENTING AUTHOR: ***Chang-Yin Li, NIH West Coast Metabolomics Center, University of California Davis, United States***

CO-AUTHORS: ***Oliver Fiehn***

Currently, MSDIAL, MZmine and XCMSonline are three free software tools widely used for preprocessing untargeted LC-MS metabolomics data. Considering that feature detection from raw data is the first key step for data processing, and that different softwares usually generate different results of feature list owing to the use of various algorithms by different workflows, it is necessary to compare the feature detection ability of all these three softwares, and to clarify which of them are relatively more reliable to generate the true feature list. Data from 24 technically replicate QC plasma samples in the untargeted LC-MS metabolomics batch analysis were employed for this comparison. For each software, parameters were optimized according to the number of IS and identified features in the list results, and feature intensity threshold was determined according to the minimum intensity of all IS features obtain from the software, while the accurate mass retention time library from Fiehn's Lab was utilized for compound identification. Despite more features were screened out, XCMSonline showed higher positive false rate with 3 IS features missing. While both MSDIAL and MZmine presented relatively lower positive false rate for feature detection and could recover all 22 IS features. MSDIAL and MZmine shared much more common features than XCMSonline. Correlation analysis on the intensity of IS, identified and unidentified features indicated that MSDIAL showed relatively better correlation with both MZmine and XCMSonline. Collectively, MSDIAL is likely a more reliable and robust option for feature detection in untargeted LC-MS metabolomics data processing.

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SAMPLE PREP AND QUALITY

P-323 Exploring the use of DESI to detect drugs in ancient hair

PRESENTING AUTHOR: ***Isabel Vincent, Glasgow Polyomics, United Kingdom***

CO-AUTHORS: ***Steven Bowie, Karl Burgess***

The analysis of hair samples in the forensic sciences to detect drugs of abuse involves the destruction of the hair sample to extract the metabolites of interest followed by LC-MS to detect these metabolites. To analyse drugs in ancient hair a non-destructive technique is required. Desorption electrospray ionisation (DESI) is an ambient technique used to analyse samples in situ with minimal sample preparation. DESI also offers an advantage over LCMS as it can scan along the length of a hair, giving a time line for the drug use. In this study, we aimed to optimise DESI parameters for detection of drugs in hair, followed by analysis of ancient hair from museums around Glasgow, UK. Sample preparation techniques were explored to efficiently wash off environmental contaminants and to open the cuticle to access the cortex of the hair shaft. Confirmation that DESI did not damage modern or ancient hair was obtained by microscope imaging. Using our optimised technique, drug metabolite standards spiked onto naïve hair were readily detected. Detections on samples of hair from known drug users were negative using DESI, but positive using LCMS. Work is continuing to explore alternative ways of accessing the drug metabolites in hair samples using DESI.

P-324 A robust biomarker for evaluating blood sample quality after room temperature exposure in metabolomics study

PRESENTING AUTHOR: ***Xinyu Liu, CAS Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China***

CO-AUTHORS: ***Xinyu Liu, Miriam Hoene, Peiyuan Yin, Rainer Lehmann, Guowang Xu***

Mass spectrometry (MS) based metabolomics is a powerful tool for biomarker discovery and mechanism interpretation. Blood samples are frequently used, while the sample quality is a critical but easily ignored factor. The real sample quality is still a question due to room temperature (RT) exposure and possible delay for processing of whole blood. Up to now, there is no universal accepted biomarker to assess the quality of blood sample that suffered to RT exposure. The purpose of our study was to identify and validate a marker applicable for high throughput quality control of biobank samples. Firstly, non-targeted metabolomics was used for biomarker screening, (4E,14Z)-sphingadienine-C18-1-phosphate (S1P-d18:2) was found suitable for assessing the significant changes in the quality of serum and plasma caused by delayed centrifugation of whole blood. Then this biomarker was rigorously validated by targeted UHPLC-MS analyses, which included the use of practicality tests on more than a thousand of plasma and serum samples randomly selected from 11 biobanks (including single- and multi-center trials) in three different countries. And cut-off values for sample assessment were defined based on target quantification. Furthermore, life-threatening disease states and strenuous exercise induced metabolic challenges were investigated, results demonstrated that these factors wouldn't affected the concentration of S1P-d18:2. To conclude, the quantification of this metabolite biomarker offers a valid method for evaluating quality of serum and plasma samples prior to storage. This strategy not only provides security for sample selection, but also significantly improves the outcome of clinical metabolomics.

P-325 Comprehensive metabolomics profiling of plasma and serum by GC×GC-TOFMS. A comparison of extraction solvents

PRESENTING AUTHOR: ***A.Paulina de la Mata, University of Alberta, Canada***

CO-AUTHORS: ***Seo Lin Nam, James J. Harynuk***

The active research for biomedical purposes involves the unbiased qualitative and quantitative analysis of metabolite profiles in body fluids. Blood is a valuable biofluid for metabolomics analysis. Blood serum, known as the gold biofluid, is usually analyzed due to its ease of collection and storage; however, plasma is considered more representative of circulating blood, making it a more attractive target for analysis. In addition, the collection tubes for serum are also possible sources of error, potentially compromising the quantification of some amino acids and sugars.1 Quantification of analytes by GC is also influenced by the complex biological fluids with the responses of some analytes depending highly on sample composition.2 This constitute a challenge for accurate quantification of different families of metabolites using the same solvent extraction. In this study, a comparison of different extraction methods for comprehensive metabolomics profiling of plasma and serum will be reported as well as options for the absolute quantification of different families of metabolites using GC×GC-TOFMS.

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SAMPLE PREP AND QUALITY

P-326 Metabolite analysis in primary cell samples obtained from fluorescence activated cell sorting

PRESENTING AUTHOR: ***Kristaps Klavins, CeMM - Research Center for Molecular Medicine, Austria***

CO-AUTHORS: ***Bettina Guertl, Thomas Krausgruber***

The fluorescence activated cell sorting (FACS) are commonly used to separate different cell populations from complex biological samples e.g. blood and tissue. The separated cell subpopulation can be used to elucidate cell type specific metabolite profiles and their changes upon perturbations. One of the major challenges for this experimental strategy is the limited amount of selected primary cells in certain tissue types, which is especially relevant for samples obtained from mouse models or human biopsies. In this work, the workflow based on liquid chromatography coupled to Orbitrap mass spectrometer for quantitative metabolite analysis of FACS samples with limited cell number was established. The Jurkat and K562 cell culture samples were used to optimize FACS sampling and sample preparation procedures as well as to estimate the lower number of cell necessary for quantitative metabolite analysis. The developed method enabled robust quantification of more than 30 metabolites in samples with 100000 cells. Additionally, employed measurement technique provided relative quantification of more than 50 metabolites. Next, the established workflow was applied for the quantitative metabolite analysis in several primary cell type samples obtained from different animal tissues. In order to increase the metabolite coverage and even further reduce the number of cells necessary for analysis; the analysis method based on a triple quadrupole mass spectrometer will be tested.

P-327 Stability of polyamine concentrations in saliva

PRESENTING AUTHOR: ***Atsumi Tomita, Tokyo Medical University, Japan***

CO-AUTHORS: ***Atsumi Tomita, Eri Yamaguchi, Masahiro Sugimoto***

Polyamine concentrations in saliva have exhibited potentials as non-invasive tools for screening various types of systematic diseases including cancers. However, the standard of protocol for the use of saliva has not still established and therefore the stability of the quantified values of these metabolites are still unknown. Therefore, we evaluated the stability of salivary polyamines and several other metabolites, such as amino acids, under various storage various storage conditions, including duration and temperature. Unstimulated saliva samples were collected, and these metabolites were analyzed by liquid chromatography-mass spectrometry. Upon the saliva collection, ethanol was added and their effects on the quantified values were evaluated. Based on the coefficient of variation, we computationally generated noise and added salivary polyamine concentrations to evaluate the discrimination ability of pancreatic cancer from the controls (Asai et al, Cancers, 2018), which showed high robustness. These data would contribute to the use of saliva as a clinical utility.

P-328 Analysis of Serum Metabolites in Diabetic Study Subjects by a Standardized and Targeted Quantitative Assay Coupled to Orbitrap™ Mass Spectrometer

PRESENTING AUTHOR: ***Anastasia Kalli, Thermo Fisher Scientific, United States***

CO-AUTHORS: ***Manuel Kratzke, Markus Langsdorf, Stephen Deart, Hai Tuan Pham, Therese Koal, Andreas Huhmer***

Type 2 Diabetes (T2D), the most prevalent form of diabetes, is a metabolic disorder characterized by decreased insulin sensitivity and abnormal hepatic glucose production. Metabolomics creates new capabilities for understanding metabolic disorders as it enables gaining insights into physiological and pathophysiological processes. However, obtaining reproducible and accurate quantitation through method standardization represents a challenge in metabolomics analysis. Here we employ a standardized analytical method for targeted metabolic profiling of 408 metabolites in serum samples obtained from T2D study subjects and healthy controls, aiming to monitor changes in metabolite concentrations between the two study groups. Sample preparation was performed with the AbsoluteIDQ® p400 HR Kit and sample analysis was performed on a Thermo Scientific™ Q Exactive™ HF Orbitrap™ mass spectrometer. We have identified several metabolites that were significantly altered between the two study groups (p values< 9.97E-04). For instance, we observed increased concentrations of acylcarnitine AC(2:0), cholesterol ester CE(22:6), diglycerides DG(41:1, DG(36:4), TG(56:7), sphingomyelins SM (36:1), SM(42:3), and phosphatidylcholines PC(38:6), PC(40:6) in the T2D serum samples compared to the control subjects. The concentration of serotonin, triclecyride TG(52:6), and phosphatidylcholines PC(40:4) were lower in the T2D serum samples. Acetylcarnitine AC(2:0), phosphatidylcholines PC(38:6) and PC(40:6) have been previously reported to be associated with higher likelihood of T2D, in agreement with our findings. We also evaluated the reproducibility of the analytical method by performing triplicate analysis of the serum samples. Overall, our results demonstrate that the method provides standardized, reproducible and accurate quantitation and allows for monitoring and quantifying changes between different study groups.

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SAMPLE PREP AND QUALITY

P-329 Metabolomics Quality Assurance and Quality Control Materials (MetQual): A NIST Interlaboratory Study

PRESENTING AUTHOR: *Christina Michele Jones, National Institute of Standards and Technology, United States*

CO-AUTHORS: *Tracey B. Schock, Yamil Simón-Manso, David A. Sheen, Blaza Toman*

As the metabolomics field matures, measurement harmonization efforts must be undertaken to confirm and validate biological discoveries that can be clinically translated and further precision medicine initiatives. A key challenge of metabolomics research is limiting technical variance such that the primary source of investigated variance results from true biological differences. Numerous sources of technical variance are introduced throughout metabolomics studies because workflows are vast, encompassing several analytical steps. As such, the development and utilization of quality control (QC)/reference materials (RMs) to understand and reduce technical variance is vital for translation of metabolomics findings. The National Institute of Standards and Technology (NIST) is currently developing a suite of new plasma RMs with spectral library data that will be available soon for the metabolomics community. As many metabolomics stakeholders agree that qualitative data will be most useful for metabolomics RMs, NIST will administer an interlaboratory study (in partnership with the Metabolomics Quality Assurance and quality Control Consortium (mQACC)) for community characterization of these materials. The information that will be obtained from this exercise is twofold: (1) consensus characterization of the materials (i.e., identification of detected metabolites and discriminant metabolite fold changes between the different phenotypes) and (2) an assessment of measurement variability within the metabolomics community (i.e., chemometric scoring metric to assess interlaboratory metabolic profile similarities and differences in multivariate space). The intended use for the suite will be as QC materials for metabolomic measurements/research, interlaboratory comparisons, laboratory and instrument qualification, and training for the metabolomics community.

P-330 Metabolomics Quality Assurance and quality Control Consortium (mQACC): Reference and Test Material Working Group

PRESENTING AUTHOR: *Krista A. Zanetti, National Cancer Institute, United States*

CO-AUTHORS: *Christina M. Jones, Warwick B. Dunn, Daniel Raftery, Thomas Hartung, Ian D. Wilson, Matthew R. Lewis, Fariba Tayyari, Baljit K. Ubhi, Amanda Souza, Ioanna Ntai, Katrice A. Lippa*

The Reference and Test Material Working Group emerged from the recently formed Metabolomics Quality Assurance and quality Control Consortium (mQACC). The metabolomics community urgently needs reference and test materials that can be used for measurements across laboratories and data standardization from different instrumental platforms to ensure translation of biological discoveries. Accordingly, we are actively working to develop measurement designs and prototype materials that can be utilized across most, if not all, instrumentation platforms and employed for interlaboratory comparisons. Additionally, we are defining the measurement challenges that different types of reference and test materials have the potential to address, as well as establishing best use practices for test and reference materials. Prototype materials currently underway, in partnership with the National Institute of Standards and Technology (NIST), include plasma and urine reference material suites. Synthetic metabolite solutions are also being considered. NIST–RTMWG interlaboratory comparison exercises will be administered to obtain community consensus data of developed materials. Moreover, surveys will be disseminated to more clearly define the test and reference material needs of the broader metabolomics community.

P-331 Plasma and Serum-based Metabolomics are Comparable in Children with Liver Disease

PRESENTING AUTHOR: *Jennifer K Frediani, Emory University, United States*

CO-AUTHORS: *Douglas I. Walker, Miriam B. Vos*

Objectives: Given the variety of biological samples used for metabolomics analysis, we aimed to determine metabolite differences between serum and plasma samples in a convenience sample of children with liver disease. Methods: This secondary analysis was performed on 178 children with serum, collected in red top tube with clotting factor, and plasma, collected in EDTA tube with anticoagulant, samples recruited for various liver disease studies. Mean, standard deviation, and frequencies were used to describe demographics. Paired two-tailed student t-tests were performed to determine differences in metabolites. Results: Average age was 12.6 years (SD 4.8) and the children were 54% male. The sample was racially diverse with 31% African American, 38% Caucasian, 26% Hispanic, and 5% other. Average body mass index Z-score was 1.29 (SD 1.37). These subjects had various liver diseases, 44% nonalcoholic fatty liver disease. Serum and plasma samples were not inherently different in this cohort. Correlations were too high for traditional modeling. Student t-tests ($p<0.05$) produced 460 significant differences out of 12804 detected mass to charge ratios, less than 4%. The top 20 most significant differences included many pesticides, food additives and plant compounds and some lipid molecules. Conclusions: There is no significant difference in metabolomics analysis between serum and plasma samples in children with liver disease. Most metabolites detected were comparable between samples. It can be concluded that studies with similar sample and data extraction but different blood samples can be compared. Researchers should be cautious when comparing lipids or environmental chemicals from serum and plasma-based metabolomics.

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SAMPLE PREP AND QUALITY

P-332 Development of Human Liver Quality Control Materials for Metabolomics, Lipidomics and Proteomics

PRESENTING AUTHOR: ***Tracey B. Schock, National Institute of Standards and Technology, United States***

CO-AUTHORS: ***Tracey B. Schock, John A. Bowden, Benjamin A. Neely, Lisa Kilpatrick, Deb Ellisor, Clay Davis***

Robust analytical measurements are the keystone to biomarker discovery. Measurements must be repeatable, reproducible, and comparable to lend confidence to the distinguishing phenotypic markers. Metrics of analytical accuracy and precision stem from the use of quality control (QC) materials, an essential component in an analytical workflow. QC materials evaluate and control systematic variance due to analyst, batch, temporal variation, and intra-laboratory studies. While it is common and important to incorporate QC materials that are specific to an experimental study, such as a matrix-matched experimental sample pool, it is also imperative to include a well-defined, stable, homogeneous sample that enables measurement harmonization and comparability across instrumentation, protocols and laboratories. The National Institute of Standards and Technology (NIST) is currently developing both biofluid (see C. Jones et al, NIST) and tissue- based quality control materials that suit multiple omics disciplines and platforms , including metabolomic (NMR and MS), lipidomic and proteomic measurements. Currently, there are no tissue-based materials available in quantities necessary to provide institutes with a sustainable, commercially accessible, harmonization material. NIST is developing a series of human liver materials (3 health states) to improve deep biomolecular profiling, differential analysis and data analytics for untargeted tissue assessments. These materials will serve as a tool for intra- and inter-laboratory quality control, characterization of new technology and protocols, as well as, a training tool for analyst proficiency. In addition, reference datasets including highly confident identifications of molecular components are being generated to validate data processing workflows and software.

P-333 Towards establishing quality assurance and quality control metrics and reporting standards through the mQACC efforts and the scientific community

PRESENTING AUTHOR: ***Claire O'Donovan, EMBL-EBI, United Kingdom***

CO-AUTHORS: ***Annie Evans, mQACC Consortium***

In the era of Big Data, it is critical that the data is reproducible and interpretable across different laboratories to ensure the full aims of any scientific endeavor are achieved. The goal of the Metabolomics Quality Assurance and quality control Consortium (mQACC) is to develop a collaborative effort between important stakeholders in academia, industry and government institutions to address key quality assurance (QA) and quality control (QC) issues in the untargeted metabolomics field. Towards achieving that goal, a number of consortium laboratories (both commercial and academic) have agreed to share their QA/QC procedures focused on research use only (RUO) metabolomics practices. This will enable us to identify the most useful metrics for assessing study and data quality and the most appropriate reporting of QA/QC data. We would like to present the summary of this activity, the lessons learned, the challenges identified and the preliminary metrics and standards at Metabolomics 2018 to prompt further discussions about this critical topic with the conference attendees to benefit from their insights and to drive this project forward.

P-334 Metabolomics Profiling and Identification of Cell Culture Media

PRESENTING AUTHOR: ***Xuejun Peng, Bruker Daltonics, United States***

CO-AUTHORS: ***Guillaume Tremintin, Anjali Alving, Heiko Neuweger, Aiko Barsch***

Metabolomics profiling and identification ofcell culture media is ofincreasing interest. Comprehensively profiling up to 95 compounds in culture media including amino acid, monosaccharide, vitamins, nucleic acid, antibiotics, and other primary metabolites was conducted by targeted and non-targeted metabolomics simultaneously, aiming to establish a media profile that can be correlated to cell growth and quality. Proteins in cell culture medium and product were crashedby acetonitrile addition, mixed and centrifuged. A polar-embedded reversed-phase column (3µm, 120A0, 3.0x100mm) was used in a 20-min gradient elution for ultrahigh resolution LC-QTOF-MS analysis in both positive and negative modes. Commercial cell culture media NCTC-109, HAM's F-10, HAM's F-12, McCoy's 5A, and six lots of in-house CHO culture supernatant (2 different media)using the same cell line were analyzed (n=6). T-Rex3D algorithm in MetaboScapewas performed for automatic data calibration; retention time alignment; combination of isotopes, charge states, adducts, and neutral losses; data normalization, recursive feature extraction; and MS/MS data import. Annotation MRSQ, formula, and database searching were used for compound verification. PCA and T-test applied to evaluate and compare cell culture media quality from different brands, batches and vendors. Additional metabolites including lipids, fatty acids and small peptides were identified. A robust, sensitive and reproducible analytical method was established to fingerprint and profile culture media quality; nutrients depletion between culture medium and product were observed; unknown identification leads to detailed understanding of cell culture process. Further investigation would determine media components to quantitatively describe the relationship of nutrients and product.

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SAMPLE PREP AND QUALITY

P-335 Label-free and Non-destructive Tissue Segmentation Application of FTIR Imaging for Disease Research

PRESENTING AUTHOR: ***Carolina B Livi, Agilent, United States***

CO-AUTHORS: ***Mustafa Kansiz, Peter Gardner***

Spectral HistoPathology allows for analysis of tissue samples fordiscovery and validation disease research studies using spatially resolved molecular spectroscopy.Fourier Transform Infrared (FTIR) chemical imaging has been an important tool in many facets of analytical chemistry for 20 years and recently has found several applications in life science research. FTIR imaging allows for the spatial analysis of heterogeneous tissue samples using the chemical spectra to differentiate among different cell types as an automated, label-free, non-destructive and objective tissue segmentation solution for the purpose of “upstream omic” qualification. Relying on chemical signatures rather than contrast from exogenous dyes and stains, infrared chemical imaging has the potential to revolutionize how histopathology analysis is performed for disease research. Here we show an example of automated tissue segmentation in formalin fixed paraffin embedded (FFPE) tissue microarrays (TMAs) from prostate cancer samples.Tissue components includingEpithelium, Blood,Lymphocytes, Smooth muscle and Extracellular matrix (ECM) are classified and results are displayed as a pseudo-colored image. The results highlight the heterogeneity of tissue cores used to build TMAs and the importance of quality controls when assessing biomarker assays. FTIR microscopy can be performed on a range of samples including live cells, fresh or frozen tissue sections. With the appropriate calibration and classificationalgorithms, various cell typescan be determined in an automated fashion based on the inherent chemical contrast within the sample. This automation removes the human subjectivity in classification and provides a more quantitative assessment. Research Use Only. Not for use in diagnostic procedures.

P-336 Correction of Ion Suppression for Improved Quantitative Rigor and Reproducibility

PRESENTING AUTHOR: ***Philip L. Lorenzi, The University of Texas MD Anderson Cancer Center, United States***

CO-AUTHORS: ***Marc Warmoes, Lucas Veillon, John N. Weinstein, Chris Beecher***

Ion suppression and ion enhancement are well-known phenomena in mass spectrometry, but spiking stable isotope-labeled internal standards into a sample at known concentrations is generally thought to represent the only method for dealing with the problem. Here we show that a chemically complex Internal Standard (IS) containing hundreds of stable isotope-labeled metabolites can correct for loss or gain of corresponding targeted metabolite signals during sample preparation and data acquisition, yielding more accurate measurements. We examined a range of chromatographic systems and conditions including HILIC, C18, ion chromatography (IC), and positive and negative ionization modes on an Orbitrap Fusion mass spectrometry system. Notably, we demonstrate that the strategy is effective over a wide range of IS:analyte ratios. The resulting, suppression-corrected measurements, in most cases, correlated strongly ($r2 > 0.98$) with the concentration of each metabolite, whereas the uncorrected values yielded weaker correlations ranging from $r2 = 0.65$ to $r2 = 0.75$. In summary, incorporation of a chemically complex IS into targeted and non-targeted metabolomic workflows provides a viable strategy for increasing quantitative rigor and reproducibility.

P-337 Fully Automated Online Trimethylsilyl (TMS) Derivatization Protocol for Global Metabolomics Using Orbitrap GC-MS and a High Resolution-Accurate Mass Metabolomics Library

PRESENTING AUTHOR: ***Jason Cole, Thermo Fisher Scientific, United States***

CO-AUTHORS: ***Xin Zheng, Giulia Riccardino, Paul Silcock***

GC-MS has been used for metabolomics analysis for decades, because it allows for comprehensive analysis of amino acids, sugars, sugar alcohols, fatty acids, and sugar phosphates in various sample types. Many of these compounds have to be derivatized before introduction into a GC-MS to prevent breakdown and increase volatility. Silylation is the classic derivatization method used in metabolomics. However, most published derivatization protocols are manual methods that are time-consuming and labor-intensive. In this study, we introduce a fully automated online TMS derivatization system coupled to an Orbitrap-GC/MS system. A 24-hour non-stop sample preparation cycle has been tested. The relative standard deviations (RSD%) of all the analytes are lower than 10%, which is considerably lower than the 20% RSD that is commonly accepted for metabolomics studies. The comparison between online derivatization and manual derivatization was also evaluated. The average RSD% of the manual derivatization protocol was significantly higher than the online protocol. Compound identification was achieved using spectral deconvolution software equipped with a high-resolution accurate mass metabolomics library. This online derivatization protocol significantly simplified the metabolomics workflow in terms of reducing sample preparation time, increasing reliability, and maximizing throughput. Furthermore, it allows two incubators to be used simultaneously for two-step derivatization at different temperatures.

POSTER SESSIONS 1 AND 2 – Monday and Tuesday – all odd number presenters will be at their posters.
POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

SAMPLE PREP AND QUALITY

P-338 Development, validation and clinical application of a simplified QuEChERS Approach for the multiresidue analysis of polycyclic aromatic hydrocarbons in low-volume human plasma, serum or urine

PRESENTING AUTHOR: ***Marc Raymond Elie, University of Colorado, Anschutz Medical Campus, United States***

CO-AUTHORS: ***Joe D. Gomez ,Michael L. Armstrong, Michael Wempe, Nichole Reisdorph***

Polycyclic aromatic hydrocarbons (PAHs) are a family of environmental toxicants that accumulate in the body over time as a result of inhalation of cigarette smoke, vehicle exhaust, processed fossil fuels, or through ingestion of grilled and charred foods, contaminated water and other processed foods. It is well documented that these pollutants have several adverse health effects including cancer and cardiovascular diseases. Recently, some studies in children and adolescents have documented the association between PAHs and obesity; oxidative stress; inflammation; asthma; renal and thyroid function impairment. However, the causal relationship is unclear. Therefore, it is important to monitor PAHs concentrations in biological samples. Nevertheless, the use of such biological matrices demands an extraction/purification pre-treatment before chromatographic analysis and also calls for large amount of samples (> 1 mL) for analysis, which can be difficult to access, especially dealing with children (sampling). The present study reports a simple, rapid and cost-effective method for the simultaneous determination of sixteen polycyclic aromatic hydrocarbons (PAHs) in plasm, serum and urine by gas chromatography-mass spectrometry (GC-MS). A modified approach of the QuEChERS methodology was used to extract PAHs from plasm, serum or urine without any clean-up. The proposed method showed excellent linearity (R2>0.99) with no significant matrix effect. The mean recovery ranged from 88 to 115 % for all the analytes. Furthermore, the proposed method was successfully applied to actual clinical sample of asthmatic children and shows potential for the analysis of other toxicants of interest in small-volume samples for biomonitoring and clinical applications.

METABOLITE ID

P-339 Optimisation and Validation of a High Throughput Semi-Targeted Method by GC-MS with Metabolite Libraries for Large Scale Molecular Epidemiological Research

PRESENTING AUTHOR: ***Antonis Myridakis, Imperial College of London, United Kingdom***

CO-AUTHORS: ***Marc-Emmanuel Dumas***

Gas chromatography-mass spectrometry (GC-MS) approaches combine the coverage of the untargeted pipelines, with the library-facilitated, rapid metabolite identification. We developed a new holistic and high-throughput GC-MS profiling protocol with the use of metabolite libraries and validated for human, mouse and cell samples. We present a detailed analytical and data processing pipeline, from sample aliquoting to the generation of the final assigned metabolite/intensity table which includes the optimization of key steps as, I) sample clean-up, II) peak integration, III) expansion of the commercially available metabolite libraries, IV) quality control and IV) batch stitching. We compared several sample clean-up techniques and conditions, optimized the chromatography and tested several commercial and in-house software packages for the data extraction and preprocessing. The method was validated for cell and cell culture media and for urine, plasma, serum, fecal, pericardial fluid samples from human and mice. Finally, the method was applied in over three thousand (3000) samples from a human non-alcoholic fatty liver disease, cardiovascular disease, diabetes, obesity etc. cohorts. Moreover, a strategy was designed for the instrument maintenance and the day-to-day routine analysis of large numbers of samples in order to ensure the acquisition of high quality data and minimize the instrument and sample preparation drifts without compromising the throughput.

P-340 A Comprehensive Strategy to Construct Intra-laboratory Database for Accurate and Batch Identification of Small Molecular Metabolites

PRESENTING AUTHOR: ***Xinjie Zhao, Dalian Institute of Chemical Physics, Chinese Academy of Science, China***

CO-AUTHORS: ***Zongda Zeng, Aiming Chen, Chunxia Zhao, Xin Lu, Guowang Xu***

Identification of the metabolites is an essential step in metabolomics study to interpret regulatory mechanism of pathological and physiological processes. However, it is still a big headache in LC-MSn-basedstudies because of the complexity of mass spectrometry, chemical diversity of metabolites, and deficiency of standards database. In this work, a comprehensive strategy is developed for accurate and batch metabolite identification in non-targeted metabolomics study. First, a well defined procedure was applied to generate reliable and standard LC-MS2 data, including experimental SOP, determination of tR, MS1 and MS2 information, and enhancement of data quality. Then, a series of systematic and automated approaches were proposed to handle the standard metabolites data for constructing an intra-laboratory database. This includes retention time calibration using internal standards, precursor ion alignment and ion fusion, estimation of noise level, auto-MS2 information extraction and selection, and database batch searching and scoring. Finally, a software system, OSI/SMMS, was realized in terms of the procedure and issues introduced above for rapid metabolites identification. Asan applicationinstance, serumsamples were analyzed. 190 metabolites involved in 37 metabolic pathways were identified in serum with ESI postive ion mode by using OSI/SMMS system. The result proved the strategy is effective for LC-MSn-based non-targeted metabolomics study.

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METABOLITE ID

P-341 Simultaneous quantification of TCA cycle intermediates using UPLC-MS/MS

PRESENTING AUTHOR: ***Feifei Lin, Shanghai Institute of Materia Medica, China***

CO-AUTHORS: ***Qingli Zhang, Jia Liu***

The tricarboxylic acid (TCA) cycle is a key metabolic pathway that connects glycolysis, fat, and protein metabolism. The TCA cycle provide the important information about the changes in energy metabolism of cells. And it plays an important role in cancer metabolism that cancer cells utilize the TCA cycle differently from those of normal cells. Thus, a rapid, selectivity and sensitivity method to quantify TCA cycle intermediates in bio-samples become urgently for the importance of aberrant TCA cycle function in tumorigenesis. An ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) method has been developed. In this method, the bio-samples were derivatized at room temperature with O-benzylhydroxylamine (O-BHA) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) as co-reagent. A robust method within 4.5 min was developed for quantification of TCA cycle intermediates, including 2-hydroxyglutarate, citrate, isocitrate, α-ketoglutarate, succinate, fumarate, malate, oxaloacetate, pyruvate and lactate. The validated method has been used to detect and quantify changes in metabolite levels of TCA cycle intermediates in cancer cells, tumor tissues, plasma sample and endothelial cells.

P-342 The 13C mouse: a valuable source for stable isotope labeled metabolite reference standards

PRESENTING AUTHOR: ***Frederik Dethloff, Department of Translational Research in Psychiatry, Max Planck Institute of Psychiatry, Germany***

CO-AUTHORS: ***Christoph Bueschl, Hermann Heumann, Rainer Schuhmacher, Christoph W. Turck***

Untargeted metabolomics is challenged by two major problems. One is related to the ability to distinguish between sample derived signals, contaminants, and chemical noise, the other is relative quantification. The use of stable isotope-labeled reference standards in the sample is an established method to alleviate both problems. In order to get the most relevant reference standard material the organism of interest can be labeled with stable isotopes. While this can be achieved in a rather straightforward manner for lower organisms by culturing them in stable isotope labeled media, labeling mammalian organisms is more challenging. For the untargeted metabolomics analysis of mouse and human specimens no comprehensively 13C-stable isotope-labeled material is currently available. With the aim to 13C label mice we generated 13C-labeled algae and bacterial feed. Tissue and blood metabolites from these mice fed for 10 days had quite different 13C enrichment levels. With the implementation of a correction factor we were able to use this partially 13C-labeled material to improve relative quantification accuracy as shown for amino acids in a dilution series. Furthermore, with the MetExtract II software we were able to automatically detect sample derived metabolic feature pairs in mouse samples and to some extend also in human samples. This allowed us to reduce the number of metabolic features by removing contaminants and redundant features for the same metabolite. We have successfully labeled mice with the 13C isotope and have shown that the generated reference material can assist in metabolite annotation and relative quantification in untargeted metabolomics studies.

P-343 Construction and application of a high-resolution MS/MS library including retention time information for rapid identification of endogenous metabolites

PRESENTING AUTHOR: ***Ulrike Schweiger-Hufnagel, Bruker Daltonik GmbH, Germany***

CO-AUTHORS: ***Shuang Zhao, Xian Luo, Wan Chan, Aiko Barsch, Liang Li***

High-resolution LC-MS is an important platform for metabolite detection and quantitation. However, for untargeted Metabolomics rapid, unambiguous and universal compound identification is still challenging. In this work, we report the construction of a library for relevant endogenous metabolite and its' successful application to human biofluids. Standards were obtained from the Human Metabolome Database (HMDB). They were injected into an Intensity Solo 2 C18 column via an Elute LC system and detected by a QTOF-MS (impact, compact; all Bruker Daltonics) for acquiring MS/MS library spectra and the retention time determination (RT). For the analysis of biofluids the same setup was used following a standard operating protocol (SOP) for consistency. Automatic metabolite identification was performed in the MetaboScape software based on matching of multiple parameters: precursor mass accuracy, isotopic pattern, RT, and MS/MS spectrum quality. In this study a library from over 800 endogenous metabolites was created. It contains MS/MS spectra of 635 compounds acquired in positive mode and 474 negative mode spectra. Up to 5 collision energy levels were applied for each standard giving more than 6000 MS/MS spectra in total. For each metabolite, library fragment spectra were manually curated by confirming each fragment via a molecular formula. For unambiguous identification we determined the RT of each standard. An RT correction method was applied to balance effects by variations in experimental conditions like instrument brands, LC columns and gradients. The described procedure was applied to biofluids, e.g. plasma and urine, and metabolite identification results will be presented.

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METABOLITE ID

P-344 Evaluation of the high-field Orbitrap Fusion mass spectrometer for compound annotation and metabolite identification

PRESENTING AUTHOR: ***Christophe Junot, Pharmacology and Immunoanalysis Unit (SPI), CEA, INRA, Paris Saclay University, France***

CO-AUTHORS: ***Pierre Barbier Saint-Hilaire, Katleen Rousseau, Ulli M. Hohenester, Benoit Colsch, Jean-Claude Tabet, François Fenaille***

Despite the constant improvements of mass spectrometers, identification of unknown metabolites remains challenging. The first level of investigations when using high resolution mass spectrometry (HRMS) data relies on the determination of an elemental composition (EC) for a given MS peak. Altogether, mass accuracy, mass resolution and isotopic pattern accuracy directly influence the ability of HRMS instruments to perform accurate EC determination. Relative natural isotope abundance (RIA) has already been studied on different mass spectrometers but these studies deal essentially with natural 13C1 isotope. Examining the M+2 isotope can also bring valuable information for refining EC list. As FT-ICR instruments, the newly introduced Orbitrap Fusion instrument is now capable of achieving mass resolutions higher than 500,000 (at m/z 200, FWHM), rendering 18O and 13C2 isotopic peaks distinguishable and exploitable. In this work, we evaluate the performance of Orbitrap fusion for metabolomics by using a set of 50 standard molecules diluted in solvent and then being spiked into human plasma. Our data emphasize the need to accurately control the number of trapped ions to avoid space charge effects, thus ensuring reliable measurement of isotopic patterns at 500,000 resolution. From these results, we propose standard optimized experimental conditions for performing accurate untargeted metabolomics on the Orbitrap Fusion. We also report on the implementation of a data dependent acquisition protocol enabling to confirm the structure of more than 2/3 of the 50 compounds spiked in a plasma extract and to get meaningful MS/MS spectra on 50% of non annotated features in a single injection.

P-345 Limiting the manual verification of metabolomics data processing from DIA data

PRESENTING AUTHOR: ***Stephen Tate, SCIEX, Canada***

CO-AUTHORS: ***Matthew Huebsch, Adam Lau***

DIA offers unbiased extraction of thousands of compounds in a single analysis, utilizing the enhanced separation in the MS2 space; improvements in data quality have been shown. However, this level of data extraction requires intense manual review and inspection which is impossible for large scale longitudinal studies. Proteomics overcame manual review by development of a False Detection Rate (FDR) which is applied to identification and quantitation. Adoption in metabolomics is limited with primary evidence of identification coming from MS2 spectral library searching. Here we present work on the development of an FDR methodology for the extraction of metabolites from data independent acquisition (DIA) approaches minimizing data review in large quantitative studies. Multiple methods were used for creation of an FDR for the peak group assignment of the different metabolites, as well as optimization of the peak group assignment. Results show that there is a significant improvement in the detection rates with the inclusion of MS1 into the scoring algorithm but if this is made as a mandatory mass for peak groups assignment then their efficiency decreases. The number of transitions for the extraction of each compound was also assessed. The method for extraction of masses which simulate a false compound are also shown and the effectiveness of each method is displayed. The simplistic approach of randomizing masses is shown to be an ineffective strategy and a final method which utilizes complete spectral library masses and structural knowledge to determine the optimal decoy set of masses for extraction is demonstrated.

P-346 iTree: MSn Mass Spectral Tree Library of Plant Natural Products

PRESENTING AUTHOR: ***Bennett Haffner, West Coast Metabolomics Center, UC Davis, United States***

CO-AUTHORS: ***Arpana Vaniya, Sajjan Singh Mehta, Celeste Felix, Dinesh Barupal, Oliver Fiehn***

Small compound identification in biological samples can be difficult due to the sheer volume and diversity of species. Isomers often share similar fragment ions and therefore share similar MS/MS spectra. Using MSn data collected from natural product standards, we construct iTree; a mass spectral tree library. iTree can of course extend beyond these standards to other compounds, allowing the use of a pairwise paradigm for any two MSn levels (e.g., any MSn to MSn+1 spectra in comparison is equivalent to comparing an MS/MS spectrum of a non-derivative compound or authentic structure). MSn data (2£n£5) were collected on a Thermo LTQ in (+) and (-) electrospray ionization (ESI) by direct infusion. Breadth and depth of the data were achieved by triggering several ions at each stage up to MS5. Each entry's spectra include substructural annotations generated by in silico fragmentation using Mass Frontier 7.0 and CFM-ID. Spectra were uploaded to MassBank of Northern America (MoNA); a novel public mass spectral database. We validated our methods by comparing MS/MS spectra of flavonoid aglycones to MS3 spectra of flavonoid glycosides. Since then, we have collected data for over 2,500 natural product standards, resulting in more than 13,000 ion trees. Using iTree, we screened for polyphenols and flavonoids in Bilberry extract standard reference material 3291 from the Nation Institute of Standards and Technology. LC-MSn data for Bilberry were acquired on a Thermo LTQ and a high resolution Thermo Q Exactive HF mass spectrometer in (+) and (-) ESI mode.

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METABOLITE ID

P-347 Comparison of DI-FTICR and UPLC-qTOF for the comprehensive profiling of myxobacterial secondary metabolomes

PRESENTING AUTHOR: *Chantal Bader, Helmholtz-Institute for Pharmaceutical Research, Saarland (HIPS), Germany*

CO-AUTHORS: *Patrick Haack, Matthias Witt, Fabian Panter, Dr. Daniel Krug, Prof. Dr. Rolf Müller*

Myxobacteria are a prolific source of natural products featuring chemical novelty as well as promising bioactivites. Follow-up of these bioactivites found in crude extracts using various analytical techniques already led to the discovery of numerous valuable molecules including epothilone1 —a cytostatic drug with approval of the FDA. Currently, investigation of the myxobacterial secondary metabolome is based mostly on screening approaches using UPLC-hrMS and subsequent dereplication for the discovery of new natural products. However, the genomic revolution strikingly has shown that current methods likely fall short of uncovering the full complement of myxobacterial secondary metabolite diversity. In this study we aim to evaluate the potential of DI-FTICR for extending the scope of myxobacterial metabolites detectable under laboratory conditions. Along these lines we conduct a comprehensive comparison with our UPLC-qTOF standard platform and characterize the chemical space covered by both methods. This comparison is motivated by the notion that UPLC-qTOF is a robust platform available in most microbial natural product laboratories, whereas high-resolution mass spectra combined with retention time information achieves identification with increased confidence - making fast scan rates during data acquisition irreplaceable. The defining feature of DI-FTICR on the other hand is the capability to generate spectra with a resolution up to 1000000 and thus supporting compound identification solely based on precise mass even from complex mixtures like bacterial crude extracts. Results from the two-platform contest are presented and implications for natural products discovery are discussed. 1 K. Gerth et al., J. Antibiot. 1996, 49, 560-563.

P-348 Untargeted open access retention time HILIC- accurate mass MS/MS spectral library for plasma, tissue and cellular metabolome analysis

PRESENTING AUTHOR: *Megan Reed Showalter, UC Davis, United States*

CO-AUTHORS: *Megan R Showalter, Michael R Sa, Tomas Cajka, Tobias Kind, Dinesh Barupal, and Oliver Fiehn*

Hydrophilic interaction chromatography (HILIC) shows improved retention for polar metabolites that perform important roles in catabolism, anabolism and regulatory roles (epimetabolites). Epimetabolites are chemically modifications of canonical metabolites but are often missed in metabolomics workflows due to lack of publicly available spectra. To improve harmonization of metabolomic reports and confidence in compound identification, open access spectra are accumulated in Mass Bank of North America (http://massbank.us), including this HILIC library. Over 2,000 authentic chemical standards were analyzed using a Waters Acquity UPLC BEH Amide column (2.1 x 150mm: 1.7µm) and MS/MS analysis on three separate mass spectrometers, a ThermoScientific QExactive HF, an Agilent QTOF and a Sciex TripleTOF. Spectra were collected using MS-DIAL and R and uploaded into Mass Bank of North America. Roughly 40% of all authentic standards ionized only in negative ESI, 50% only in positive ESI, and only 20% of the compounds ionized in both modes. Retention times and peak shape parameters were validated across multiple matrices and showed high precision with drifts of less than 0.2 minutes in inter-day and inter-batch comparisons. We show a range of example studies in plasma, tissue and cells for which the new library, in combination with spectra from NIST17 and MoNA, identified up to 250 compounds on MSI levels 1 and 2. Spectra include chemical identifiers, m/z and retention time information. Specifically, a very high number of novel identifications of acetylated- and methylated epimetabolites were retrieved, along with a range of validated di- and tripeptides.

P-349 Large-scale non-targeted profiling of human cohorts to find novel predictors of disease

PRESENTING AUTHOR: *Sarah Jeanfavre, Broad Institute, United States*

CO-AUTHORS: *Daniel Hitchcock, Julian Avila Pacheco, Justin Scott, John F. O'Sullivan, Jordan E. Morningstar, Robert E. Gerszten, Clary B. Clish*

Our lab has created an LC-MS-based metabolomics platform that combines a set of complementary methods to comprehensively profile polar and nonpolar metabolites that constitute the metabolome. Each method uses a hybrid approach measuring both metabolites of known identity, confirmed using authentic reference standards, and yet to be identified LC-MS peaks, indexed by measured mass to charge ratio and retention time. In an effort to enable the application of these methods to study disease in human cohorts and thousands of samples, we have created strategies and software tools that address many of the challenges associated with large-scale metabolomics studies. These challenges include assuring data quality within batches and between batches, accurate alignment of unknown features between batches and cohorts, and identification of biologically significant unknowns. Here, we present an overview of our large-scale metabolomics workflow. Examples are provided from an on-going effort to identify indicators of cancer in a study of >7000 individuals and a recent discovery of a novel indicator of liver fat and predictor of type two diabetes.

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METABOLITE ID

P-350 **Differentiating Standard Reference Materials for Smoker's vs. Non-Smoker's Urine using Comprehensive Two-Dimensional Gas Chromatography-High Performance Time-of-Flight Mass Spectrometry**

PRESENTING AUTHOR: ***David E Alonso, LECO Corporation, United States***

CO-AUTHORS: ***Joe Binkley, Lorne Fell***

This investigation demonstrated the advantages of modern day high performance GC-MS including enhanced chromatographic resolution, and high performance mass spectral data production for characterization of urine. Rapid automated and confident compound identifications were accomplished via advanced chromatographic separations paired with automated peak find and deconvolution yielding clean mass spectra, followed by spectral similarity searches of large databases. Mass precision and retention index filtering were also influential in adding confidence to identifications. The novel analytical approach was utilized to identify and differentiate compounds in two standard reference materials (SRMs): smoker's and non-smoker's urine. These SRMs were vital in the diagnosis and setup of standard operating procedures for metabolic profiling of urine in humans. The applied methodology included a combination of comprehensive chromatography coupled to high performance time of flight mass spectrometry (GCxGC-TOFMS). Characterization of samples was conducted using both targeted and untargeted processing methods. Analyses of the standards resulted in composition maps (Contour plots) displaying wide variety of functionality (e.g., polyaromatic hydrocarbons, phthalates, phenols, pain killers, and tobacco related compounds). The contour plots were highly structured showing clustered classes of compounds and provided high quality spectral data that were searched against large, well-established databases. These full mass range, historical data archives were probed collectively to identify important classes of compounds and clearly distinguish the sample types.

P-351 **High resolution UPLC-ESI-QTOF-MS analysis confirmed secondary metabolites rhamnolipids produced by P. aeruginosa ARS - NRRL B59184 from Amazonian agroindustry residue.**

PRESENTING AUTHOR: ***Noemi Jacques Vieira, Natura Innovation and Technology, Brazil***

CO-AUTHORS: ***Rafael Cunha de Assis Castro, Renata Hannel Bueloni, Felipe Shigueru Takano, Roberta Roesler***

Glycolipids and lipopeptides constitute the most widely studied groups of biosurfactant compounds displaying broad spectrum chemical activity which allows theirapplicationinseveralfieldsin cosmetic industries. Such uniquecharacteristics allow these biomolecules to play a key role in emulsification, foam formation, detergency and dispersal, which are desirable qualities in different formulations.This compounds are classified as secondary metabolites that are non ribosomally synthesized by actively growing microbial cells (bacteria, fungi and yeast). In this work, a residue of oil production from a Brazilian Amazonian seed was evaluated as carbon source for rhamnolipid production by Pseudomonas aeruginosa ARS-NRRL B59184. The obtained fermentation extract was analyzed by ultra-performance liquid chromatography coupled with electrospray ionization-quadrupole time-of-flight mass spectrometry (UPLC-ESI-QTOF-MS). The presence of di- and monorhamnolipid congeners were confirmed, specifically Rha–C8–C12/Rha–C12–C8, Rha–C8–C10/Rha–C10–C8, Rha–Rha–C10–C10 and Rha–C10–C10 as well as Rha–Rha–C12–C10/Rha–Rha–C10–C12 and Rha–Rha-C14–C14. Dimeric structures were also confirmed as 2Rha-Rha- C10–C10, 2Rha–C10–C10. The results showed that the majoritarian compound was Rha-Rha–C10–C10 as a sodiated specie ion 673.38 m/z. High resolution mass spectrometry analysis is simple and powerful technique to provide fast, sensitive and highly detailed data on the characterization of complex fermentation broth, therefore providing specific industrial applications.

OBESITY, DIABETES AND CVD

P-352 **Metabolomics Identifies Predictive Biomarkers Cardiovascular Risk of Exposure to Low Dose Space Radiation**

PRESENTING AUTHOR: ***Amrita Cheema, Georgetown University, United States***

CO-AUTHORS: ***Khyati Mehta, Vijayalakshmi Sridharan, Martin Hauer-Jensen, Allan Tackett, Marjan Boerma***

Introduction: Although exposure to ionizing radiation is known to cause normal tissue toxicity, the underlying molecular alterations and/or biological mechanisms injury are not yet fully understood. Pathophysiological manifestations after low-dose radiation exposure are therefore strongly influenced by noncytocidal radiation effects, such as changes in cellular function, metabolic pathways, epigenetics, and gene expression. Methods: Male C57BL/6 mice at 6 months of age were exposed to either oxygen ions (600 MeV/n) at doses of 0.1, 0.25, or 1 Gy or 0.5 and 1 Gy of proton radiation or 1 and 3 Gy of gamma radiation. Left ventricle tissue and plasma was collected at 14 or 90 days after irradiation. We used untargeted metabolomics and proteomics profiling of the cardiac tissue to identify pathway perturbations in response to type and dose of radiation. Preliminary Results: We see robust changes in metabolite profiles at low doses as compared to higher doses which seem to cluster with the sham irradiated groups. Interestingly perturbations in biochemical pathways correlate with proteomic profiles in these mice. To our knowledge this is the first report on low dose radiation mediated alterations in metabolite profiles in cardiac tissue. IR induces robust changes at the local tissue level implicating increase in inflammatory mediators and oxidative as well as nitrosative stress. Analyses of these data for identification of dose and time dependent as well radiation type molecular signatures are ongoing.

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OBESITY, DIABETES AND CVD

P-353 PLASMA OXIDIZED PHOSPHOLIPIDS: IDENTIFICATION AND STUDY OF THEIR ROLE IN DIABETES DEVELOPMENT

PRESENTING AUTHOR: *Joanna Godzien, Universidad SAN PABLO CEU, Spain*

CO-AUTHORS: *Federico Traldi, Jitka Siroka, Michal Ciborowski, Coral Barbas*

Oxidized lipids play an important role in many processes such as energy production through β-oxidation, signaling through eicosanoids or uncontrolled oxidative degradation provoked by free radicals. Currently, particular attention is being paid to the oxidation of phospholipids (PLs) which have been associated with inflammation, immune response and atherosclerosis. There is also a potential link between oxidized PLs (oxPLs) and progression of a range of diseases. Despite the biological importance of oxPLs, there are several analytical difficulties making their measurement through global, untargeted approaches problematic, i.e their stability, trace concentration and challenging identification. In this work, oxidized glycerophosphocholines (oxPCs) were selected as the most abundant plasma oxPLs to test the potency of global metabolomics methodology to robustly detect such type of compounds. Afterwards, a strategy for recognition of two groups of oxPCs (short and long chain oxPCs) was proposed, leading to the development of a semi-automated tool for their annotation through CeuMassMediator web platform. Finally, this established methodology was applied to track changes in the level of oxPCs related to the development of type II diabetes. With this aim, RP-HPLC-ESI-QTOF-MS analysis was conducted over healthy, insulin resistant, pre-diabetic and diabetic patients. The results, validated between two research centers, revealed interesting relationships between all groups of patients and the involvement of oxPCs in the progression of diabetes.
Acknowledgments: This work was supported by grants from the Spanish Ministerio de Economía y Competitividad (grant CTQ2014-55279-R).

P-354 Serum lipidome alterations depending on nonalcoholic fatty liver disease severity in patients with NAFLD

PRESENTING AUTHOR: *Youngae Jung, Korea Basic Science Institute, South Korea*

CO-AUTHORS: *Min Kyung Lee, Geum-Sook Hwang*

Nonalcoholic fatty liver disease (NAFLD) is characterized by abnormal lipid accumulation, and is a progressive disease range from the simple accumulation of fay in the liver (simple steatosis) to the more severe necroinflammatory complication nonalcoholic steatohepatitis (NASH). The study about the pathogenesis of NAFLD have been focused on obese subjects with body mass index (BMI) > 25 kg/m2, because NAFLD is closely related with obesity. However, some non-obese subjects with body mass index (BMI) < 25 kg/m2 are identified to have NAFLD, especially in Asian countries. Nevertheless, pathogenesis of fatty liver disease in non-obese subjects is not fully elucidated. In this study, we confirmed the differences of lipidome alterations in non-obese (BMI < 25 kg/m2) and obese (BMI < 25 kg/m2) NAFLD patients depending on NAFLD severity. Lipid profiling of serum samples acquired from patients with biopsy-proven NAFLD were performed using ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC/QTOF MS). Lipid species including free fatty acids, glycerophospholipids, sphingolipids, and glycerolipids were confirmed in serum samples from NAFLD patients, and different patterns between non-obese and obese NAFLD patients depending on NAFLD severity were detected. The levels of several sphingomyelin were characteristically increased according to progression of steatosis, ballooning, and lobular inflammation. Our results showed that NAFLD could be differently developed between non-obese and obese NAFLD patients depending on NAFLD severity.

P-355 Metabolomics approach reveals decreased theophylline clearance correlated with poor outcome in heart failure

PRESENTING AUTHOR: *Hsiang-Yu Tang, Chang Gung University, Taiwan*

CO-AUTHORS: *Mei-Ling Cheng*

Heart failure (HF) is a complex clinical syndrome with high morbidity and mortality. Though novel pharmaceuticals and interventional therapies have been development, HF-related hospital readmissions still high and caused financial burden. To evaluate the outcome of HF, metabolomics approach may potentially provide risk stratification for HF patients. A total of 61 patients hospitalized due to acute decompensated HF, 31 developed HF-related events in one year after discharged (Event group), and the other 30 patients did not (Non-event group). The plasma were collected during hospital admission and were analyzed by UPLC-TOFMS based metabolomics approach. Eight metabolites showed significant differences between patients with and without event ($p<0.05$). Each of butyrylcarnitine, decatrienoylcarnitine, dimethylxanthine and phenylacetylglutamine was significantly higher in HF with event, but each of LysoPC(18:2), LysoPC(18:3), LysoPE(18:2), and hypoxanthine was significantly lower in HF with events. Among them, dimethylxanthine plays a pivotal divergence between event and non-event HF, and comprises 3 isomers. To clarify which isomer of dimethylxanthines contributes the diversity, we quantified the levels of theophylline, theobromine, and paraxanthine in individual subject. A total of 102 subjects were collected for validation our findings. There are about 22.5% (n=23) HF patients with higher theophylline level, and 18 HF patients (about 78.2%) in event group. These findings suggest metabolic profiling have the potential to identify patients with HF-related events and provide insights related to HF outcome. The disturbances in dimethylxanthines also indicate the status of medication.

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OBESITY, DIABETES AND CVD

P-356 Dynamics of Plasma Lipidome in Progression to Islet Autoimmunity and Type 1 Diabetes

PRESENTING AUTHOR: ***Santosh Lamichhane, University of Turku, Finland***

CO-AUTHORS: ***Linda Ahonen, Thomas Sparholt Dyrlund, Esko Kemppainen, Heli Siljander, Heikki Hyöty, Jorma Ilonen, Jorma Toppari, Riitta Veijola, Tuulia Hyötyläinen, Mikael Knip, Matej Oresic***

Type 1 diabetes (T1D) is the most prevalent autoimmune diseases among children in Western countries. Current clinical practice for T1D disease prediction in preclinical phase is based on islet autoantibodies determination. Earlier metabolomics based studies suggest that children who progress to T1D are specifically characterized by dysregulation of lipid and amino acid metabolism. However, further studies are needed to disclose the clinical and pathogenic aspects of metabolite-based markers in development of islet cell autoimmunity and overt T1D. Here we used a lipidomics approach to analyze molecular lipids in a prospective series of 428 plasma samples from 40 children who progressed to T1D (PT1D), 40 children who developed at least a single islet autoantibody but did not progress to T1D during the follow-up (P1Ab) and 40 matched controls (CTR). Sphingomyelins were found to be persistently downregulated in PT1D when compared to the P1Ab and CTR groups. Triacylglycerols and phosphatidylcholines were mainly downregulated in PT1D as compared to P1Ab at early age of life before the initiation of autoimmunity. Overall, our study suggests that distinct lipidomic signatures characterize children who progressed to islet autoimmunity or overt T1D, which is of clinical relevance because these metabolic markers might be translated into the healthcare setting as a complementary tool to identify at-risk children before the initiation of autoimmunity.

P-357 Identification of Altered Metabolism in Healthy Asian Indians at Risk for Type 2 Diabetes Mellitus using Mass Spectrometry based Metabolomics

PRESENTING AUTHOR: ***Aneesh Kumar Asokan, Rajiv Gandhi Centre for Biotechnology, India***

CO-AUTHORS: ***Mahesh Chandran, G Vijayakumar, CC Kartha, Abdul Jaleel***

Studying the differential risk of normal healthy people for type 2 diabetes (T2D) is essential in Asian Indian population. We believe that there exists a distinct metabolic transition state leading to a pre-diabetes state well in advance, in those who are at the risk of developing diabetes. An equal number of healthy females and males aged between 18 and 40 years, categorized into three groups; normal healthy controls, NC (N=30), first-degree relatives of patients with type 2 diabetes, FDR (N= 30), overweight (N=30), and pre-diabetic positive controls (N=20). Each study subjects consumed a mixed meal (25 kcal/ kg body weight) in the study day after an overnight fast. Blood samples were collected before the meal and then every 30 minutes after meal ingestion till 120 minutes. Blood samples were subjected to both clinical as well as mass spectrometry-based untargeted metabolomics. We found markers of diabetes and inflammation such as Insulin, adiponectin, Ghrelin, and PAI-1 were significantly at different levels in participants who are having diabetes risks when compared to control. Metabolite profiling was performed using ultra-performance liquid chromatography, ACQUITY UPLC System (Waters) coupled to a Quadrupole- Time of Flight (Q-TOF) mass spectrometer (SYNAPT-G2, Waters). Progenesis QI (Non-linear dynamics, Waters) was employed for peak/feature picking and raw data deconvolution. The putative identification of those features was identified by HMDB, and MASSBANK database search. Our study shows that metabolites belong to sphingolipid metabolism and linolenic acid metabolisms were altered in subjects those who are at risk for T2D.

P-358 Perturbation in circulating carbohydrates and amino acids profile in type II diabetes

PRESENTING AUTHOR: ***Maryam Goudarzi, Cleveland Clinic Lerner Research Institute, United States***

CO-AUTHORS: ***Belinda Willard, Stanley L. Hazen***

Type 2 diabetes mellitus (T2DM) is a risk factor for heart disease and stroke and accounts for 95% of all diagnosed cases in adults according to Center for Disease Control and Prevention. While several studies have pointed to fatty acid metabolism, the underlying mechanism of T2DM is unclear. To this end we performed an untargeted metabolomic study of plasma from 40 patients with T2DM and 40 healthy individualsusing a UHPLC Q-Exactive HF orbitrap LC/MS system. A total of 12 discriminating metabolites were identified between the cases and the controls using an in-house comprehensive data treatment workflow. This analysis revealed that carbohydrate metabolism, glycerolipid metabolism, and amino acid metabolism were among the most perturbed pathways in T2DM subjects. In fact, increased plasma levels of glutamic acid, aspartic acid, N-acetyl-phenylalanine, and hydroxyadipic acid were strongly associated with T2DMwhile decreased levels were observed withLysoPCs containing even-chain fatty acids. Together, these changes constitute a unique disease signature in plasma which may be useful in pathway inhibitor discoveries and disease management in the future.

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OBESITY, DIABETES AND CVD

P-359 Time-course Metabolomics Reveals Differential Effects of Diabetes in Human Pregnancy

PRESENTING AUTHOR: ***Jacquelyn Walejko, University of Florida, United States***

CO-AUTHORS: ***Jacquelyn Walejko, Anushka Chelliah, Anthony Gregg, Maureen Keller-Wood, Arthur S. Edison***

Diabetes during pregnancy results in significant maternal and fetal morbidity and mortality. Yet, there is little evidence on how diabetes affects normal metabolic alterations that occur during gestation. The goal of this study is to identify altered metabolic transitions in women with diabetes during pregnancy and determine if these metabolic alterations are present in cord blood at term. Subjects were consented for participation in an IRB approved study. 281 urine and blood specimens were collected at first, second, and third trimester (n=221 controls; n=60 diabetics), and cord blood at delivery (n=24 controls; n=8 diabetics). Nuclear magnetic resonance spectroscopy (1H-NMR) was conducted on a 600 MHz spectrometer. A 1-way ANOVA was used to determine metabolites that were significantly altered throughout gestation in control specimens and two-way MANCOVA was utilized to determine if metabolic trends were significantly altered between diabetic and control specimens. Amino acids and TCA cycle intermediates were diminished over normal gestation, adding to evidence of increased utilization by the fetus. In addition, metabolites involved with glycolysis/gluconeogenesis, microbiome, and lipids were elevated throughout gestation. These metabolic trends were not found in women with pre-gestational diabetes, while women with gestational diabetes retained normal trends until mid-gestation. However, metabolites present in the cord blood at term were not reflective of altered maternal blood metabolites, suggesting placental regulation of transport or metabolism. A better understanding of how diabetes during pregnancy alters maternal and fetal metabolism can lead to better treatments, ultimately leading to decreased fetal and maternal morbidity and mortality at term.

P-360 Metabolomic profiles of rat offspring depending on maternal diet during pregnancy

PRESENTING AUTHOR: ***Jeongae Lee, Korea Institute of Science and Technology, Korea, South***

CO-AUTHORS: ***Yoonhwan Kim, Minseon Kim, Eun Jin Kwon, Ji-Eun Du, Young-Ah You, Young Ju Kim, Insook Rhee, Hesson Chung, Bong Chul Chung***

The FetalOrigins of Adult Disease (FOAD) hypothesis holds that events during important tissue and organ formation periods in the fetal life could impact on long-term or lifetime effects on an organism. It is hypothesis that, especially after adulthood, it is susceptible to various adult diseases such as hypertension, diabetes and obesity. In this study, the pregnancy rat model consisted of three groups: control, 50% dietary group and 45% high fat dietary group. The offspring plasma was collected. A non-targeted UPLC-ORBITRAP/MS and multivariate analysis was applied using reversed-phase liquid chromatography (RPC). The partial least-squares discriminant analysis (PLS-DA) model was generated from metabolic profiling data and the score plots showed a significant difference on each group. The most differential metabolites were detected from four modes (a total of 146 (VIP > 1) out of 945 variable ions from RPC positive ionization mode. Based on pathway analysis, we found strong correlation with phenylalanine, D-glutamine and D-glutamate, retinol, tyrosine, taurine, hypotaurine, retinol and phenylalanine pathways. Thus, the changes of these metabolites can be used to assess the risk of FOAD impact and further study need to these associations.

P-361 Metabolic signature to predict future diabetes susceptibility

PRESENTING AUTHOR: ***Yashwant Kumar, Translational Health Science and Technology Institute, India***

Diabetes mellitus is a metabolic syndrome characterized by high levels of blood glucose levels. The current established pharmacological therapy in type-2 diabetes is metformin, sulfonylurea agents and insulin along with lifestyle management. However, diabetes is complicated and not easy to manage once the progression of the disease sets in. Predictive metabolic signatures for diabetes can go a long way in identifying the susceptible individuals and managing the disease early. If the disease can be predicted in the susceptible individuals, simple lifestyle management and diet control can further delay the onset of the disease and make a marked difference to the quality of life of the susceptible individuals. In this case control study, normal and diabetes each of 250 in in number has been analyzed using untargeted LCMS metabolomics and predictive modelling. We have used machine learning approaches to accurately predict the future diabetic patients.Novelty of this study is using large dataset from Indian subject to predict future diabetic using mass spectrometry based metabolomics

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OBESITY, DIABETES AND CVD

P-362 Germinated Soy Germ Extract ameliorates obesity via inhibition of adipogenesis and beige fat activation

PRESENTING AUTHOR: ***Woo Duck Seo, National Institute of Crop Science, South Korea***

CO-AUTHORS: ***Woo Duck Seo, Sik Won Choi, Hyeon Jung Kang, Mi Ja Lee, Hyun-Young Kim, Hyun Mi Ham, Han-Jun, Eun Ji Choi, Sun Hee Do, Ki Do Park***

In this study, we investigated the effects of germinated soy germ extracts (GSGE) on 3T3-L1 pre-adipocytes and high-fat diet induced obese animal models. GSGE treatment suppressed 3T3-L1 cells to differentiate into mature adipocytes, along with the reduced lipid accumulation and lipid droplet formation. In vivo studies also showed that GSGE treatment reduced weight gain, decreased adipocyte area, reduced serum level of triglyceride and LDL-cholesterol in HFD-fed mice. Gene expression analysis of adipogenic factor (C/EBP-α, SREBP-1c) and lipogenic factors (FAS, SCD-1) were down-regulated in both in vitro and in vivo study. In addition, GSGE treatment increased the expression levels of beige fat activation (UCP1, CIDEA, CPT1, PPAR-α), beta oxidation (ACOX1) lipolysis (ATGL, HSL) in all concentration of GSGE. However, gene expression levels of lipid droplet and triglyceride synthesis-related genes (Plin1, Caveolin, CGI-58, ASCL1, 4) were down-regulated only in low concentrations of GSGE. The present study provides evidences that GSGE is effective in inhibiting adipo- and lipogenesis and accelerating energy consumption through activation of beige fat activation even under excessive caloric intake. These results demonstrate that GSGE can be a promising dietary strategy for the prevention of obesity by promoting weight loss, reducing fat accumulation, and improving obesity-related glucose tolerance and insulin resistance.

P-363 Screening complex lipids in a large Type 2 Diabetes cohort in Native Americans

PRESENTING AUTHOR: ***Ying Zhang, University of California, China***

CO-AUTHORS: ***Brian DeFelice, Sili Fan, Jinying Zhao, Oliver Fiehn***

Native Americans, similar to other indigenous people around the world, respond more rapidly and sensitively to Western diets than Non-Hispanic White cohorts, leading to drastic increases in type 2 diabetes (T2D). A range of lipid species have been implicated in the development of insulin resistance and T2D, from mono- and diacylglycerols to lipid mediators. To test the contribution of complex lipids to the development of T2D in Native Americans, we conducted lipidomics analysis using charged surface hybrid UPLC with QTOF mass spectrometry in a nested case/control study on T2D development in 120 subjects from a cohort of 4,000 individuals. Using a modified Matyash extraction method with methyl-tert-butyl ether (MTBE), 15 spiked internal standards were used for semiquantitative comparisons of lipid profiles, yielding more than 450 unique identified lipids, using MS-DIAL vs. 2.8 and an enlarged LipidBlast MS/MS library for data processing and compound annotations. Univariate and multivariate statistics in the new MetDA software within the MetaBox suite of data analysis tools was complemented by a range of regression analyses for finding statistical differences between study groups. The new Chemical Enrichment statistics software ChemRICH was used to cluster lipidomics results with respect to all covered lipid classes, including FAHFA lipids, and was complemented to a detailed analysis of lipid changes based on acyl-chains alone. These discovery data will be validated by the much larger cohort of the full Native American parent study.

P-364 Alteration in the plasma metabolome associated with PFAS exposure and type 2 diabetes

PRESENTING AUTHOR: ***Carl Brunius, Chalmers University of Technology, Sweden***

CO-AUTHORS: ***Tessa Schillemans, Lin Shi, Ingvar A. Bergdahl, Kati Hanhineva, Hannu Kiviranta, Panu Rantakokko, Olov Rolandsson, Rikard Landberg, Agneta Åkesson***

Through various sources, people are exposed to perfluoroalkyl substances (PFAS), which are persistent, bioaccumulative and detected in almost all environments. PFAS have been suggested as risk factors for cardiometabolic diseases, but very little is known about their metabolic effects. We therefore conducted a nested case-control study to investigate associations between PFAS, plasma metabolome and risk of developing type 2 diabetes (T2D). Plasma samples from 187 case-control pairs, randomly selected from a Swedish prospective cohort, were analyzed at baseline (median 7 years before diagnosis) and at 10-year follow-up for PFAS and untargeted LC-MS metabolomics (24758 features). Principle component analysis of six detectable PFAS showed two distinct exposure clusters: i) PFOA, PFOS & PFHXS and ii) PFNA, PFDA & PFUNA. Random forest regression showed that the plasma metabolome predicted combined exposure in the 1st group (PC scores) (Q2=0.64; 10 selected features), which was associated with decreased risk of developing T2D (OR=0.63 (95% CI 0.50-0.81); adjusted for age, BMI and sample year). The 2nd exposure group was less well predicted (Q2=0.37; 23 selected features) and not significantly associated with T2D risk (OR=0.87 (0.69-1.10)). Metabolite profiles were similar within PFAS exposure clusters and the two clusters were differently associated with T2D, suggesting that clusters rather than individual PFAS may be used to assess their role in health. Mechanistic links between PFAS exposures and disease risk are yet unknown. Further analyses of potential confounders from life style as well as identification of metabolites associated with the two clusters and T2D will aid biological interpretations.

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OBESITY, DIABETES AND CVD

P-365 New Paradigms in Type 2 Diabetes Treatment

PRESENTING AUTHOR: ***Yen Chin Koay, Postdoctoral Researcher, Australia***

CO-AUTHORS: ***Dr. Dorit Somacha-Biet, Dr. PengYi Yang, Dr. John O'Sullivan***

Background: The prevalence of type 2 diabetes mellitus (T2D) has dramatically increased worldwide with insulin resistance (IR) as the major driver. IR is predominantly determined by muscle and liver, and drugs target either organ to varying degrees. T2D therapy is a stepwise approach starting with metformin to which ~40% of patients do not or poorly respond to. Knowing whether IR is driven by the liver or muscle could circumvent ineffective treatments and unnecessary side effects. Aim. 1. Discover novel biomarkers that discriminate between liver and muscle IR. 2. Use a machine-learning approach to obtain optimal combined biomarker score to distinguish liver vs muscle IR/insulin sensitivity (IS). Method. Plasma was obtained from 64 obese patients undergoing hyperinsulinemic-euglycemic clamp, who were categorized as: MuscleIR and LiverIR (n = 33); MuscleIR and LiverIS (n = 10); MuscleIS and LiverIR (n = 9); and MuscleIS and Liver IS (n = 12). Using non-targeted lipidomics, >5,000 lipid species were measured using a C18 column on an Agilent 1290 HPLC-6550 QTOF system. Results and Conclusions. A circulating lipid species was significantly elevated in plasma of obese individuals with hepatic IR vs obese hepatic IS individuals (fold change = +4; $p < 1.1E-16$), which we elucidated as N-palmitoylsphingomyelin, SM(d18:1/16:0). We then identified three m/z features (m/z: 275.0457, 565.0879, and 1134.8870) that identified group assignment with an overall accuracy of 95.2% using machine-learning approaches. Ultimately, we plan to develop a collective diagnostic based on biomarker and patient characteristics.

P-366 Therapeutic Targeting TPL2 (Tumor Progression Locus-2)/ATF4 (Activating Transcription Factor-4)/SDF1α (Chemokine Stromal Cell-Derived Factor-α) Axis Suppresses Diabetic Retinopathy.

PRESENTING AUTHOR: ***Meei-Ling Sheu, National Chung Hsing University, Taiwan***

RATIONALE: Diabetic retinopathy (DR)is characterized by vasopermeability, vascular leakage, inflammation, blood-retinal barrier breakdown, capillary degeneration, and neovascularization. However, the mechanisms underlying the association between diabetes mellitus and progression retinopathy remain unclear. OBJECTIVE: TPL2 (tumor progression locus 2), a serine-threonine protein kinase, exerts a pathological effect on vascular angiogenesis. This study investigated the role of Nε-(carboxymethyl)lysine, a major advanced glycation end products (AGEs), and the involved TPL2-related molecular signals in diabetic retinopathy using models of in vitro and in vivo and human samples. METHODS AND RESULTS: Serum Nε-(carboxymethyl)lysine levels and TPL2 kinase activity were significantly increased in clinical patients and experimental animals with diabetic retinopathy. Intravitreal administration of pharmacological blocker or neutralizing antibody inhibited TPL2 and effectively suppressed the pathological characteristics of retinopathy in streptozotocin-induced diabetic animal models. Intravitreal VEGF (vascular endothelial growth factor) neutralization also suppressed the diabetic retinopathy in diabetic animal models. Mechanistic studies in primary human umbilical vein endothelial cells and primary retinal microvascular endothelial cells from streptozotocin-diabetic rats, db/db mice, and samples from patients with diabetic retinopathy revealed a positive parallel correlation between Nε-(carboxymethyl)lysine and the TPL2/chemokine SDF1α (stromal cell-derived factor-α) axis that is dependent on endoplasmic reticulum stress-related molecules, especially ATF4 (activating transcription factor-4). CONCLUSIONS: This study demonstrates that inhibiting the Nε-(carboxymethyl)lysine-induced TPL2/ATF4/SDF1α axis can effectively prevent diabetes mellitus-mediated retinal microvascular dysfunction. This signaling axis may include the therapeutic potential for other diseases involving pathological neovascularization or macular edema.

P-367 mTORC1 Regulates Mitochondrial Integrated Stress Response and Mitochondrial Myopathy Progression

PRESENTING AUTHOR: ***Vidya Velagapudi, University of Helsinki, Finland***

CO-AUTHORS: ***Nahid A. Khan, Joni Nikkanen, Shuichi Yatsuga, Christopher Jackson, Liya Wang, Swagat Pradhan, Riikka Kivela, Alberto Pessia, Vidya Velagapudi, Anu Suomalainen***

The highlights of our recently published article (Khan et al., 2017, Cell Metabolism, 26, 419-428) about inherited metabolic disorders, mitochondrial myopathy,are as follows, 1. mtDNA replication defect activates mTORC1 and integrated mitochondrial stress response. 2. mTORC1 upregulates mitochondrial One-Carbon cycle, FGF21, and UPRmt in mitochondrial disease. 3. mTORC1 contributes to ragged-red fiber formation in mitochondrial myopathy. 4. Rapamycin reverts progression of mitochondrial myopathy in mice. Summary Mitochondrial dysfunction elicits various stress responses in different model systems, but how these responses relate to each other and contribute to mitochondrial disease has remained unclear. Mitochondrial myopathy (MM) is the most common manifestation of adult-onset mitochondrial disease and shows a multifaceted tissue-specific stress response: (i) transcriptional response, including metabolic cytokines FGF21 and GDF15; (ii) remodeling of one-carbon metabolism; and (iii) mitochondrial unfolded protein response. We show that these processes are part of one integrated mitochondrial stress response (ISRmt), which is controlled by mTORC1 in muscle. mTORC1 inhibition by rapamycin downregulated all components of ISRmt, improved all MM hallmarks, and reversed the progression of even late-stage MM, without inducing mitochondrial biogenesis. Our evidence suggests that (a) chronic upregulation of anabolic pathways contributes to MM progression, (b) long-term induction of ISRmt is not protective for muscle, and (c) rapamycin treatment trials should be considered for adult-type MM with raised FGF21.

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OBESITY, DIABETES AND CVD

P-368 Roles of the Histone Methyltransferase MLL4/KMT2D in Metabolic Syndrome

PRESENTING AUTHOR: ***Seunghee Lee, Seoul National University, South Korea***

The pathophysiologic continuum of non-alcoholic fatty liver disease begins with steatosis. Despite recent advances in our understanding of the gene regulatory program directing steatosis, how it is orchestrated at the chromatin level is unclear. PPARg2 is a hepatic steatotic transcription factor induced by overnutrition. Here, we report that the histone H3 lysine 4 methyltransferase MLL4/KMT2D directs overnutrition-induced murine steatosis via its coactivator function for PPARg2. We demonstrate that overnutrition facilitates the recruitment of MLL4 to steatotic target genes of PPARg2 and their transactivation via H3 lysine 4 methylation because PPARg2 phosphorylated by overnutrition-activated ABL1 kinase shows enhanced interaction with MLL4. We further show that Pparg2 (encoding PPARg2) is also a hepatic target gene of ABL1-PPARg2-MLL4. Consistently, inhibition of ABL1 improves the fatty liver condition of mice with overnutrition by suppressing the pro-steatotic action of MLL4. Our results uncover a murine hepatic steatosis regulatory axis consisting of ABL1-PPARg2-MLL4, which may serve as a target of anti-steatosis drug development.

CANCER

P-369 Differential utilization of glutamine by IDH1-mutant cell lines produces sensitivity or resistance to glutaminase inhibition by CB839

PRESENTING AUTHOR: ***Victor Ruiz Rodado, Neuro-Oncology Branch, NCI, NIH, United States***

CO-AUTHORS: ***Adrian Lita, Alejandra Cavazos-Saldana, Tyrone Dowdy, Mark R. Gilbert, Kylie Walters, Mioara Larion***

Mutant IDH1 (IDH1mut) gliomas have characteristic genetic and metabolic profiles, in addition to exhibit a markedly-different phenotype compared with their wild-type counterparts. One of such metabolic routes, the glutamine/glutamate pathway, has been reported as one of those selective therapeutic potential targets in IDH1mut gliomas. However, little information exists on the contribution of this pathway to the formation of D-2-hydroxyglutarate (D-2HG), a hallmark of IDHmut cells, and the metabolic consequences of inhibiting such pathway. Through a combined NMR and LC-MS 13C metabolic tracing analysis, we show that glutamine/glutamate pathway contributes differentially to D-2HG formation in a cell-line dependent fashion on a panel of IDHmut cell lines. Interestingly, we have observed different effects of CB839 on cellular proliferation, driving us to define them as GLS-inhibition super-sensitive, -sensitive or -resistant. Our data indicates a decrease in the production of downstream metabolites of glutamate, including those involved in the TCA cycle, when treating the sensitive cells with CB839 (glutaminase -GLS- inhibitor). Notably, CB839-sensitive IDH1mut cells respond to GLS inhibition by upregulating glycolysis and lactate production. Contrastingly, CB839-resistant IDH1mut cell line does not rely only on glutamine for the sustenance of TCA cycle. In these cells, glucose contribution to TCA is enough to compensate the downregulation of glutamine derived TCA-metabolites. Additionally, CB839-resistant cell line responds to the treatment by increasing glutamate intake via EAAT1 transporters. This investigation reveals a heterogeneous landscape of IDH1mut metabolic phenotypes, and underscore the importance of detailed metabolic profiling of IDH1mut patients prior to the decision to target glutamine/glutamate pathway clinically.

P-370 Metabolomic analysis of cancer cell-derived extracellular vesicles

PRESENTING AUTHOR: ***Ryosuke Hayasaka, Keio University, Japan***

CO-AUTHORS: ***Akiyoshi Hirayama, Sho Tabata, Tomoyoshi Soga, Masaru Tomita***

[Introduction] Extracellular vesicles (EVs) are released from any kind of cell and are capable of entering other cells. In cancer field, many studies demonstrated that cancer-derived EVs contribute to cancer progressions such as angiogenesis, immunosuppression, and distant metastasis. The EVs contain functional cellular components including DNA, mRNA, microRNA, and protein. However, metabolites in EVs are largely unexplored. [Purpose] The purpose of this study is to elucidate comprehensive metabolite profiling of cancer-derived EVs. As a model for studying cancer metabolism, we evaluate the difference of metabolomic profiles in EVs obtained from cancer cells cultured normoxic vs hypoxic conditions. [Methods] Pancreatic cancer cell line, Panc1 was cultivated under normoxic (20% O2) and hypoxic (1% O2) conditions. EVs were isolated from conditioned medium using ultracentrifugation. The amount of EVs was determined by nanoparticle tracking analysis and protein level of CD9, exosomal marker, that was measured using enzyme-linked immunosorbent assay (ELISA). Metabolomic analysis was performed by using liquid chromatography-mass spectrometry and capillary ion chromatography-mass spectrometry. [Preliminary Results] We identified more than 100 metabolites in pancreatic cancer-derived EVs. Principal component analysis (PCA) of anionic metabolites showed some what differentiated the results between normoxia and hypoxia. The top 4 metabolites showing significant difference between normoxia and hypoxia were ADP-ribose, UMP, NADH, and N-Acetylglucosamine 1-phosohate. These results suggest that the metabolomic profiling of cancer-derived EVs is changed by oxygen concentration.

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CANCER

P-371 An untargeted metabolic flux analysis in human myeloma cell lines

PRESENTING AUTHOR: *Tumpa Dutta, Mayo Clinic, United States*

CO-AUTHORS: *Tumpa Dutta, Wilson I. Gonsalves, Almira Smailovic, Xuan-Mai Petterson, Ian Lanza*

Defects in regulation of mammalian cell growth lead to cancer. 2-hydroxyglutarate (2-HG) is a hallmark oncometabolite derived from α-ketoglutarate in cancers like acute myeloid leukemia and glioblastoma. However, elevated levels of 2-HG is also associated with c-MYC overexpression which controls glutamine metabolism. GC-MS based 13C-stable isotope resolved metabolomics using U[13C6]glucose and U[13C5]glutamine tracers evaluated the TCA cycle flux in human myeloma cell lines (HMCLs) which overexpress c-MYC. We found that 2-HG is derived predominantly from glutamine anaplerosis into the TCA cycle and glutamine is preferentially utilized as a substrate by the TCA cycle compared with glucose. Additionally, elevated levels of 2-HG in patient plasma samples predicts for early progression from asymptomatic to symptomatic multiple myeloma (MM). Such targeted approaches are appropriate for well-defined hypotheses but fail to provide knowledge on unknown parts of the metabolic regulation. Therefore, our aim is to determine the incorporation of U[13C5]-glutamine in an untargeted manner in order to identify known or novel metabolites and metabolic pathways in HMCLs that utilize glutamine. We have developed UPLC-QToF-MS approach for untargeted detection of isotopic enrichment using a multi-stage isotopologue extraction process. The mass spectrum of each compound from the labeled sample is paired with its unlabeled counterpart. A custom database is created to generate a list of known and unknown entities with stringent retention time lock. Newly identified metabolites derived from glutamine utilization by HMCLs can potentially serve as biomarkers for early identification of patients at high risk of progression to MM that may benefit from early therapy.

P-372 Detection of differential expression of blood proteins in glioblastoma through omics analysis

PRESENTING AUTHOR: *Dr. Rashmi Rana, Sir Ganga Ram Hospital, India*

CO-AUTHORS: *Rashmi Rana, Poonam Gautam1, Rajesh Acharya, Nirmal K Ganguly*

Gliomas are brain tumors with glial cell characteristics, and are composed of a heterogeneous mix of cells, which includes astrocytomas, oligodendrogliomas, ependymoma, and mixed gliomas. Gliomas account for 32% of all brain and central nervous system (CNS) tumors and 80% of all malignant brain and CNS tumors. Glioma among is found mostly men, often in their 50s and 60s, though it has in recent years began appearing much more frequently in 40 year old and even younger. The WHO grades IV glioblastoma (GBM) is highly invasive tumors and make up approximately three-quarters of all gliomas. The average survival period for this disease is six to eight months, pointing the need to find ways of early diagnosis and treatment. Today, the main methods of diagnosis of this disease are MRI and brain biopsy. Therefore, there is an urgent need to develop new, non-invasive methods for diagnosis. Qualitative and quantitative changes in the detected protein groups could serve as a good indicator of glioma using blood and tissue samples. Therefore, we have obtained more data using 2DE separation and iTRAQ followed by ESI-LC MS/MS, verified by ELISA/ IHC. These proteins can serve as potential targets for the development of biomarkers and predict clinical behaviour and/ or therapeutic response is identified.

P-373 Metabolomics revealed the selective requirement for inosine monophosphate dehydrogenase in a subset of small cell lung cancers

PRESENTING AUTHOR: *Zeping Hu, Tsinghua University, China*

CO-AUTHORS: *Fang Huang, Min Ni, Milind D. Chalishazar, Kenneth E. Huffman, Jiyeon Kim, Ling Cai, Xiaolei Shi, Lauren G. Zacharias, Feng Cai, Wen Gu, Abbie S. Ireland, Adi F. Gazdar, Trudy G. Oliver, John D. Minna, Ralph J DeBerardinis*

Small cell lung cancer (SCLC) is a rapidly lethal disease with few therapeutic options. We studied metabolic heterogeneity in SCLC to identify subtype-selective vulnerabilities using metabolomcis and metabolic flux analysis. Metabolomics in SCLC cell lines identified two groups correlating with high or low expression of the Achaete-scute homolog-1 (ASCL1) transcription factor (ASCL1-High and ASCL1-Low), a lineage oncogene in SCLC. Guanosine nucleotides were elevated in ASCL1-Low cells and tumors from genetically engineered mice. ASCL1-Low tumors abundantly express the guanosine biosynthetic enzymes inosine monophosphate dehydrogenase-1 and -2 (IMPDH1 and IMPDH2). These enzymes are transcriptional targets of MYC, which is selectively overexpressed in ASCL1-Low SCLC. IMPDH inhibition with clinically available drugs reduced RNA polymerase I-dependent expression of pre-ribosomal RNA and potently suppressed ASCL1-Low cell growth in culture, selectively reduced growth of ASCL1-Low xenografts, and combined with chemotherapy to improve survival in genetic mouse models of ASCL1-Low/MYCHigh SCLC. The data define an SCLC subtype-selective vulnerability related to dependence on de novo guanosine nucleotide synthesis.

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P-374 Breast Cancer Detection Using Targeted Plasma Metabolic Profiling

PRESENTING AUTHOR: ***Paniz Mohajer Jasbi, Arizona State University, United States***

CO-AUTHORS: ***Dongfang Wang, Dan Du, Qiang Fei, Daniel Raftery, Haiwei Gu***

Breast cancer (BC) is one of the most prevalent and deadly cancers in the world. Despite the important role of metabolism in the molecular pathogenesis of cancer, robust metabolic markers to enable screening, surveillance, and therapeutic monitoring of BC are still lacking. In this study, we present a targeted liquid chromatography-tandem mass spectrometry-based metabolic profiling approach for identifying metabolic marker candidates that could enable highly sensitive and specific BC detection using human plasma samples. In this targeted approach, 105 metabolites from more than 35 metabolic pathways of potential biological significance were monitored in 201 samples taken from two groups of patients (102 BC patients and 99 healthy controls) collected from two different locations. Partial least squares-discriminant analysis (PLS-DA) models proved to be powerful for distinguishing BC patients from healthy controls. A receiver operating characteristic (ROC) curve generated based on these PLS-DA models showed high sensitivity (0.80 for differentiating BC patients from healthy controls), good specificities (0.75), and excellent area under the curve (0.89). Permutation testing (cross-validation) was also applied, demonstrating the robust diagnostic power of this metabolic profiling approach. The results of our exploratory factor analysis (EFA) show significant disturbances in arginine/proline, taurine/hypotaurine, and tryptophan metabolism. Our results indicate the effectiveness of this metabolomics approach for BC diagnosis. This study considers samples from multiple clinical locations which, although critically important, is underused in metabolomics study design. Future studies should examine larger cohorts from multiple locations in addition to the altered metabolic pathways related to BC pathogenesis discovered in our study.

P-375 Loss of SETD2 induces a metabolic switch in VHL-inactivated clear cell renal cell carcinoma toward enhanced oxidative phosphorylation

PRESENTING AUTHOR: ***Jingping Liu, Key Laboratory of Transplant Engineering and Immunology, West China Hospital, Sichuan University, China***

CO-AUTHORS: ***Paul D. Hanavan, Katon Kras, Yvette W. Ruiz, Erik P. Castle, Douglas F. Lake, Keith D. Robertson, Haiwei Gu, Thai H. Ho***

SETD2, a histone H3 lysine trimethyltransferase, is frequently inactivated and highly associated with metastatic burden and reduced survival in clear cell renal cell carcinoma (ccRCC). However, the impact of SETD2 loss on metabolic alteration in ccRCC is still unclear. In this study, SETD2 null isogenic 38E/38F cells from 786-O cells were generated by zinc finger nuclease, and the metabolic, genomic, and cellular function changes were analyzed by target metabolomics, RNA-seq, and biological methods, respectively. Our results showed that compared to 786-O cells, 38E/38F cells had elevated levels of MTT/alamar blue signals, ATP, glycolytic/mitochondrial respiration capacity, fumarate hydratase (FH)/citrate synthase (CS) activities, and TCA metabolites such as aspartate, malate, succinate, fumarate, and α-ketoglutarate. 38E/38F cells also utilized alternative sources beyond pyruvate to generate acetyl-CoA for the TCA cycle. Moreover, 38E/38F cells showed disturbed gene networks mainly related to mitochondrial, fatty acids and glucose metabolism, which was associated with increased levels of PGC1α, mitochondria mass, and cellular size/complexity. Our results indicate that SETD2 deficiency induces a metabolic switch toward enhanced oxidative phosphorylation in ccRCC, which can be related to PGC1α-mediated metabolic networks. Therefore, this current study lays the foundation for the further development of a global metabolic analysis of cancer cells in individual patients, which ultimately will have significant potential for novel therapeutics discovery and precision medicine in SETD2 inactivated ccRCC.

P-376 Systematic identification and experimental validation of collateral metabolic lethality in cancer

PRESENTING AUTHOR: ***Jonas Zierer, Institute for Precision Medicine, Weill Cornell Medicine, United States***

CO-AUTHORS: ***Anna Halama, Karsten Suhre, Olivier Elemento***

The development of cancer is an evolutionary process enabled by driver mutations causing genomic instability and consequently accumulation of passenger mutations, amongst others in metabolic genes. Generally, the metabolism is robust against such gene losses through corrective feedback mechanisms. However, due to the initial gene loss, these compensatory mechanisms are essential for the affected cancer cells but not for healthy cells and, thus, might be therapeutically targetable. Here, we aimed to identify such actionable metabolic weaknesses in 3288 metabolic genes from Recon3D by combining data on copy number variants, point mutations, and gene expression from 9031 tumor samples of the TCGA. We found 45.8% of metabolic genes repeatedly and homozygously lost; 61.1% of those in multiple cancer types. For instance, HACD4, a catalyst of long-chain fatty acid synthesis, is homozygously lost in 7.7% of all lung cancer samples. To investigate the effect of the mutation in vitro, we used an untargeted metabolomics approach and confirmed a depletion of long-chain fatty acids and particularly phosphatidylcholines (enrichment $p<10^{-5}$) in cell lines bearing the mutation compared to cells with wildtype HACD4. Also, amino acids were significantly depleted in the medium of these cells, suggesting an increased uptake of alternative sources of energy, which may be potentially targeted. In summary, we established a systematic protocol to identify collateral metabolic lethalities in tumors and demonstrated their predicted effect on the metabolism in vitro. These functional differences between cancer cells and healthy cells constitute potential targets for cancer therapy, which we will explore in the future.

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POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

CANCER

P-377 Kynurenine Pathway's Metabolites Identified as Co-Biomarkers of Serum PSA Level in Prostate Cancer

PRESENTING AUTHOR: ***Youngja Hwamg Park, College of Pharmacy, Korea University, United States***

CO-AUTHORS: ***Adnan Khan***

Prostate-Specific Antigen (PSA) alone as a biomarker cannot predict every patient with the desired diagnostic and prognostic values due to instability in the level of PSA caused by heterogeneity of prostate diseases. The discovery of co-biomarker of PSA would provide a better strategy to improve the diagnosis and management of prostate cancer (PCa). This study aimed to apply high-resolution metabolomics to characterize the metabolic alterations caused by difference in the level of PSA among prostate cancer patients, and to detect potential compounds correlated to the level of PSA. PCa patients (n=50) were sub divided into two groups on the basis of their PSA level >4 (n=25) and <4 (n=25) to analyze with healthy control (n=100). Principal component analysis and hierarchical cluster analysis clearly discriminated healthy subjects from PCa groups, while no significant difference was observed among PSA <4 or >4's PCa patients. Significantly altered metabolites among groups were determined with the false discovery rate at q = 0.05. Tryptophan metabolism along kynurenine pathway was observed with the highest pathway impact and –log (p) < 0.0005. L-tryptophan, kynurenine, anthranilate, isophenoxazine, glutaryl-CoA, (S)-3-hydroxybutanoyl-CoA, acetoacetyl-CoA and acetyl-CoA, were upregulated in PCa along kynurenine pathway; in contrast, indoxyl, indolelactate, and indole-3-ethanol, involved in the alternative pathway, were downregulated in the PCa. Validation of potential metabolites by tandem mass spectrometry further concreted disturbance of tryptophan metabolism along kynurenine pathway, suggesting kynurenine pathway's metabolites as potential biomarkers of PCa. These findings may allow identification of viable target opportunities for the development of new PCa diagnostic tools.

P-378 Glucose-derived Acetate and ACSS2 as Key Players in Cisplatin Resistance in Bladder Cancer

PRESENTING AUTHOR: ***Tin Tin Manh Nguyen, Seoul National University, South Korea***

CO-AUTHORS: ***He Wen, Sujin Lee, Wei-Guo Zhu, Ok-Jun Lee, Seok Joong Yun, Jayoung Kim, Sunghyouk Park***

Cisplatin is an important chemotherapeutic agent against metastatic bladder cancer, but resistance often limits its usage. With the recent recognition of lipid metabolic alterations in bladder cancers, we studied the metabolic implications of cisplatin resistance using cisplatin-sensitive (T24S) and resistant (T24R) bladder cancer cells. Real-time live metabolomics revealed that T24R cells consume more glucose, leading to higher production of glucose-derived acetate and fatty acids. Along with the activation of general metabolic regulators, enzymes involved in acetate usage (ACSS2) and fatty acid synthesis (ACC) and a precursor for fatty acid synthesis (acetyl-CoA) were elevated in T24R cells. Consistently, metabolic analysis with 13C isotope revealed that T24R cells preferred glucose to acetate as the exogenous carbon source for the increased fatty acid synthesis, contrary to T24S cells. In addition, ACSS2, rather than the well-established ACLY, was the key enzyme that supplies acetyl-CoA in T24R cells through glucose-derived endogenous acetate. The relevance of ACSS2 in cisplatin resistance was further confirmed by the abrogation of resistance by an ACSS2 inhibitor and, finally, by the higher expression of ACSS2 in the patient tissues with cisplatin resistance. Our results may help improve the treatment options for chemoresistant bladder cancer patients and provide possible vulnerability targets to overcome the resistance.

P-379 Metabolic Phenotype Differs in Prostatic Neuroendocrine Carcinoma and Prostatic Adenocarcinoma

PRESENTING AUTHOR: ***Bei Gao, University of California, Davis, United States***

CO-AUTHORS: ***Bei Gao, Sili Fan, Hui-wen Lue, Jennifer Podolak, Kevin Kolahi, Colm Morrissey, Eva Corey, Archana Sehrawat, Joshi Alumkal, George Thomas, Oliver Fiehn***

Prostate cancer is the most common cancer in men. In benign prostate cells, zinc accumulates which inhibits the activity of mitochondrial aconitase, a TCA cycle enzyme that does not have regulatory role in other organs. In addition to prostate cancer subtypes, this highly specialized behavior of prostate cells makes prostate cancer metabolism of particular interest. However, the metabolic phenotypes of each subtype are poorly understood. To test prostate cancer metabolism for better risk stratification and treatment developments, we compared metabolic phenotypes of prostatic neuroendocrine carcinomas, an aggressive and lethal subset of prostate cancer, to prostatic adenocarcinoma, which has a more indolent behavior. Untargeted profiling of primary metabolism (including TCA metabolites) by GC-TOF MS and lipidomics by CSH-QTOF MS/MS were performed on LASCPC-01 and LNCAP cell lines and LuCaP PDX tumor samples. Among other effects, metabolomics data showed that lactate was significantly increased in LASCPC-01, suggesting a strong Warburg effect. Consistent with this finding, the Seahorse assay showed that LASCPC-01 exhibited higher glycolytic activity with lower oxygen consumption rates and higher extracellular acidification rate. The TCA metabolites citrate and isocitrate were significantly decreased in LASCPC-01, indicating that the TCA cycle was not inhibited by Zn-mediated inhibition of aconitase but by pyruvate dehydrogenase activity. Lipidomics results showed that triglycerides were significantly decreased in both prostatic neuroendocrine carcinomas cell lines and PDX tumor samples. Our findings suggest that the metabolic phenotype differs in prostatic neuroendocrine prostate carcinomas versus prostatic adenocarcinoma and provides new potential targets in each subtype.

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CANCER

P-380 Metabolic profiling of Hepatocellular Carcinoma by High-Definition Mass Spectrometry

PRESENTING AUTHOR: ***Daisuke Saigusa, Tohoku University, Japan***

CO-AUTHORS: ***Koshi Nagai, Baasanjav Uranbileg, Makoto Kurano, Hitoshi Ikeda, Yutaka Yatomi, Yoshihisa Tomioka, Junken Aoki***

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world. It is assumed that the number of HCC cases will continue to increase, and biomarkers that are related to the degree of tumor progression and cellular characteristics and that can provide clinical information linked to treatment are required. Metabolome, which is the end product in the central dogma, directly reflects the current phenotype, and metabolomics has been developed as one of the post-genomic research fields and is a promising avenue for discovery of HCC biomarkers. The global metabolomics (G-Met) can analyze metabolic pathways and obtain new knowledge, such as the detection of unknown metabolites. However, it is required that the comprehensiveness and reproducible method to cover a wide range of metabolites, and the distribution analysis of metabolites in tissue with accurate identification. Therefore, we applied a high-definition mass spectrometry (HDMS) technology to identify the metabolites in HCC tissue using UHPLC-QTOF/MS and desorption electrospray ionization (DESI)-MS imaging. The detected features obtained from the G-Met analysis equipped with a Scherzo SS-C18 column in tumor regions (n=38) and non-tumor regions (n=72) of human liver were analyzed by means of principal component analysis and orthogonal partial least square discriminant analysis. From the result, m/z 904.83 and m/z 874.79 were shown to be significantly high and low in the tumor regions, and identified as triglyceride (TG) (16:0/18:1/20:1(11Z)) and TG (16:0/18:1(9Z)/18:2(9Z, 12Z)) using HDMS, respectively. Finally, we demonstrated that the TGs were localized in the tumor and non-tumor regions by DESI-MSI analysis.

P-381 The metabolic comparison between normal and lung cancer cell lines in the anoikis condition

PRESENTING AUTHOR: ***MUNKI CHOO, Seoul National University, South Korea***

Lung cancer is a fatal disease with a low survival rate because it has little symptoms in the advanced stage. Generally, when normal cells lose their interaction with the matrix, it results in a phenomenon called anoikis as a defense mechanism of the living body, and the cells die. Therefore, by studying the anoikis resistance of cancer, we can prevent cancer metastasis and improve the existing cancer treatment. In this study, we compared the difference between the metabolism of normal and cancer cells; further, we analyzed how cancer cells can be resistant to anoikis. First, we compared the viability of the lung cancer cell (A549) and the normal cell (MRC5) in the anoikis condition. Using metabolic analyses, the ratio of GSH/total glutathione in the normal dipped notably. This indicates that normal cells die because they cannot overcome the increased oxidative stress. Furthermore, the 13C-glucose flux data of the energy metabolites showed a significant reduction of M+5 of energy metabolites, especially in normal cells. In other words, it was possible to deduce that some specific step of purine synthesis was down regulated from glucose to ATP. Purine biosynthetic enzyme PPAT was decreased in the normal using real-time PCR. In terms of cancer, pyruvate carboxylase of the cancer was significantly increased in the anoikis condition. Studies have shown that metabolic differences between the normal and cancer cells might play an important role in anoikis resistance. This could provide a new perspective for future studies of metastasis and resistance to lung cancer.

P-382 Machine learning and urinary polyamines to detect colorectal cancers

PRESENTING AUTHOR: ***Masahiro Sugimoto, Tokyo Medical University, Japan***

Colorectal cancer (CRC) is one of the most daunting diseases due to its increasing worldwide prevalence. Urinary polyamines have shown as potential markers to detect CRC. However, the single polyamine metabolite showed a limitation of its low sensitivity and specificity. Therefore, we utilized liquid chromatography mass spectrometry to quantify various polyamines, such as spermine and spermidine with their acetylated forms. Urinary samples from 201 CRCs and 31 non-CRCs were used for the analyses. Overall, 59 samples were analyzed to evaluate the reproducibility of quantified concentrations, acquired by collecting three times on three days each from each healthy control and the stability of the observed quantified values were confirmed. Adtree, one of machine learning methods, was utilized to combine polyamines for obtaining high discrimination ability to differentiate CRC from non-CRCs. Computational validations confirmed the generalization ability of the models. Polyamines and a machine-learning method showed potential as a screening tool of CRC.

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CANCER

P-383 Identification of patients with brain metastases using NMR-biofluid metabolomics

PRESENTING AUTHOR: ***James R Larkin, University of Oxford, United Kingdom***

CO-AUTHORS: ***Vanessa A Johanssen, Timothy D W Claridge, Miranda Payne, Geoff S Higgins, Nick dePennington, Nicola R Sibson***

Introduction Secondary tumours, or metastases, underlie around 90% of cancer deaths. Over 20% of cancer patients develop brain metastases. This is particularly problematic because brain metastases are hard to diagnose early; their gold-standard MRI-based diagnosis depends on late-stage blood-brain barrier breakdown. We have previously shown that brain metastases can be detected early in mice using urine NMR metabolomics [1]. Here, we hypothesised that this approach would translate to patient populations. Methods We collected urine from patients with primary brain tumours or with primary lung tumours or malignant melanomas, as these are prone to developing brain metastases. Patients were classified as having either brain or systemic metastases, a combination of both, or no metastases. Samples were mixed with buffer and analysed by 1H NMR spectroscopy on a Bruker AVIII 700MHz spectrometer using a 1D NOESY water presaturation sequence. Spectra were aligned and post-processed before model-building using PLS-DA. Results In both melanoma and lung cancer populations, patients with metastases in the brain could be separated from patients with purely systemic metastases. In melanoma, patients with metastases (any location) were distinct from patients without. Comparisons between patients with primary or secondary tumours in the brain revealed significant separations dependent on tumour type. Conclusions Categorisation of cancer patients as a function of metastatic burden and/or metastatic location appears possible, and it appears highly likely that profiles specific to brain metastasis can be developed. Follow-up of patients will reveal whether metabolic profiling is more sensitive than traditional MRI-based detection. [1] Larkin et al. Theranostics, 2016

P-384 Metabolic characterization of FLCN deficient renal cancer cells and tumor tissues using Stable Isotope-Resolved Metabolomics

PRESENTING AUTHOR: ***Ye Yang, National Institutes of Health, China***

CO-AUTHORS: ***Ye Yang, Daniel R. Crooks, Geetha M. Cawthon, Richard M. Higashi, Teresa W.-M. Fan, Andrew Lane, Laura S. Schmidt, W. Marston Linehan***

Birt-Hogg-Dube syndrome (BHD) is caused by germline mutations in the folliculin gene (FLCN), and patients are at risk of developing bilateral, multifocal renal tumors. Loss of heterozygosity of the FLCN locus and somatic “second hit” mutations are found in 17% and 53% of the renal tumors from patients with BHD syndrome, respectively [1]. While multiple potential roles of FLCN have been uncovered in diverse pathways, such as AKT-mTOR pathway signaling, AMPK activation regulation, TFE3/TFEB transcriptional activation, etc. [2], there are few studies that have directly investigated the activity of central metabolic pathways such as glycolysis and the Krebs cycle in FLCN-deficient tumors and cells. In this study we utilized Stable Isotope Resolved Metabolomics (SIRM) to investigate and characterize the altered metabolic pathways in patient derived FLCN deficient cell lines and renal tumor slices obtained intra-operatively in patients undergoing surgery at the NIH Clinical Center. Both NMR and Ultra-high-resolution mass spectrometry were used to analyze the polar/non-polar metabolites extracted from BHD tumor cells and tissues. Our preliminary data reveal that metabolic pathways including glycolysis, the Krebs cycle, pentose phosphate pathway, nucleotide synthesis and amino acid metabolism are altered in the FLCN deficient renal cancer cell lines compared with non-transformed human primary renal epithelial cells. These findings will provide a novel perspective for selection of therapeutic agents for treatment and/or prevention of FLCN deficient renal cancer.

P-385 LC-MS/MS based discovery of GCA and TCDCA as precise metabolic biomarker in Cholangiocarcinoma

PRESENTING AUTHOR: ***Won-Suk Song, Seoul National University, Korea, South***

CO-AUTHORS: ***Han-Gyu Park, Da-Hee Ann, Yun-Gon Ki,***

Several biomarkers can be used to distinguish from cholangiocarcinoma (CCA) and healthy controls, but it is not easy to distinguish it from benign biliary disease (BBD) or pancreatic cancer (PC). Conventional CCA biomarkers were not associated with low specificity or with respect to the biological effects of CCA. In this study, among patients in CCA, BBD and PC, biliary bile acids were quantitatively analyzed to determine specific CCA biomarkers for glycocholic acid (GCA) and taurochandeloxycholic acid (TCDCA). As a result, the mean concentration of total bile acid in the CCA patients was quantitatively less than the other patient groups. The mean composition ratio of bile acid and conjugated bile acid was the highest in patients with CCA. The mean composition ratio of GCA of CCA patients was significantly higher than that of other patients. Conversely, the mean composition ratio of TCDCA in CCA patients was significantly lower in the patient group. To confirm the biological effects of GCA and TCDCA, gene expression of bile acid receptors related to the development of CCA was analyzed in CCA cell lines. The gene expression of S1PR2 and TGR5 in the CCA cell line treated with GCA was 8.6 times higher than that in the control group (the group not treated with bile) by 3.4 times. each. Gene expression of TGR5 and S1PR2 in TCDCA - treated cell lines was not significantly different from the control group. Taken together, this study confirmed that GCA and TCDCA are phenotype-specific biomarkers for CCA.

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CANCER

P-386 Glutamine-derived 2-hydroxyglutarate is associated with disease progression in plasma cell malignancies

PRESENTING AUTHOR: ***Wilson Gonsalves, Mayo Clinic, Rochester, United States***

CO-AUTHORS: ***Vijay Ramakrishnan, Taro Hitosugi, Toshi Ghosh, Dragan Jevremovic, Tumpa Dutta, Dhananjay Sakrikar, Xuan Mai Petterson, Linda Wellik L, Shaji Kumar, Sreekumaran Nair***

he production of the oncometabolite 2-hydroxyglutarate (2-HG) has been associated with c-MYC overexpression. c-MYC also regulates glutamine metabolism and drives progression of asymptomatic precursor plasma cell (PC) malignancies to symptomatic multiple myeloma (MM). However, the presence of 2-HG and its clinical significance in PC malignancies is unknown. By performing 13C stable isotope resolved metabolomics (SIRM) using U[13C6]Glucose and U[13C5]Glutamine in human myeloma cell lines (HMCLs), we show that 2-HG is produced in clonal PCs and is derived predominantly from glutamine anaplerosis into the TCA cycle. Furthermore, the 13C SIRM studies in HMCLs also demonstrate that glutamine is preferentially utilized by the TCA cycle compared with glucose. Finally, measuring the levels of 2-HG in the BM supernatant and peripheral blood plasma from patients with precursor PC malignancies such as smoldering MM (SMM) demonstrates that relatively elevated levels of 2-HG are associated with higher levels of c-MYC expression in the BM clonal PCs and with a subsequent shorter time to progression (TTP) to MM. Thus, measuring 2-HG levels in BM supernatant or peripheral blood plasma of SMM patients offers potential early identification of those patients at high risk of progression to MM, who could benefit from early therapeutic intervention.

P-387 Circulating metabolites, lipids, IGF axis and breast cancer risk: causal estimates from Mendelian randomisation.

PRESENTING AUTHOR: ***Vanessa Y. Tan, University of Bristol, United Kingdom***

CO-AUTHORS: ***Kalina M. Biernacka, Caroline Bull, Diana D. Ferreira, Charleen D. Adams, Robert C. Kaplan, Qi Qibin, Alexander Teumer, Claire M. Perks, the BCAC consortium, Jeff M.P. Holly, Nicholas J. Timpson***

Circulating metabolites are modifiable risk factors for breast cancer (BC) and it is possible for the metabolome to affect insulin-like growth factors (IGFs) and subsequently cancer risk. We use a combination of two-step Mendelian randomization (MR) undertaken using data from the IGF-I/IGFBP-3 (IGF-I:n=30,884;IGFBP-3:n=18,995) GWAS by the IGF working group of the CHARGE consortium, metabolite GWAS (n=24,925) by the Magnetic consortium, lipids GWAS (n=188,577) by the GLGC Consortium and BC risk GWAS (n=122,977cases;105,974controls) by the BCAC Consortium and observational analysis undertaken in the ProtecT study to (i) confirm anticipated associations between IGF-I/IGFBP-3 and BC; (ii) assess the causal relationship between the metabolome and BC; (iii) assess the relationship between the metabolome and IGF-I/IGF-II/IGFBP-2/IGFBP-3. MR analyses found that the OR for BC per SD unit increase in IGF-I and IGFBP-3 was 1.12 (95%CI:1.02,1.22) and 0.99 (95%CI:0.96,1.03), respectively. MR analyses indicated that BC risk was increased by HDL (OR:1.07;95% CI:1.01,1.14), LDL (OR:1.06;95%CI:1.01,1.12) and total-cholesterol-in-large-HDL (OR:1.08;95%CI:1.03,1.12) but reduced by triglycerides (OR:0.93;95%CI:0.86,1.00) and VLDL metabolites (OR:0.89;95%CI:0.86,0.93). Observational analyses indicated that the IGFs were associated with numerous metabolites. MR analyses found that a SD unit increase in triglycerides decreased IGF-I by 0.12SD (95%CI:-0.18,-0.06) and a SD increase in LDL increased IGFBP-3 by 0.08SD (95%CI:0.02,0.14). Findings here suggest a causal effect of elevated IGF-I and HDL on BC risk. For triglycerides, results suggest that the inverse association with BC risk has a coincident and predicted negative effect on IGF-I, implying that lipid moiety profile and relative abundance may be important for disease outcome.

P-388 High-content metabolomics screening of the prostate tumor induced by natural compound library using direct-infusion mass spectrometry

PRESENTING AUTHOR: ***Xiyuan Lu, The University of Texas at Austin, United States***

CO-AUTHORS: ***Achinto Saha, Alessia Lodi, John DiGiovanni, Stefano Tiziani***

Natural products are well known as acting on multiple targets in vivo to induce pharmacodynamic responses. The high failure rate of preclinical reagents entering the clinical trialshighlights the weak understanding of the network pharmacology. The new disciplines of high-content metabolomics screening will offer a cost-effective and comprehensive in vitro assay, facilitating the investigation of compound combination mechanism, and limiting the drug failure during further development. In this study, a natural product library of 150 compounds was screened on cultured mouse prostate tumor cells from HiMyc mice (HMVP-2 cells) to identify synergistic combinations of natural compounds effective in prostate cancer cells. High-throughput ATP bioluminescence measurements were performed as a primary screen to identify the top combinatory treatments. Based on the outcome of this primary screen, combinations of ursolic acid with 21 natural compounds in the library were selected as top hits and further evaluated using high-content isotope metabolomic analyses. During treatment, cells were cultured with stable isotopically labeled nutrients (e.g. 13C5, 15N2-glutamine, 13C6-glucose) to monitor the metabolic fate of these nutrients. High-resolution mass spectrometry coupled with nanoelectrospray direct-infusioninstrumentation was used for the large-scale high-content untargeted profiling of both intra- and extra-cellular metabolites in prostate cancer cells after the combination treatments. By investigating the dysregulated fractional distribution of the labeled isotopologues, we are able to elucidate the metabolic shifts and perturbations occurring upon combined treatments. Overall, this approach can be widely utilized for the exploration of synergistic combinations of agents for chemopreventive and therapeutic interventions.

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CANCER

P-389 Multiplatform urinary metabolomic profiling to discriminate cachexia and pre-cachexia in colorectal cancer patients: Results from the ColoCare Study

PRESENTING AUTHOR: *Jennifer Ose, Huntsman Cancer Institute, United States*

CO-AUTHORS: *Tengda Lin,David Liesenfeld, Jürgen Böhm, Biljana Gigic, Johanna Nattenmüller, Lin Zielske, Petra Schrotz-King, Nina Habermann, Martin Schneider, Alexis Ulrich, Hans-Ulrich Kauczor, Cornelia M. Ulrich*

BACKGROUND: Cachexia is a multifactorial metabolic syndrome with high morbidity and mortality. The precise molecular mechanisms and key biological pathways involved in cachexia remain poorly characterized. This study aims to investigate urinary metabolic profiles in pre-cachectic and cachectic colorectal cancer patients. METHODS: Urine samples from n=52 newly diagnosed colorectal cancer patients (stage I-IV) recruited from the ColoCare Consortium site in Heidelberg, Germany, were collected prior to surgery and at 6- and 12-month follow-up. Samples were analyzed using state of the art GC-MS and 1H-NMR. Patients were classified as cachectic (n=16) pre-cachectic (n=13) or non-cachectic (n=23) based on standard criteria on weight loss over time. We calculated differences in metabolites across groups, performed orthogonal projections to latent structures discriminant analysis as well as metabolite enrichment analysis. RESULTS: We detected 152 features with GC-MS and 154 features with 1H-NMR, respectively. Thirty-six compounds were significantly different across the three groups. Of these, 18 compounds could be identified, including glycerol and glycine. We observed significantly lower levels in cachectic patients compared to pre-cachectic patients for hydroquinone (Fold Change (FC)=2.3, p=0.006), glycine (FC=1.1, p=0.004) and several glucuronides. These metabolites were discriminating between (1) cachexia and pre-cachexia and (2) pre-cachexia and non-cachexia. Metabolite set enrichment analysis revealed glycerol phosphate shuttle metabolism and glycerol lipid metabolism as the two pathways with enriched differences between cachectic and pre-cachectic patients. CONCLUSIONS: Multiplatform metabolic profiling of urinary samples from colorectal cancer patients revealed differences in glycerol phosphate and glycerol lipid pathways in cachectic, pre-cachectic and non-cachectic colorectal cancer patients.

P-390 Prospective metabolomics study identifies potential novel blood metabolites associated with pancreatic cancer risk

PRESENTING AUTHOR: *Xiang Shu, Vanderbilt University Medical Center, United States*

CO-AUTHORS: *Xiang Shu, Wei Zheng, Danxia Yu, Hong-Lan Li, Qing Lan, Gong Yang, Hui Cai, Xiao Ma, Nathaniel Rothman, Yu-Tang Gao, Wei Jia, Yong-Bing Xiang, Xiao-Ou Shu*

Using a metabolomics approach, we systematically searched for circulating metabolite biomarkers for pancreatic cancer risk in a case-control study nested within two prospective Shanghai cohorts. Included in this study were 226 incident pancreatic cancer cases and their individually-matched controls. Untargeted mass spectrometry platforms were used to measure metabolites in blood samples collected prior to cancer diagnosis. Conditional logistic regression was performed to assess the associations of metabolites with pancreatic cancer risk. We identified 10 metabolites associated with pancreatic cancer, after accounting for multiple comparisons (the Benjamini-Hochberg false discovery rate < 0.05). The majority of the identified metabolites were glycerophospholipids (ORs per SD increase: 0.44 to 2.32; p values: 7.2×10-4 to 1.0×10-6), six of which were associated with decreased risk and one with increased risk. Additionally, levels of coumarin (OR = 1.96, p = 3.7×10-6) and picolinic acid (OR = 2.53, p = 5.0×10-5) were positively associated with pancreatic cancer risk, while tetracosanoic acid was inversely associated with risk (OR: 0.48, p = 7.16×10-7). Four metabolites remained statistically significant after mutual adjustment. This study provides novel evidence that the dysregulation of glycerophospholipids may play an important role in pancreatic cancer development.

P-392 Gradient Boosting Feature Selection and Classification of Metabolomic Signatures in Urine from Renal Cell Carcinoma (RCC) Patients.

PRESENTING AUTHOR: *David A. Gaul, Georgia Institute of Technology, United States*

CO-AUTHORS: *Harsh Shrivastava, Srinivas Aluru, Olatomiwa Bifarin, Arthur S. Edison, Rebecca S. Arnold, John A. Petros, Facundo M. Fernández*

Typical discriminant models used in metabolomics emphasize the common elements among sample groups to drive importance of features. Noise, variation and bias within sample groups inversely affect these models' performances. Often, outliers are simply removed to boost performance, but valuable variance may be lost that reduces the model's robustness. A random forest model is an ensemble of independent decision trees that reduce error by reducing variance. Gradient boosting is a collection of sequential decision trees, where each tree focuses on the shortcomings of the previous tree. Our strategy incorporates both approaches to handle overfitting while reducing variance and bias to shorten the time and iterations needed to optimize the prediction of RCC from metabolomics signatures derived from UPLC-MS analysis of urine samples. Using orthogonal partial least squares discriminant analysis, a model built using 7097 features with random subset cross validation produced an area under the curve (AUC) of 0.8854. A model based on bagging and a gradient boost strategy with five-fold cross validation reached an overall AUC of 0.9931. This dataset is a typical case of a "large p, small n" problem and requires variable selection to achieve a more acceptable metabolite panel. Simple extraction of most useful features from an oPLS-DA model rarely maintains predictive performance when used alone; however, our gradient boosting approach allowed extraction of 34 features critical for classification, with those features maintaining high predictive performance. Gradient boost machine learning shows great promise for selecting robust biomarkers for detection of RCC using a patient's urine sample.

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CANCER

P-393 Using liquid chromatography-mass spectrometry in combination with PCI-IS strategy to analyze biogenic amines in urine and its application in investigating chemotherapeutic response to breast cancer patients

PRESENTING AUTHOR: *Ching-Hua Kuo, School of Pharmacy, College of Medicine, National Taiwan University, Taiwan*

CO-AUTHORS: *Ya-Lin Hsu, Han-Chun Kuo, Hsiao-Wei Liao, Ching-Hung Lin*

Breast cancer is one of the most common cancers observed among women, and its associated death rate has been on the rise. Polyamine metabolism has been indicated to be dysregulated in cancer cells. This study used a postcolumn infused-internal standard (PCI-IS) strategy to analyze polyamines for investigating potential markers in evaluation of chemotherapeutic response in breast cancer patients. Fourteen biogenic amines were selected as the target analytes, and they were derivatized with dansyl chloride to improve their detection sensitivity. The dansylated putrescine-d6 was utilized as a universal PCI-IS for the 14 target amine, and it effectively calibrated the errors caused by matrix effect in urine samples. The developed method was applied to analyze urine samples collected from 60 breast cancer patients at three time points before and during the chemotherapy process. It was found that N1,N12 diacetylspermine (N1N12) and N1, N8-diacetylspermine (N1N8) in the responder group (n=43) were significantly higher ($P<0.05$) than the nonresponder group (n=17) prior to the chemotherapy. An increase of N-acetylputrescine (NAP) and a decrease of N1N12 were also observed in the responder group after chemotherapy, and this change was assumed to be caused by the inhibition of polyamine biosynthesis after receiving chemotherapy. Although further studies with larger validation cohort are required to confirm this finding, this study provided valuable information for future studies in elucidating the roles of polyamines in cancer treatment.

P-394 Investigating the effects of dietary and physical activity interventions on the metabolome of men with prostate cancer: The PrEvENT randomised controlled trial.

PRESENTING AUTHOR: *Meda Sandu, University of Bristol, United Kingdom*

Background Lycopene, plant-based diets(PBD) and physical activity(PA) have been previously associated with reduced risk and slower progression of prostate cancer(PC), however, the potential mechanisms are not completely understood. Methods We explored the effects of a randomised controlled trial with a 6-month dietary (lycopene supplementation and PBD advice) and brisk walking(BW) intervention on 155 serum metabolites in 74 men with PC who had undergone prostatectomy using linear regression and instrumental analysis(IV). The causal effect of the metabolites on PC was assessed by Mendelian Randomization(MR) on 44,825 cancer cases and 27,904 controls in the PRACTICAL consortium. Results The effects of lycopene supplementation and PBD advice on the serum metabolic profile were comparable(R2=.64). There were no strong differences in metabolite levels in either the BW or the dietary intervention before adjustment for baseline metabolites. After adjustment, there was evidence for decreases of triglycerides in intermediate-density lipoproteins(IDL), large, medium and small low-density lipoproteins(LDL) and saturated fatty acids($p<0.00385$) in the BW arm. When accounting for the effect of the dietary intervention on serum lycopene (IV analysis), pyruvate decreased, and acetate increased (p-value<0.05). In MR analysis using genetic instruments, there was evidence for a causal effect of pyruvate on PC (OR 1.29, 95% CI 1.03-1.62). Conclusion The interventions to increase lycopene, PBD and PA altered the serum metabolome of men with PC. BW improved the cardiometabolic profile, and lycopene and PBD advice lowered the levels of pyruvate, which is known to be involved in cancer mechanisms and had an increased risk of PC in MR analysis.

P-395 Discovery of Potential Biomarkers for Renal Cell Carcinoma via Urine NMR Metabolomics

PRESENTING AUTHOR: *Olatomiwa O. Bifarin, University of Georgia, United States*

CO-AUTHORS: *Fariba Tayyari, Goncalo Gouveia, David A. Gaul, Rebecca Arnold, John A. Petros, Facundo M. Fernández, Arthur S. Edison*

The best chance of curing Renal Cell Carcinoma (RCC) is in the early diagnosis, primarily because it is characterized by an asymptomatic progress. Currently, RCC is identified through cross-sectional imaging, followed by renal mass biopsy, which is invasive and riddled with sampling errors. Hence, the need for a non-invasive diagnosis method. Given that RCC is a disease of altered cellular metabolism and the proximity of urine to the kidney, we studied the metabolic profile of RCC patients using urine NMR based metabolomics.There were 106 patients (cases) and 179 controls in the study. Untargeted metabolic profiling was carried out using both 1D and 2D NMR experiments: 1D 1H NOESY, J-RES, HSQC, and HSQC-TOCSY. Our methodology includes multivariate analysis, statistical total correlation spectroscopy (STOCSY), and COLMARm data matches to databases of standards via Human Metabolome Database (HMDB), and Biological Magnetic Resonance Bank (BMRB). Multivariate analysis revealed NMR feature differences between controls and RCC patients. PLS-DA discriminated between controls and RCC (Q2: 0.58 R2: 0.86), and controls vs other types of RCC (Q2: 0.58 R2: 0.87). We are currently identifying discriminating metabolites between controls and RCC patients.In conclusion, it appears that a metabolic profile of RCC patients' urine could be developed as a diagnosis method for the detection of RCC.

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POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

CANCER

P-396 Targeted plasma metabolite profiling to identify markers for colorectal cancer detection

PRESENTING AUTHOR: ***Stefanie Brezina, Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria***

CO-AUTHORS: ***Andreas Baierl, Anne J.M.R. Geijsen, Eline van Roekel, Pekka Keski-Rahkonen, David Achaintre, Martijn Bours, Fränzel J.B. van Duijnhoven, Biljana Gigic, Tanja Gumpenberger, Andreana N. Holowatyj, Dieuwertje E.G. Kok, Annaleen Koole, Arve Ulvik, Nivonirina Robinot, Jennifer Ose, Alexis B. Ulrich, Per Magne Ueland, Ellen Kampman, Matty Weijenberg, Nina Habermann, Augustin Scalbert, Cornelia M. Ulrich, Andrea Gsur***

BACKGROUND. Colorectal cancer is a major public health concern worldwide, remaining the third leading cause of cancer-associated mortality. Novel blood-based biomarkers for colorectal cancer detection and risk prediction could complement existing screening methods. Metabolomics is a powerful approach to unravel metabolic changes associated with carcinogenesis and is increasingly applied as the method of choice for biomarker discovery. METHODS. Targeted metabolomics analysis was performed on plasma samples from 669 newly diagnosed colorectal cancer patients, and 628 cancer-free controls, using mass spectrometry and the Biocrates AbsoluteIDQ p180 Kit, covering 188 endogenous metabolites. Samples included in this study derive from five colorectal cancer cohorts embedded in the European MetaboCCC consortium aiming to investigate metabolic profiles across the continuum of colorectal carcinogenesis. We applied a comprehensive data analysis strategy establishing and validating a random effects model using an independent discovery (518 cases / 382 controls) and validation data set (151 cases / 246 controls). RESULTS. Data on 131 metabolites were applicable for further analysis in all study samples. The established prediction model comprised a panel of five metabolites (serotonin, glutamate, trans-4-Hydroxyproline, lysophosphatidylcholine(18:0), C22:2-OH sphingomyelin) able to differentiate colorectal cancer patients and cancer-free controls. The model obtaining an area under the curve of 0.82 in the validation set. CONCLUSION. Our findings suggest that blood-based metabolic markers have the potential to be utilized as non-invasive clinical markers for colorectal cancer detection and may offer a promising diagnostic tool to complement existing screening strategies.

P-397 Monitoring Lipid Droplet Formation Induced by Apoptosis and Necrosis by HRMAS 1H NMR Spectroscopy

PRESENTING AUTHOR: ***Marta Wylot, University of Cambridge, United Kingdom***

CO-AUTHORS: ***David Whittaker, Steven AC Wren, Les Hughes, Jules L Griffin***

Apoptosis has a major role in cancer and understanding its regulation may trigger new therapeutic strategies. The accumulation of lipid droplets (LDs) in cells that follow apoptosis has been previously reported but it has not been mechanistically explained. In this project, High Resolution Magic Angle Spinning (HRMAS)1H Nuclear Magnetic Resonance (NMR) spectroscopy-based metabolomics was used to monitor abnormal formation of LDs in C2C12 myotubes exposed to apoptotic and necrotic agents. Lipid profiles of C2C12 myotubes were investigated under five treatment conditions: 1) control, 2) cisplatin (apoptosis), 3) etoposide (apoptosis), 4) hydrogen peroxide (apoptosis + necrosis), 5) hot media (necrosis). Diffusion rates of LDs and T2 relaxation times were also investigated. Flow cytometry (Annexin V) and caspase activity assays were used to confirm the induction of apoptosis and/or necrosis. HRMAS 1H NMR spectra showed the highest lipid concentration for the peroxide exposed cells that were 40% apoptotic. The lowest lipid concentration was observed for the heat exposed cells that were 33% necrotic. LDs in necrotic cells (hot) diffused slower than LDs in apoptotic cells. Faster diffusion in apoptotic cells may be related to the smaller size of LDs, the changes in cytoplasmic viscosity and/or damaged cytoskeletal structures that direct the LD movement. Similarly, T2 relaxation times of LDs in apoptotic cells were faster than in necrotic cells implying changes in micro compartment size. In conclusion, the results showed that apoptotic and necrotic cells could be discriminated by HRMAS NMR Spectroscopy and the NMR results correlated well with molecular biology.

P-398 Investigating lactate metabolism in cancer cells using stable isotope labelling, metabolomics and an extracellular oxygen probe

PRESENTING AUTHOR: ***Emily Barnes, Imperial College London, United Kingdom***

CO-AUTHORS: ***Anke Nijhuis, Adrian Benito, Hector C Keun***

Background: Conditions under which cancer cells switch from aerobic glycolysis to consume lactate andto the extent lactate can contribute to growth and survival is unclear. Methods: We characterised HCT116, LS174T and LS174TMCT4-/- (deficient in the lactate transporter MCT4, Marchiq et al. Cancer Res 2015) cancer cell lines using the MitoXpress® Xtra Oxygen Consumption Assay (Luxcel Biosciences), 1H NMR spectroscopy and GC-MS with stable isotope labelling under differing nutrient availability. Results: NMR analysis of culture media confirmed lactate consumption under conditions of glucose and glutamine deprivation. Survival under these conditions was conditional on MCT-mediated uptake of lactate as demonstrated by the sensitivity of LS174TMCT4-/- cells to the MCT1 inhibitor AZD3965. Glutamine alone could also maintain survival, and in combination with lactate growth could be maintained under glucose deprivation. The addition of lactate significantly enhanced oxygen consumption rate (OCR) and the capacity for respiration under FCCP-decoupled conditions. Tracing the fate of U-13C-lactate in HCT116 cells, we observed that lactate entry into the TCA cycle was primarily through PDH flux. Interestingly, we observed 13C labelling in PEP from glutamine but not lactate under glucose deprivation suggesting that glutamine carbon was being used for gluconeogenesis. Conclusions: Lactate or glutamineis sufficient for survival under glucose deprivation in the cells examined, but both are required for growth under the same conditions. Further, our data suggests that lactate increases oxidation capacity, whereas glutamine is required for anabolism and anaplerosis. Acknowledgements:MetaCell-TM H2020 FastTrack to Innovation(737978) and Cancer Research UK Imperial Centre(C17375/ A19482).

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CANCER

P-399 Early detection of prostate cancer by using proton nuclear magnetic resonance spectroscopy

PRESENTING AUTHOR: ***M. Arjmand, Metabolomics Lab, Pasteur Institute of Iran, Iran***

CO-AUTHORS: ***Z. Akbari, M. Abdolalipour, Z. Zamani, R. Haj_Hossiani***

Introduction: Prostate cancer is the second leading causes of death among men over the age of forty years. Early diagnosis and appropriate clinical managements are considered to be important for its successful treatment. Metabolomics is a new approach for early detection of diseases with discovery of a new biomarkers and metabolic profile fingerprint modeling. Using 1H NMR spectroscopy and feed forward neural network modeling it is possible to detect and predict the disease. In this investigation, we examined the chance of neural network modeling as tools in early diagnosing this disease. Material and methods: In this study serum from 50 men with prostate cancer and 50 healthy males with same range of age were collected. 1 H NMR spectroscopy with CPMG protocol were recorded by Bruker 400 MHZ and data were processed and analyzed. Feed forward neural networking was run with seventy percent of samples. Model was tested with other thirty percent of samples. ROC test was also run to find out the robustness of this model. Results and discussion: Analyzing the data showed that there was variation in metabolite concentration and biochemical pathways in both healthy and cancer patients. Using ROC test showed 83% of sensitivity in discrimination of two groups with 100 metabolites and 0.2875 error rate by using artificial neural network. Thirty-one biochemical pathways showed changes but the major one was seen in the aminoacyl tRNA biosynthesis, nitrogen metabolism, arginine and proline metabolism, alanine, aspartate glutamate metabolism.

P-400 Metabolome profile comparison of cisplatin sensitive and resistance in ovarian epithelial cells (A2780S & A2780CP) by NMR spectroscopy

PRESENTING AUTHOR: ***Ziba Akbari, Metabolomics Lab, Pasteur Institute of Iran, Iran***

CO-AUTHORS: ***M. Arjmand, A. Mellati, A. Amanzadeh***

Introduction: Epithelial ovarian carcinomas accounts for more than 85% of ovarian carcinomas. The chemotherapeutical treatment of choice is cisplatin. However, long-term use of this drug mostly results in drug resistance phenomenon. Proton nuclear magnetic resonance spectroscopy (1H-NMR) is non-invasive and high reproducible technique used in metabolomics. In the present investigation, we tried to find out biochemical pathways and its metabolites alterations in epithelial cells of ovarian carcinoma and study the mechanism involved in cisplatin drug resistance. Materials and Methods: The cell lines A2780 and A2780CP were cultured. Cell metabolites extraction was performed and 1H-NMR spectroscopy were applied on a Bruker spectrometer operating at 400 MHz. After processing the data, outlier metabolites were identified and their biochemical pathways were worked out by Metaboanalyst (a web server for metabolomics data analysis and interpretation) and Human Metabolome Database (HMDB). Results: Our finding indicated that cisplatin drug resistance results in decreases in lipophilic metabolites such as progesterone, aldosterone, 2-Methoxyestradiol, sphingosine and sphingosine, oleic acid in cell lines A2780 and A2780CP. Meanwhile, hydrophilic metabolites such as galactonite, mannose, sorbitol, fucose were showed increases and mannitol and glucuronate registered increases in these cells. Conclusion: Significant alteration in cell lines A2780 and A2780CP metabolome profile and changes in steroid hormones, sphingolipids, fatty acid, amino sugar, galactose, fructose and mannose metabolome pattern probably were resulted by Cisplatin drug resistance in ovarian carcinomaand hence require further investigation to confirm these valuable findings.

P-401 Fructose metabolic priming in the regulation of breast cell reprogramming and 3D growth

PRESENTING AUTHOR: ***Fionnuala Morrish, Fred Hutchinson Cancer Research Center, United States***

CO-AUTHORS: ***Li Huang, Laura Gaydos, Martin Sadilek, David Hockenbery***

Altered cellular metabolism is one of the “hallmarks” of cancer. Substrate supply and partitioning within cellular metabolic networks are regulated by oncogenes but excessive supply of certain nutrients, in particular sugars, may facilitate cellular reprogramming and the development of a cancerous state. Here we present the effects of fructose on glucose uptake, cell growth in 3D, epithelial to mesenchymal transition (EMT), and cell dormancy in breast cancer cells expressing the fructose transporter, Glut5. Mechanistic studies include gene, transcription factor and metabolomic analysis with 13C1,2 fructose and 13C1,2 glucose isotope flux measurements. The data indicate the presence of fructose stimulates the uptake of glucose, EMT transition, cell growth and recovery from dormancy in 3D, the expression of metabolic genes and the flux of glucose in metabolic circuits. In particular, the production of glucose-derived TCA intermediates, and serine, glutamate and alanine are influenced by the presence of fructose. The transcription factor ChREBP/MLXIPL is activated by fructose and studies are underway to determine the contribution of this factor to the alterations in glucose metabolism and cell fate induced by fructose supplementation in breast cancer cells. Together these studies provide a foundation for future work to explore the effects of inhibiting fructose uptake or metabolism on tumor growth.

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CANCER

P-402 Cancer and Chemotherapy-Induced Cachexia Yield Distinct Metabolic Perturbations

PRESENTING AUTHOR: ***Fabrizio Pin, Department of Anatomy and Cell Biology, Indiana University School of Medicine, United States***

CO-AUTHORS: ***Rafael Barreto, Marion E. Couch, Andrea Bonetto, Thomas M. O'Connell***

Cancer patients frequently suffer from cachexia, a condition characterized by muscle and adipose tissue wasting. Chemotherapy, one of the primary treatment options for cancer can leads to cachexia. In this study, we elucidate the metabolic perturbations of these two drivers of cachexia. Our study included four experimental groups: (1) vehicle, (2) Folfiri-treated mice (5-FU, leucovorin, irinotecan), C26 tumor-bearing animals (3) treated or (4) not with Folfiri. The multi-platform metabolomics approach utilized both NMR and mass spectrometry analyses on serum, muscle and liver tissue. The data showed differences between the metabolic phenotypes of the C26 and chemotherapy groups. The C26 group displayed reduced levels of TCA cycle intermediates, branched chain amino acids and short chain acylcarnitines while the same were unchanged in the Folfiri group. The number of low density lipoprotein particles (LDL-P) increased by more than five-fold in the C26 group while a more modest two-fold increase was observed in the Folfiri group suggesting differences in lipid and cholesterol metabolism. In muscle, the ATP levels were more significantly reduced in the Folfiri group compared with the C26 suggesting a more dramatic impact on oxidative metabolism. In the C26 group treated with Folfiri, the effects of each treatment were sometimes additive and sometimes appeared to be dominated by one condition over the other. Overall, our results show that cancer- and chemotherapy-induced cachexia are characterized by different metabolic perturbations indicating different pathophysiological mechanisms. New therapeutic interventions to treat cachexia will have to account for these differences in order to provide effective treatment.

P-403 Development of a Metabolic Biomarker Panel for the Early Detection of Cancer Cachexia

PRESENTING AUTHOR: ***Thomas OConnell, Indiana University School of Medicine, United States***

CO-AUTHORS: ***Fabrizio Pin, Rafael Barreto, Marion E. Couch, Andrea Bonetto***

Currently there are no mechanism based tools for the early detection & diagnosis of cachexia, a debilitating condition that typically progresses through three phases, namely pre-cachexia, cachexia and refractory cachexia. Unfortunately the classically emaciated patient is often in the refractory stage where the decline is irreversible. In this study we employed a comprehensive, multi-platform metabolomics including untargeted high field NMR, targeted LC/MS and NMR-based lipoprotein analysis. Our study design involved implanting mice s.c. with C26 colorectal tumor cells and sampling serum and muscle tissue every other day for two weeks. In this model the mice generally develop significant weight loss starting day 10. The metabolomics analyses of serum revealed that the trajectories of several biomarkers, among which the branched-chain amino acids (BCAAs), significantly diverged from controls in advance of detectable weight loss. In particular, leucine levels significantly dropped by day 6, well in advance of detectable weight loss and even in advance of a palpable tumor. Other metabolic markers that diverged at or before day 10 include low density lipoprotein particles, taurine and GlycA (an inflammatory marker associated with acute phase response). These initial biomarkers represent a set of critically altered pathways in the development of cancer cachexia including protein catabolism, lipolysis/dyslipidemia, oxidative stress and inflammation. This work provides the foundation for the development of a biomarker panel for the early detection and stratification of cachexia based upon a unique constellation of metabolic disturbances. Further studies will be conducted on different cancer models and eventually in human cohorts.

P-404 Metabolic Reprogramming with β-Alanine to Overcome Chemotherapy Resistance in Pancreatic Cancer

PRESENTING AUTHOR: ***Danny Yakoub, University of Miami, United States***

CO-AUTHORS: ***Smitha T.Totiger, Alexandra Morán, Sujit Suwal, Julio Pimentel, Lauren O'Donnell, Jamie Walls, Nipun Merchant, Alan Livingstone***

Introduction: Chemoresistance in Pancreatic Ductal Adenocarcinoma (PDAC) is partly mediated by the Warburg effect with tumor microenvironment acidification giving cancer cells a survival advantage and compromising intracellular drug delivery of weak base chemotherapeutic agents such as gemcitabine (Gem). We profiled over 40 metabolites using 1H-NMR spectroscopy of Gem sensitive(BXPC-3) and resistant(PANC-1) PDAC cell lines. In resistant cell lines, Gem treatment was associated with a significant decrease in the intracellular concentration of β-alanine (βA), unlike in sensitive cell lines, suggesting that βA may have a role in chemotherapy response. We aimed to evaluate the role of β-alanine supplementation in improving gemcitabine efficacy for the pancreatic cancer treatment. Results: βA supplementation in gemcitabine resistant cells resulted in: (1) decreased glycolytic and proton production rates as measured by basal extracellular acidification rates and oxygen consumption rate (measured by Seahorse analyzer); (2) decreased protons and lactic acid levels in the conditioned media resulting in increased pH; (3) suppression of genes which promote glycolysis (e.g. Aldolase-A, GAPDH, Enolase-2 and LDH-A)(assessed by Next Generation Sequencing). Additionally, βA supplementation enhanced cell sensitivity to gemcitabine treatment, as seen with decreased IC50 of Gem, decreased cell migration, proliferation and increased apoptosis (2.5 folds;P<0.05). Conclusion: We have demonstrated that βA supplementation reprograms cell metabolism via reversing the Warburg effect to a more normal metabolic phenotype; this results in enhancement of gemcitabine efficacy with decreased proliferation and increased apoptosis. Beta alanine may be considered for use as a co-therapeutic and metabolic enhancer in pancreatic cancer.

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CANCER

P-405 The Influence of Omega-3 Fatty Acid Supplementation on the Brain Lipidome After Chemotherapy

PRESENTING AUTHOR: ***Rachel E. Kopec, The Ohio State University, United States***

CO-AUTHORS: ***Melissa Solano, Tonya S. Orchard, A. Courtney DeVries, Rachel E. Kopec***

Background: Solid tumor chemotherapy produces long-term cognitive side effects well beyond treatment, but the structural changes on brain chemistry are unknown. A diet supplemented with omega-3 fatty acids (EPA+DHA) before and during chemotherapy partially protects the cerebral tissue against some of the chemo-induced modifications. We hypothesize that EPA+DHA supplementation results in a greater neuroinflammation-resolving response mediated by specialized pro-resolving mediators (SPMs i.e. omega-3 derived metabolites which attenuate inflammation), and reduces oxidation of structural brain lipids. Methods: For four weeks, ovariectomized mice were fed a 2% kcal EPA+DHA supplemented (n=60) or control diet (n=60), followed by two treatments with vehicle (n=30 per dietary group) or doxorubicin(n=30 per dietary group). Animals were sacrificed at 4, 7, and 14 days post-treatment, and samples extracted and purified with SPE. Targeted analyses (LC-MS/MS) were performed on extracts, using stable isotope internal standards for SPM quantitation (i.e. resolvin E1, D1, D2, D3, D5, maresin 1, protectin D1). Untargeted LC-HRMS metabolomics analyses were performed on the hippocampal extracts of follow-up set of animals, to determine changes in the brain lipidome Results: Resolvin D1 was quantifiable in all samples regardless of dietary or treatment group, and correlations were observed with orthogonal measures of inflammation in chemo-treated animals. Resolvin D3, maresin 1, and protectin D1 were detected in a subset of animals. Untargeted data analysis is ongoing and will be presented. Conclusions: The protective effects of EPA+DHA supplementation on chemo-induced cerebral damage appear to be only partially correlated with SPM synthesis over the time course observed.

RARE DISEASES

P-406 UPLC-MS/MS Analysis of Fabry Patients Gb3 Isoforms/Analogues in Unfractionated Leukocytes, B Lymphocytes and Monocytes

PRESENTING AUTHOR: ***Christiane Auray-Blais, Université de Sherbrooke, Canada***

CO-AUTHORS: ***Amanda Toupin, Pamela Lavoie, Marie-Françoise Arthus, Mona Abaoui, Michel Boutin, Carole Fortier, Claudia Ménard, Daniel G. Bichet***

Fabry diseaseis an X-linked lysosomal storage disorder caused by a deficiency in the alpha-galactosidase A enzyme activity, leading to storage of glycosphingolipids, such as globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3), in tissues and biological fluids. Recent metabolomic studies performed in our laboratory revealed different Gb3 isoforms/analogues as Fabry disease biomarkers. This study aimed at the development/validation of a novel UPLC-MS/MS method to perform a relative quantitation of Gb3 isoforms/analogues in unfractionated white blood cells, B lymphocytes and monocytes and to assess these biomarkers in a cohort of Fabry patients and healthy controls. Various correlations were established. Blood samples from 21 Fabry patients and 20 healthy controls were analyzed. Leukocytes, B lymphocytes and monocytes were isolated, and purified using different selection and depletion kits. Liquid-liquid extraction of glycosphingolipids was performed to investigate 4 groups of Gb3 isoforms/analogues: saturated, unsaturated with one-double bond, unsaturated with two-double bonds and methylated. A 20-minute chromatographic-run showed a good separation using a UPLC-Acquity coupled to a Xevo TQ-S mass spectrometer system (Waters Corp.). A positive electrospray ionization mode and a multiple reaction monitoring (MRM) signal acquisition were selected. Our results showed that some Gb3 isoforms/analogues in leukocytes were higher for Fabry males compared to control males. The severity of mutations in Fabry patients seemed to be related with the blood cell biomarker levels. Moreover, this study revealed the presence of methylated Gb3 isoforms/analogues at the blood cell level, which to our knowledge, has never been reported. This emphasizes the possible metabolic pathway between Gb3 and lyso-Gb3.

P-407 NMR Metabolomic Profiles of an Emerging Genetic Disease

PRESENTING AUTHOR: ***Houda BOUMAZA, Univ Lyon, CNRS, Université Claude Bernard Lyon 1, Ens de Lyon, Institut des Sciences Analytiques, France***

CO-AUTHORS: ***Suzy Markossian, Gilles Rautureau, Karine Gautier, Frédéric Flamant, Bénédicte Elena-Herrmann***

Resistance to thyroid hormone (RTHα) due to mutations in the thyroid hormone receptor alpha (TRα1), which is encoded by the THRA gene, is a recently discovered genetic disease. Patients present a high variability in clinical features (skeletal dysplasia, growth retardation, intellectual disability, etc...), and the absence of reliable biochemical markers make the diagnosis of this disease difficult. Since its first description in 2012, 31 cases of RTHα have been reported worldwide, corresponding to 20 different mutations of TRα1. Considering that the mouse Thra and human THRA genes display extensive sequence similarities, mouse lines with Thra mutations are highly relevant animal models for RTHα. We used CRISPR/Cas9 genome editing to introduce 4 different germline mutations in the mouse Thra gene.1 These animal models were used to test the capacity of 1H nuclear magnetic resonance (NMR) metabolomics to serve as a diagnostic tool for RTHα in humans. Urine and plasma samples from adult mice carrying these Thra mutations were analyzed by NMR with associated wild-type controls. Multivariate statistical analysis (OPLS-DA models) showed that samples collected from specific groups of mice carrying human-like mutations can be discriminated from control samples collected from wild-type littermates. Our results reveal the presence of changes in the metabotypes induced by Thra mutations, which provides a proof-of-principle that NMR metabolomics fingerprints can be used to diagnose RTHα in humans. References: 1. Markossian S, Guyot R, Richard S, Teixeira M, Aguilera N, Bouchet M, Plateroti, Guan W, Gauthier K, Aubert D, Flamant F Thyroid. 2018 Jan;28(1):139-150.

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RARE DISEASES

P-408 Metabolomic changes in oxidatively stressed iPSC derived hepatocyte-like cells

PRESENTING AUTHOR: ***Anna Artati, Helmholtz Zentrum Muenchen, Germany***

CO-AUTHORS: ***Alexander Cecil, Anwar Palakkan, Jyoti Nanda, James A. Ross, Jerzy Adamski***

Oxidative stress contributes to the initiation and progression of various liver diseases. Monitoring oxidative markers in hepatocytes offers more insights to study the diseases. However, due to limited availability of human hepatocytes, models used in the studies mostly rely on animal cells, which do not completely reflect the real human models. Recently human hepatocyte-like cells differentiated from induced-pluripotent stem cells (iPSC) have been gaining attention due to, among others, its potential for modeling drug metabolism in vitro. In this study, BCMNC iPSC lines, an iPSC derived hepatocyte-like cells were challenged with compounds which are known as oxidative stress causing agents, i.e., 80 µM potassium bromate (KBrO3), 2.5 µM Camptothecin (CPT) or 50 µM Acetaminophen. Cell lysates were harvested by scraping them with 80% methanolic extraction solvent. The culture supernatants were also collected for analysis. Metabolic changes were monitored and relatively quantified with UHPLC-MS/MS. The MS2 spectra data was matched to Metabolon's database library for metabolite identification. PLS-DA analysis of cell samples showed overlap of all oxidatively-stress treatments ($p < 0.05$) with the control group being separate, whereas PLS-DA analysis on supernatant samples yielded distinct clusters of each sample group ($p < 0.001$) corresponding to the treatments. Some metabolites involved in glycolysis, amino acids, lipids, peptides, cofactors and vitamins metabolism contribute to the clustering. Variables of importance indicate a shift of gamma-glutamyl metabolites from media into the cells. Biomarker derived from these analyses can be used to monitor metabolic changes during oxidative stress process in hepatocyte-like cells.

P-409 Plasma metabolomics of myalgic encephalomyelitis/chronic fatigue syndrome implicates redox imbalance in disease symptomology

PRESENTING AUTHOR: ***Arnaud Germain, Cornell University, United States***

CO-AUTHORS: ***David Ruppert, Susan Levine, Maureen Hanson***

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is an incurable disease of enigmatic origin. Its constellation of symptoms has silently ruined the lives of millions of people around the world. A plethora of hypotheses have been vainly investigated over the past few decades so that the biological basis of this debilitating condition remains a mystery. In this study, we investigated whether there is a disturbance in homeostasis of metabolic networks in plasma of a 32-patient cohort compared to 19 healthy controls. Extensive analysis of the concentrations of an 832-metabolite dataset generated by Metabolon®, covering eight biological classes, generated compelling insight into the underlying mechanisms of ME/CFS. We report on a dozen metabolites with significant difference in abundance, allowing us to develop a theory of broad redox imbalance in plasma of ME/CFS patients, which is consistent with findings of prior work in the ME/CFS field. Moreover, enrichment analysis exploration using www.MetaboAnalyst.ca returns compelling disease-associated metabolite sets in blood, while the biomarker analysis unit yielded prospective biomarkers for ME/CFS using blood testing. We cross-validated and compared our dataset to publicly available datasets from other teams who used different populations and equipment platforms, and found statistically similar behaviors of metabolites between studies. This work contributes key elements to development of ME/CFS diagnostics, a crucial step required for discovering a therapy for any disease of unknown origin.

P-410 Lipidomic Changes Associated with Knock-outs of the vraSR Two-Component Regulatory System in Methicillin-Resistant Staphylococcus aureus (MRSA)

PRESENTING AUTHOR: ***Tianwei Shen, University of Washington, United States***

CO-AUTHORS: ***Kelly M. Hines, Brian J. Werth, Libin Xu***

Methicillin-resistant Staphylococcus aureus (MRSA) is the leading cause of wound and hospital-acquired infections, and multidrug-resistant MRSA has emerged. Studies have shown that cell wall synthesis and lipid synthesis in S. aureus are metabolically closely connected. Since increase in cell wall thickness is commonly observed in the resistant strains, we hypothesized that upregulation of cell wall synthesis might lead to downregulation of fatty acid and lipid synthesis. The two-component regulatory system vraSR has been demonstrated to regulate cell wall synthesis. Because cell-wall thickening has been found in resistant strains containing vraTSR mutations, we sought to examine the changes to lipid metabolism when vraS or vraR is knocked out (KO) in a MRSA strain, JE2, via transposon insertion. Through a complete lipidomics study, we found that long-chain fatty acids and phosphatidylglycerols increased in the vraR KO, which might correlate with increased susceptibility to β-lactams. We also showed that the overall lipid content did not change significantly in either vraS or vraR KO. Furthermore, we quantified the resistance of the vraS or vraR KO against glycopeptides, lipopeptides, lipoglycopeptides and β-lactams by MIC measurement and population analysis profiling, and found that the KOs improved susceptibility to vancomycin and dalbavancin. These findings might indicate two things: 1) KO of the vraSR system is not sufficient to lead to lipid changes, but gain-of-function mutations might; or 2) the other regulatory system yycGF, which has also been shown to be involved in resistance in S. aureus, could be more important in governing lipid metabolism than vraSR.

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RARE DISEASES

P-411 Probing the Immunometabolic Regulatory Networks of Neutrophils Following Phagocytosis and Activation

PRESENTING AUTHOR: ***Wyatt Keegan, Montana State University, United States***

CO-AUTHORS: ***Kyler Pallister, Brian Eilers, Julie Anne Morgan, Amanda L. Fuchs, Brian P. Tripet, Jovanka Voyich, and Valérie Copié***

Neutrophils are the most abundant white blood cell in the human body and are one of the first responders to bacterial pathogens. Following phagocytosis of pathogens, neutrophils become activated and respond by generating reactive oxygen species (ROS), and inflammatory mediators. These immune functions are intimately connected to metabolic changes in the neutrophils, a re-emerging concept referred to as immunometabolism. However, studies investigating immunometabolism in human primary cells are currently limited. We hypothesize that Staphylococcus aureus takes advantage of the metabolic reprogramming of neutrophils to survive phagocytosis and evade killing. Supporting this idea is the published literature demonstrating the importance of metabolism in both host and pathogen. To establish “proof of principle” regarding the importance of the cross-talk between neutrophil immune function and metabolism, we have begun to investigate how neutrophil metabolic profiles correlate with their phenotypes. 1D 1H NMR spectra are currently being recorded on intracellular metabolite extracts of resting and activated neutrophils following both pathogen phagocytosis and phorbol myristate acetate (PMA) activation. Initial profiling of the intracellular metabolome using the ChenomxTM software and its corresponding small molecule library for 600 MHz (1H Larmor frequency) magnetic field strength NMR instruments suggests that intriguing changes in central metabolism occur, and support the notion that central metabolism is an important modulator of neutrophil immune cell function

P-412 Serum metabolite analysis of patients with Orientia tsutsugamushi infection

PRESENTING AUTHOR: ***Hyuk Chu, Korea National Institute of Health, South Korea***

CO-AUTHORS: ***Sangho Choi, Jae-yon Yu, Dongmin Kim, Geum-sook Hwang***

Scrub typhus (tsutsugamushi disease) is a tick-borne bacterial disease and one of the febrile diseases caused by Orientia tsutsugamushi in Korea and East Asia. Due to the high incidence rate in Korea, numerous research studies have been preferentially focused on the development of diagnostic tools and therapeutic agents for treating this disease. However, reports on the pathogenesis of O. tsutsugamushi in humans are few. In this study, we identified metabolic changes in the sera from patients with tsutsugamushi disease and other febrile diseases (non-tsutsugamushi diseases) to understand the metabolic pathways involved during infection. O. tsutsugamushi infections were confirmed by PCR using buffy coats from 125 patients who came to the hospital with febrile illnesses. All the samples were processed and subjected to nuclear magnetic resonance (NMR) spectroscopy to identify the metabolic changes. To identify metabolites that are differentially expressed between PCR-positive and -negative samples, we performed the Students t-test for parametric analysis. In addition, the Mann-Whitney U test was performed for non-parametric statistical analysis. Forty-eight tsutsugamushi-infected patients were confirmed from the 125 febrile patients. Metabolites that varied between the O. tsutsugamushi-positive and -negative sera included glutamate, isoleucine, leucine, valine, lactate, and lysine. Glutamate and branched-chain amino acids (BCAAs), which include isoleucine, leucine, and valine, were significantly lower in tsutsugamushi-positive patients. These results may provide a useful guide for understanding the metabolic pathways of tsutsugamushi disease and identifying distinct biomarkers of febrile diseases. This study was supported by a research grant (2018-NI002-00) of Korea Centers for Disease Control and Prevention.

P-413 Characterisation of the metabolic impact of a rare genetic variant within APOC3

PRESENTING AUTHOR: ***Laura J Corbin, MRC Integrative Epidemiology Unit at the University of Bristol, United Kingdom***

CO-AUTHORS: ***David A Hughes, Amy E. Taylor, Alix Groom, Catherine L. Winder, Andris Jankevics, Andrew Chetwynd, Warwick B Dunn, Nicholas J Timpson***

Apolipoprotein C-III (apoC-III) is a key regulator of plasma triglyceride levels, elevated levels of which are associated with a risk of adverse cardiovascular events. ApoC-III has been recognised as a potential therapeutic target with work ongoing in the area of both antisense inhibition and monoclonal antibody targeting of apoC-III. In this work, we exploit a rare loss of function variant in APOC3 (rs138326449) to characterise the likely on-target and potential off-target effects of targeting apoC-III directly. In the first phase of this study (Drenos et al. 2016), a high-throughput serum NMR metabolomics platform was used to quantify 225 metabolic measures in 13,285 participants from two European population cohorts. Eighty-one metabolic measures showed evidence of association with APOC3(rs138326449). In the second phase, stored plasma samples were mobilised from one of the cohorts in a recall-by-genotype study design where all available samples from carriers of the rare variant APOC3(rs138326449) along with samples from age and sex-matched controls were analysed by UPLC-MS to acquire non-targeted metabolomics data. Results from the analysis of 63 fasted samples from 57 unique individuals revealed that changes in lipid metabolism are most prevalent, specifically in the lipid classes of ceramides and glycerophospholipids. The next step in the analysis will see the incorporation of a further 52 samples. To summarize, we were able to refine the biological signature of APOC3(rs138326449) by a combination of metabolomics approaches. Our results illustrate the possible use of such approaches as a relatively quick and low-cost tool in the evaluation of drug targets.

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POSTER SESSIONS 3 AND 4 – **Wednesday and Thursday –** all even number presenters will be at their posters.

RARE DISEASES

P-414 Untargeted urine NMR metabolomic profiling of Zika virus-infected pregnant women

PRESENTING AUTHOR: ***Sicong Zhang, The University of Georgia, United States***

CO-AUTHORS: ***Jacquelyn M. Walejko, Maria T. Arévalo, Ted M. Ross, Arthur S. Edison***

Zika virus infection in pregnant women can lead to stillbirth and increased incidence of fetal encephalopathy. Despite the popularity of studies in this area recently, there is currently still no sufficient treatment for it. We conducted an untargeted metabolomics study on Zika virus infected versus non-infected mothers to aid in etiological mechanism understanding and in vitro diagnostic biomarker discovery. 275 pregnant women from Puerto Rico were recruited in an IRB approved study. An NMR metabolomics study was conducted using urine samples collected up to 6 monthly time-points throughout pregnancy from 10 presumptive Zika-positive women and 10 controls matched for gestational age. At least 4 women were infected during pregnancy, after study entry. Thirty-eight features exhibited significant differences in intensity between the two groups. Furthermore, orthogonal signal corrected-partial least squares discriminant analysis (OSC-PLSDA) produced a good model (R2=0.3388; Q2=0.7570) and indicated several metabolites discriminating Zika-positive from Zika-negative subjects. Trajectories of these discriminating features will be evaluated as a function of gestational age for each study participant to understand the time-course of infection. The identification of more metabolites will help us identify pathways involved in disease progression, ultimately leading to a better understanding of mechanisms that lead to poor fetal outcomes.

P-415 Metabolomics for finding potential biomarkers in serum samples for the diagnosis of Behcet's disease

PRESENTING AUTHOR: ***Yu Eun Cheong, Korea University, Korea, South***

CO-AUTHORS: ***Jungyeon Kim, Kyoung Heon Kim***

Currently, there is a lack of available biomarkers to diagnose Behcet's disease (BD). In this study, metabolomics was performed to identify potential biomarkers in sera for the reliable diagnosis of BD. A total of 104 metabolites were identified from 45 BD patients and 45 healthy controls (HC) using gas chromatography with time-of-flight mass spectrometry (GC/TOF–MS). A partial least squares discriminant-analysis (PLS-DA) model using 104 metabolites was employed to discriminate metabolite profiles of BD patients from HC. Based on univariate analyses, a total of 13 metabolites were suggested as potential biomarkers for the diagnosis of BD. To suggest a diagnosis panel using multiple biomarkers, a PLS-DA model was created by using decanoic acid, fructose, tagatose, linoleic acid, and oleic acid. This PLS-DA model was validated by using a permutation test, an receiver operating characteristic (ROC) curve, and external samples. Based on a metabolite set enrichment analysis (MSEA), significant perturbation in linoleic and oleic acid metabolism and other fatty acid metabolic pathways, was observed. This is the first report of metabolite profiles and potential metabolite biomarkers in sera for the reliable diagnosis of BD using GC/TOF–MS.

P-416 Serum Metabolome Changes in Zika and Dengue virus infection reveals potential targets for assertive clinical diagnosis

PRESENTING AUTHOR: ***Fabio Neves dos Santos, University of Campinas, Brazil***

CO-AUTHORS: ***Aline Maria Araújo Martins, Kelly Grace Magalhães, Marcos Nogueira Eberlin.***

Introduction: Zika (ZKV) and Dengue (DENV) Virus have been a major public health challenge in the world due to large number of underreporting, inaccurate and false diagnoses. The metabolomics-based method can detect biomarkers of the physiological variations at all stages of the infection and immunological responses. Objectives: Herein we showed that metabolomics-based mass spectrometry can be applied for molecular diagnostic of Zika and Dengue and co-infection of infected serum. Material and Methods: The serum samples were separated in four groups through ELISA (IgG and IgM) protocol: 30 ZKV, 30 DENV and 30 controls. The method of metabolites extraction from 50 µL serum infected was Bligh-Dyer. The upper phase was collected for metabolites analysis and 1 µL was analyzed using an Agilent 1290 Infinit LC-MS system coupled with an Agilent 6550 Q-TOF/MS mass spectrometer equipped with an electrospray source, in positive and negative modes. The chromatograms were processed by XCMS software and multivariate analysis (PLS-DA) was applied to classify the serum samples according to the type of virus infection. Results and Discussion: The serum infected by ZKV and DENV were clearly distinguishable into two clusters by PSL-DA plot. The majors metabolites identified as responsible for the differentiation of the two clusters, by variable importance in projection (VIP plot), were phosphatidylcholine (PC), lysophosphatidylcholine (LysoPC), acylcarnitine, amino acids and derivatives. Angiotensin (1-7) and Angiotensin I were upregulated only under infection of the ZKV. Conclusions: The metabolites identified allude to possible novel diagnostic targets and advance our understanding on the mechanisms of arbovirus pathogenesis.

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RARE DISEASES

P-417 HUMAN BABESIOSIS: COMPARATIVE MULTIPLATFORM METABOLOMIC FOOTPRINTING AND FINGERPRINTING OF AN IN VITRO DISEASE MODEL

PRESENTING AUTHOR: ***Luka Andrisic, Universidad SAN PABLO CEU, Croatia***

CO-AUTHORS: ***Luka Andrisic, Miguel Fernandez-Garcia, Luis Miguel Gonzales, Fernanda Rey-Stolle, Estanislao Nistal, David Rojo, Elena Sevilla, Coral Barbas, Estrella Montero, Antonia Garcia***

Babesiosis is a tick-borne hemolytic malaria-like disease caused by protozoan parasites from the genus Babesia (Phylum Apicomplexa, Family Babesiidae). From over 100 species ofBabesia, human babesiosis is primarily caused by B. microti, B. duncani andB. divergens [1]. Upon human infection, this parasite progresses through different intraerythrocytic morphological stages. Ultimately, merozoites, the infective forms, are released from erythrocytes. The clinical manifestations range from asymptomatic to mild-moderate in immunocompetent patients, although severe symptoms can be observed in asplenic and immunocompromised patients, children and elderly. B. divergens infections have the most severe symptoms with much higher fatality rate (42%) [2]. In this study, our goal was to determine the footprint of target metabolites which were found significant in a previous untargeted, multiplatform GC-QTOF-MS, CE-TOF-MS and LC-QTOF-MS fingerprinting study of an in vitro model of B. divergens-infected erythrocytes (iRBC). We obtained a comparative profile of the metabolic changes found in both iRBC intracellular and extracellular media. For the purpose of distinguishing metabolites reflecting host-pathogen interactions, Overrepresentation Analysis (ORA) and Pathway analysis (PA) revealed relevant metabolic alterations related to several processes, including nitrogen, glycerophospholipid, and amino acid metabolism. Such changes are suggested to be related to the erythrocyte and parasite cross-talk as well as extracellular signal transduction pathways with potential to support activation of the immune system. 1. Vannier, E. et al. Babesiosis. Infect Dis Clin N Am. 2015,29,357–370. 2. Jeffrey A. Gelfand, JA.; Vannier, E. Babesiosis. In Harrison's Principles of Internal Medicine; Fauci, AS. et al; 17ed. McGraw-Hill's Access Medicine, 2008.

P-418 Metformin inhibits Branched Chain Amino Acid (BCAA) derived ketoacidosis and promotes metabolic homeostasis in Maple Syrup Urine Disease (MSUD)

PRESENTING AUTHOR: ***Sonnet S. Davis, Buck Institute for Research on Aging, United States***

CO-AUTHORS: ***Yuehmei Hsu Shereen Chew Chenyu Liao Vidur Kailash Samuel Richard Larson Vanessa A nova Josef Flores Junying Wang Brian K Kennedy Arvind Ramanathan***

Branched-chain amino acids (BCAA) leucine, valine, and isoleucine are essential amino acids that are not only building blocks for protein synthesis but also mediate nutrient signaling (i.e. mTOR) and play an important role in metabolism and homeostasis. Dysregulation of BCAA metabolism caused by the branched-chain keto acid dehydrogenase (BCKDH) complex has been associated with a range of diseases including Type II Diabetes, obesity and is the direct cause of MSUD. MSUD is an in born error in metabolism that arises from mutations in the E1 or E2 subunits BCKDH complex, a multi-subunit protein complex localized to the mitochondrial inner membrane. BCKDH catalyzes the first irreversible step in BCAA catabolism and its' dysfunction leads to the incomplete metabolism of BCAAs resulting in a toxic accumulation of branched-chain keto-acids (BCKA) and BCAAs in bodily fluids and tissues. Using liquid chromatography-mass spectrometry (LC-MS) based metabolomics and a murine model of MSUD; we have developed a comprehensive view of the role of BCKDH in tissue (brain, liver, muscle) homeostasis and metabolism. Our findings show reduced BCKDH activity leads to loss of TCA intermediates and ketoacidosis in multiple tissues (brain, liver, gastrocnemius muscle). Chronic treatment with Metformin, a FDA-approved anti-diabetic drug, alleviates the over-accumulation of BCAA and BCKAs caused by BCKDH dysfunction. Metformin treatment partially restores metabolic homeostasis and significantly increases the survival of BCKDH deficiency in vitro and in vivo murine modelproviding an innovative therapeutic strategy for managing MSUD and related diseases of BCAA-catabolism.

PEDIATRICS

P-419 Untargeted UPLC-MS analysis of potential pesticide and biomarkers of fetal growth restriction

PRESENTING AUTHOR: ***Aude-Claire MORILLON, Infant Centre / University College Cork, Ireland***

CO-AUTHORS: ***Shirish Yakkundi, Gregoire Thomas, Martin Wells, Philip Baker, Louise Kenny***

Fetal growth restriction (FGR) is defined as impaired fetal growth compared with normal growth potential for the fetus. This pregnancy complication is estimated to affect 10% of pregnancies and leads to short and long-term complications for the baby. Pesticides are commonly used in agriculture, and exposure during pregnancy has been linked with FGR, birth defects and impaired child neurodevelopment. The present study assessed exposure to pesticides and their impact on fetal growth. A nested case-control study used urine samples collected at 20 weeks' gestation from pregnant women participating in the SCOPE study (www.scopestudy.net). Cases were women with severe FGR (customised birthweight ≤ 8th centile, n=40) matched by age, body mass index and ethnicity to controls (n=40) who had uncomplicated pregnancies. Urine samples (100µL) were diluted with MQ water (200µL) before analysis. Data were collected for all samples in triplicate in untargeted positive and negative ion mode using UPLC (BEH C18 column, 2.1x100mm, 1.7µm) coupled with mass spectrometry (Synapt G2-S, Waters), using data independent analysis approach. Data were normalised, processed and database searched (Human Metabolite Database and pesticide library) using Progenesis QI (Nonlinear dynamics, UK). Statistical analysis was performed on non-normalised data, using non-parametric tests, and features were ranked based on adjusted p-values from empirical Bayes analysis. One pesticide, had significantly increased intensity (p-value 9.28x10-4) in cases compared to controls in negative ion mode. This study suggests exposure to this pesticide in early pregnancy may impair in utero growth trajectories. Further research is needed to identify the precise association with FGR.

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PHARMACOMETABOLOMICS

P-422 **Metabolomics of alum adjuvant**

PRESENTING AUTHOR: ***Sakda Khoomrung, Mahidol University, Thailand***

CO-AUTHORS: ***Sakda Khoomrunga,b, Intawat Nookaewa,c, Partho Sena, Thorunn A. Olafsdottird, Josefine Perssond, Thomas Moritze, Peter L. Andersenf, Ali M. Harandid, Jens Nielsena***

The advent of "omics" as genomics, transcriptomics, proteomics, lipidomics, metabolomics alongside with advanced bioinformatic tools are promising technologies for a better understanding of the mechanisms of action of vaccines. Alum has been clinically used as a vaccine adjuvant over past 80 years, however, the impact of alum on host metabolism remains largely unknown. Herein, we have applied MS-based metabolite profiling to study the effects of alum adjuvant on mouse serum at 6, 24, 72 and 168 hours following an immunization with the Mycobacterium tuberculosis vaccine antigen H56 in combination with alum. We employed a novel strategy for class-wise identification of LC-MS-based untargeted metabolomics data called Subclass Identification and Annotation method for Metabolomics (SIAM). Using this approach, we have identified and validated that lipids were the major class of metabolites that significantly changed at 24 hr after alum administration. The majority of these lipids existed in the form of triglycerides alongside with several other unidentified metabolite species. These results may provide new insights into the mode of action of alum adjuvants.

P-423 **Non-targeted metabolomics analysis of Oseltamivir action on Leishmania parasites to discover the metabolomics changes**

PRESENTING AUTHOR: ***Maheshkumar Kharat, Savitribai Phule Pune University, India***

CO-AUTHORS: ***Shyam Sundar, Kanchankanhoja Kharat, Milind Patole, Kalpana Pai***

Global population increased day-by-day and emerging infectious disease burden with multidrug-resistance pathogens to require essentially therapeutic drugs to improve health. Although, nowadays tremendously increased drug necessity. On that, basis increases the cost of innovative drug development and tackled the rational drug discovery. Drug repurposing is emerging discipline, which assists to utilize the previously developed drug to the new purpose. Repurposing of drug applied to the recycling of novel therapeutic uses or formulations for a well-known drug, or the two or more certify drug's combination, formerly used individually. With these considerations and with the aim of repurposing of drugs, an attempt was made to investigate the effect of Oseltamivir along with known antileishmanial drugs Miltefosine on two Leishmanial strain. However, we implement the untargeted metabolism to interpret the metabolomic changes elaborated in the action of Oseltamivir with Amphotericin-B relapsed parasite strain. Throughout, 500 metabolites were discovered, 15% of which were modified considerably. Oseltamivir treated cells appeared exhausted proportion of most metabolites, showing that compromising the outer membrane of cell's integrity, and internal metabolites will be disappearing upon cell death. In the amphotericin-b relapsed cells, the drug was attributed and established the changes on the strength of metabolite.

P-424 **Effect of PHMG, a humidifier disinfectant, on eye-dryness in rat model using 1H-NMR-based metabolomics.**

PRESENTING AUTHOR: ***Jung Dae Lee, College of Pharmacy, Sungkyunkwan University, Korea, South***

Humidifier was used to relieve eye-dryness symptom and polyhexamethylene guanidine (PHMG) is contained in humidifier as a preservative. PHMG is known to induce severe pulmonary fibrosis in Koreans. This study was performed to elucidate effect of PHMG on eye-dryness in rat model using 1H-NMR-based metabolomics. PHMG was instilled to rat's eye showing eye-dryness induced by scopolamine and dry condition. In order to induce eye-dryness, scopolamine (3 mg/kg for 7 days) was subcutaneously injected then positive control (benzalkonium chloride 0.1%) and PHMG (0.1% or 1%) were instilled in the eye for 5 days. The amount of tears and tear film breakup time levels showed a tendency to decrease in eye-dryness-induced group (PHMG and positive control group) compared with control. However, there was no significant difference between PHMG-treated and eye-dryness-induced groups. The levels of corneal damage, IL-6, IL-1β and TNF-α in cornea neutrophil and lacrimal gland neutrophil were significantly higher in PHMG treated group (1%) than eye-dryness group. Metabolomics study showed that PCA and OPLS-DA score plot were separated by each group in plasma and urine. Metabolites (VIP> 1.0) showing differences among groups in plasma and urine. Among them, the levels of 3-hydroybutyrate and glucose were significantly different between control and PHMG 1% group in plasma. The levels of 2-oxoglutarate, 3-indoxylsulfate and malonate were significant difference between control and PHMG 1% group in urine. These results contribute to understand the metabolic alteration and can be useful for potential biomarkers related to eye-dryness and deteriorating effect of PHMG on eye-dryness.

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PHARMACOMETABOLOMICS

P-425 Short-Term Fasting alters Acetaminophen Metabolism in Humans

PRESENTING AUTHOR: ***Laureen Lammers, Academic Medical Center, University of Amsterdam, Netherlands***

CO-AUTHORS: ***Roos Achterbergh, Hans (J.A.) Romijn, Ron Mathot***

Introduction. Acetaminophen (APAP) hepatotoxicity is caused by the metabolite N-acetyl-p-benzoquinone imine (NAPQI) formed by Cytochrome P450 (CYP)-mediated metabolism. Preclinical studies have shown that fasting is a predisposing factor for acetaminophen-induced hepatotoxicity. Furthermore, previous studies in humans have demonstrated that short-term fasting can alter the activity of CYP enzymes. Aims. The aim of our study was to assess the effect of short-term fasting (STF) on the pharmacokinetics of acetaminophen and its metabolites. Methods. In a randomized controlled cross-over trial, nine healthy subjects received a single oral administration of 1000 mg acetaminophen after (1) an overnight fast (control) and (2) 36h of fasting. Pharmacokinetics of acetaminophen and its metabolites (acetaminophen-glucuronide (APAP-Glc), acetaminophen-sulfate (APAP-Sul), 3-cysteinyl-acetaminophen (APAP-Cys), acetaminophen-mercapturate (APAP-Cys-NAC) and 3-methoxy-acetaminophen (APAP-OMe) (Figure 1) were analyzed by non-linear mixed-effects modeling (NONMEM). Apparent clearances of acetaminophen (CLapap/Fapap) and metabolites (CLmet/(Fapap x fmet)) were estimated, where F represents bioavailability and f represents the fraction APAP converted to the metabolite. Results. Short-term fasting decreased the apparent clearance of APAP (DCLapap/Fapap =10%, $p<0.01$), APAP-Sul (DCLSul/(Fapap x fSul) =17%, $p<0.01$), APAP-Cys (DCLCys/(Fapap x fapap-Cys) =12%, $p<0.01$) and APAP-OMe (DCLOMe/(Fapap x fOMe) =15%, $p<0.01$) whereas apparent clearance of APAP-Cys-NAC increased by 15% (DCLapap-Cys-NAC/(Fapap x fapap-Cys-NAC x fapap-Cys), $p<0.01$). Fasting did not affect apparent APAP-Glc clearance. Discussion. The study demonstrates that STF increases acetaminophen exposure and the exposure of its CYP-mediated metabolites in humans. Although NAPQI was not determined directly, this implies that fasting increases the risk of acetaminophen induced hepatotoxicity in humans.

NEUROLOGY AND PSYCHIATRY

P-426 Does human milk metabolome reflect maternal psychological distress and stress hormone secretion differently?

PRESENTING AUTHOR: ***Maaria Kortesniemi, University of Turku / University of California, Davis, Finland***

CO-AUTHORS: ***Carolyn Slupsky, Anna Aatsinki, Jari Sinkkonen, Linnea Karlsson, Kaisa Linderborg, Baoru Yang, Hasse Karlsson, Henna-Maria Uusitupa***

Human milk is a dynamic matrix subject to substantial compositional variation due to e.g. maternal phenotypes and physiological conditions. Early nutrition can play a role in modulating the transmission of maternal stress to the offspring. The aim of this study is to see how maternal psychological distress and high human milk cortisol levels are reflected in human milk metabolome, and to assess the biochemical potential of human milk to function as a resilience factor in stress transmission in early life. The present study is part of the Finnish population-based FinnBrain Birth Cohort Study, designed to prospectively investigate the effects of early life stress on child development [1]. Human milk samples (n = 150) were collected at 2.5 months postpartum and analyzed with 1H NMR metabolomics [2]. Maternal distress was determined by using standardized self-report questionnaires (including depressive and anxiety symptoms) and human milk cortisol levels. Untargeted, unsupervised overview showed no apparent trends or clusters within NMR data. Supervised multivariate analysis (OPLS-DA) of the metadata and the milks classified according to 1) high milk cortisol, 2) high prenatal psychological distress, and 3) low distress and low milk cortisol (control) demonstrated class discrimination. The data suggests N-trimethyl compounds and short-chain fatty acids as potential biomarker candidates for maternal psychological distress, and fumarate and urea for high and low milk cortisol levels, respectively. Targeted analysis of individual metabolites and their statistical significance in light of the stress and other maternal factors are evaluated to further validate and determine biological relevance.

P-427 Serum Metabolic Profiling using Small Molecular Water-Soluble Metabolites in Individuals with Schizophrenia: A Longitudinal Pre- and Post-Treatment Study

PRESENTING AUTHOR: ***Cao Bing, Peking University, China***

CO-AUTHORS: ***Bing Cao, Lailai Yan, Elisa Brietzke, Roger S. McIntyre, Dongfang Wang, Joshua D. Rosenblat, Renee-Marie Ragguett, Chuanbo Zhang, Xiaoyu Sun, Jingjing Yan, Rodrigo B. Mansur, Carola Rong, Jingyu Wang***

Abnormalities in cellular bioenergetics are implicated in the pathophysiology of disparate brain-based disorders, including schizophrenia. Herein, we sought to compare alterations in serum bioenergetic markers within a well-characterized sample of adults with schizophrenia at baseline and after 8 weeks of pharmacological treatment. We hypothesized that treatment would be associated with significant changes in bioenergetic markers given the role of bioenergetic dysfunction in schizophrenia. We recruited 122 adults with schizophrenia who had not received pharmacological treatment for at least one month prior to enrollment, including drug naïve (i.e., firs-episode) participants and treatment non-adherent participants. Serum samples were analyzed using liquid chromatography-tandem mass spectrometry. Metabolites with the greatest change, when comparing pre- and post-treatment levels, were identified revealing fourteen water-soluble metabolites of interest. The composition of these metabolites were amino acids (n=6, i.e., L-Proline, D-Glutamic acid, L-Arginine, Ornithine, L-Cystine and L-Lysine), carnitines (n=4, i.e., Oleoylcarnitine, L-Palmitoylcarnitine, Linoleyl carnitine and L-Acetylcarnitine), polar lipids [n=3, i.e., Lysophosphatidylcholine (LPC) (14:0), LPC(15:0) and LPC(16:0)], and organic acid (n=1, i.e., 2,5-Dichloro-4-oxohex-2-enedioate). All above amino acids, LPCs and the organic acid were increased, while the four carnitines were decreased post-treatment. Taken together, the current study identified several bioenergetic markers that consistently change with pharmacological treatment, especially involving fatty acid metabolism and amino acid metabolism. These changes may provide further insights into the pathophysiology of schizophrenia along with furthering our understanding of the mechanisms subserving both the effects (e.g., antipsychotic effects) and side effects (e.g., metabolic syndrome) of antipsychotics .

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NEUROLOGY AND PSYCHIATRY

P-428 Finding of new diagnostic biomarkers for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) using metabolome analysis

PRESENTING AUTHOR: ***Emi Yamano, RIKEN Compass to Healthy Life Research Complex Program, Health Metrics Development Team, Japan***

CO-AUTHORS: ***Emi Yamano, Masahiro Sugimoto, Akiyoshi Hirayama, Satoshi Kume, Yasuyoshi Watanabe, Tomoyoshi Soga, Yosky Kataoka***

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a persistent and unexplained pathological state characterized by exertional and severely debilitating fatigue, with/without infectious or neuropsychiatric symptoms, lasting at least 6 consecutive months. Its pathogenesis was not fully understood. Because of incomplete understanding of aetiology and diagnostic uncertainty of ME/CFS, there are no firmly established objective diagnosis or treatment recommendations. In the present study, we performed comprehensive metabolomic analyses of plasma samples obtained from ME/CFS patients and healthy controls using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) to establish an objective diagnosis of ME/CFS. ME/CFS patients exhibited significant differences in intermediate metabolite concentrations in the tricarboxylic acid (TCA) and urea cycles. This revealed the decreased activity in these two cycles, which may reflect the pathophysiological state of ME/CFS. The combination of ornithine/citrulline and pyruvate/isocitrate ratios discriminated ME/CFS patients from healthy controls yielding high area under the receiver operating characteristic curve values of 0.801 (95% confidential interval [CI]: 0.711–0.890, P < 0.0001) and 0.750 (95% CI: 0.584–0.916, P = 0.0069) in training and validation datasets, respectively. These findings provide compelling evidence that a clinical diagnostic biomarker could be developed for ME/CFS based on the ratios of metabolites in plasma.

P-429 An UHPLC-ESI-HRMS metabolomics workflow for brain neurotoxic biomarkers investigation: evaluation of racemic Mefloquine versus pure enantiomers of Mefloquine analogues in a murine model

PRESENTING AUTHOR: ***Desoubzdanne Denis, French Armed Forces Biomedical Research Institute, France***

CO-AUTHORS: ***Desnouveaux Laura, Dormoi Jérôme, Taudon Nicolas***

In this study, we present a metabolomics strategy to investigate brain neurotoxic biomarkers in a mouse model treated with racemic Mefloquine (MQ) or with pure enantiomers of MQ analogues. Indeed, malaria is still one of the most widespread parasitic tropical diseases: the pharmacological need of new or improved antimalarial drugs is real. Among these drugs, MQ has been widely used, whereas secondary neurological effects have sometimes been observed due to the (-)-MQ form. These effects can drive to dramatic situations for treated patients: sleep disorders, anxiety, depression or suicide in few cases. In our study, two hours after intra-peritoneal administration, different mouse brain regions (cortex, olfactory bulb, cerebellum, sub-cortical area, and hippocampus) were collected and homogenized. Metabolites were extracted according to Folch protocol and hydrophilic phase was evaporated to dryness for concentration. After re-suspension, metabolites were separated and analyzed in both ESI+ and ESI- modes with an UHPLC-ESI-HRMS instrument (1290 + 6550A QTOF, Agilent). A Kinetex® 1.7 µm Biphenyl 100 Å LC column (150 mm x 2.1 mm, Phenomenex) was used for its interesting retention properties of aromatic polar and mild-polar compounds. Metabolomics data were processing and normalized thanks to the open-source web-based Galaxy platform developed for metabolomics community. Further multivariate and univariate biostatistical analyses were also realized in order to highlight putative neurotoxic biomarkers of different MQ treatments. During this investigation, a particular focus was given to bioamines (Catecholamines, Trp metabolites...) and neurotransmitters (GABA, Glu...) which are ubiquitous neurological stress biomarkers.

P-430 Plasma metabolites on first-onset psychosis: schizophrenia and bipolar disorder biomarkers

PRESENTING AUTHOR: ***Helena P G Joaquim, Laboratory of Neuroscience - LIM27, Brazil***

CO-AUTHORS: ***Alana C Costa, Leda L Talib, Wagner F Gattaz***

Background:Schizophrenia (SCZ) and bipolar disorder (BD) are severe psychiatric disorders and share many characteristics and symptoms since the first-onset. Identify molecular biomarkers for psychiatric disorders can assist in the diagnosis of disease and treatment and monitoring of patients. Methods: Plasma metabolites were quantifiedusing the AbsoluteIDQ® p180 Kit (BIOCRATES Life Science) followed by mass spectrometer operating in the MRM mode. Data analysis was performed using the MetIDQ software (Biocrates) and Metaboanalyst version 3.0. Results:37 metabolites were different between the groups: 5 Lyso- phosphatidylcholines, 11 phosphatidylcholines, 6 acylcarnitines, 1 sphingomyelin, 10 amino acids and 4 biogenic amines. Analyzing the pathways, we found three metabolites alterated: Nitrogen metabolism (FDR = 4.8 x 10-3), Arginine and proline metabolism (FDR = 4.8 x 10-3) and Aminoacyl-tRNA biosynthesis (FDR = 1.66 x 10-6). In order to determine the 5 main metabolites able to differentiate the diagnosis, ROC curves were used. Considering SCZ patients and healthy controls, the area under the curve (AUC) was 0.534, applying metabolites Met-SO, Gly, LysoPCaC26:1, C16-OH; PCaaC40:3. Considering BD patients and healthy controls the AUC was 0.947, applying metabolites t4-OH Pro, Creatinine, PCaaC24:0; PCaaC26:0 and LysoPCaC26:0. Considering SCZ and BD patients the AUC was 0.921, applying metabolites t4-OH-Pro; C16:2-OH; C3-OH; PCaaC36:1 and Met. Conclusion: Our results clearly show that different classes of metabolites are implicated in both schizophrenia and bipolar disorders comparing to healthy controls. Besides that, we observed that the metabolites implicated in each disorder are not the same since the first onset psychosis.

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POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

NEUROLOGY AND PSYCHIATRY

P-431 Plasma levels of metabolites differentiate first episode psychosis in schizophrenia and bipolar disorder patients

PRESENTING AUTHOR: ***Alana C. Costa, Laboratory of Neuroscience - Institute of Psychiatry USP, Brazil***

CO-AUTHORS: ***Helena P. G. Joaquim, Leda L. Talib, Wagner F. Gattaz.***

Background: Schizophrenia (SCZ) and bipolar disorder (BD) are serious psychiatric disorders that affect young adults and lead to disability, psychosocial functioning impairment and premature death. These disorders share several characteristics and symptoms and the diagnosis yet is mainly clinical. The development of sensitive and accurate biomarkers is highly required. Our aim was to determine plasma levels of metabolites of subjects in first episode psychosis and controls and find cutoff values that differentiate each group. Methods: We analyzed 55 drug-naïve patients (28 SCZ and 27 BD) and 30 controls. Lipid quantification was performed by mass spectrometry - Flow injection analysis, using AbsoluteIDQ p180® kit (Biocrates Life Sciences). Statistical analyzes were performed using Classification and Regression Tree. Results: We observed that there the combination of four metabolites are able to differentiate the diagnoses studied: PC aa C26:0, PC aa C38:4, PC aa C34:3 and C16-OH. The accuracy of the method is 87,1%. Discussion: Levels of some plasma metabolites differentiate subjects in FEP in SCZ, BD and controls, which can be a potential biomarker for psychosis, as well as a diagnostic marker. The findings from this study require further validation in BD and SCZ subjects, but suggest that the metabolome is a good tool to understand the pathophysiology of these disorders and presents potential diagnostic biomarkers for the diseases studied.

P-432 Non-targeted metabolic profiling analysis for diagnosis of internet/smartphone addiction disorder

PRESENTING AUTHOR: ***Sung-Hee Cho, Center for Chemical Analysis, Korea Research Institute of Chemical Technology (KRICT), South Korea***

CO-AUTHORS: ***Hyuck Ho Son***

Smartphone addiction, sometimes called nomophobia, is often fueled by an Internet overuse problem or internet addiction disorder. The internet/smartphone addiction is related to various kinds of psychiatric problems such as depression, attention deficit hyperactivity disorder (ADHD), impulse control disorder, and low self–esteem, but the precise cause of this is still unknown. In vivo metabolic studies to elucidate the pathogenesis of Internet/smartphone addiction, only a fractional study of single markers such as DHEA and cortisol has been published, and systematic studies on their alteration by metabolic pathway have not been conducted yet to be. Therefore, in order to diagnose and treat Internet/smartphone addiction, research to clarify the mechanism of onset through bio-metabolism analysis is needed. In this study, non-targeted metabolic profiling analysis for diagnosis of internet/smartphone addiction disorder was performed by ultra-performance liquid chromatography-quadrupole time of flight mass spectrometry (UPLC-Q-TOF MS) combined with multivariate statistical analysis. Urine samples were separated using Imtakt Cadenza HS-C18 column (2 x 100 mm, 3 μm) and a mobile phase consisting of eluent A (0.1% formic acid and in water) and eluent B (0.1% formic acid in methanol) with a gradient program at a flow rate of 0.4 mL/min and were monitored by Q-TOF MS. MarkerlynxTM and SIMCA were used for statistical processing. Metabolic patterns obtained from patients with internet/smartphone addiction disorder were distinguishable from controls. The alteration of metabolism in biological samples may play important role to understanding probable disorder, and the described methods could be used to evaluate and monitor patients with internet/smartphone addiction.

P-433 A global HILIC-HRMS-based approach to measure polar human cerebrospinal fluid metabolome: exploring its variation in a cohort of cognitively healthy elderly subjects

PRESENTING AUTHOR: ***Hector Gallart-Ayala, University of Lausanne, Switzerland***

CO-AUTHORS: ***Ioana Konz, Florence Mehl, Tony Teav, Aikaterini Oikonomidi, Gwendoline Peyratout, Vera van der Velpen, Julius Popp, Julijana Ivanisevic***

Cerebrospinal fluid (CSF) is a key body fluid that maintains the homeostasis in central nervous system. As a biofluid whose content reflects the brain metabolic activity, the CSF has been profiled in the context of neurological diseases to provide novel insights into the disease mechanisms. However, a global high-throughput approach to measure a broad diversity of polar metabolites present in CSF is lacking. Although still perceived as being challenging and less reproducible, hydrophilic-interaction liquid chromatography (HILIC) has recently evolved to offer the unprecedented coverage capacity of water-soluble metabolites. Considering the highly polar nature of CSF we have comprehensively characterized the CSF polar metabolome using HILIC coupled to HRMS to determine which polar metabolites can be detected and routinely quantified in CSF. To extend the coverage of CSF polar metabolome we have combined the profiling in acidic pH ESI (+) and basic pH ESI (-). This approach allowed us to identify and measure a broad range of central carbon metabolites (implicated in glycolysis, TCA cycle, nucleotide, amino acid and fatty acid metabolism) in CSF collected from a population of cognitively healthy elderly individuals (n=32), using a single extraction method. Metabolite annotation was achieved using accurate mass, RT and MS/MS identification criteria, allowing for the validation of identity of 146 measurable metabolites. Characterized CSF profiles were further explored for inter-individual, gender, BMI and age-associated variability. Altogether, this extensive polar CSF metabolic phenotyping has an added value in biomedical research to advance the understanding of different health and disease-related metabolic and regulatory processes.

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NEUROLOGY AND PSYCHIATRY

P-434 Annexin A3 and PLA2 in platelets of schizophrenia patients

PRESENTING AUTHOR: ***Leda Leme Talib, University of São Paulo, Brazil***

CO-AUTHORS: ***Helena PG Joaquim, Wagner Farid Gattaz***

Associations of disturbed neuroplasticity, increased inflammation and disturbance in phospholipid metabolism have already been described in schizophrenia. Regarding to phospholipid metabolism, one of the main changes already described in schizophrenia concerns to Phospholipase A2 (PLA2). PLA2 comprise a super-family of enzymes that have a major role in membrane phospholipid homeostasis. Dysfunction in phospholipase inhibitors proteins (PLIPs) may underlie the increased PLA2 activity in schizophrenia. PLIPs provided structural and functional information that led to identification of a large group of annexins proteins. Annexins functions comprehend initiation of membrane fusion in exocytosis and endocytosis, inhibition of PLA2, inhibition of coagulation, and calcium channel activity. In an exploratory study, using bidimensional electrophoresis followed by mass spectrometry, we found that annexin A3 (ANXA3), was differentially expressed in first-onset schizophrenia patients. We investigated annexin A3 levels and PLA2 activity in platelets of 26 drug-naïve patients in the first-onset schizophrenia, 14 chronic schizophrenia patients and 16 healthy controls. PLA2 subgroups activity was determined by radioenzimatic assay and annexin A3 levels by western blotting. We found differences in sPLA2 ($p=0.05$) activity and ANXA3 level ($p<0.001$) between groups. sPLA2 and tPLA2 activities were higher in first-onset schizophrenia compared to chronic schizophrenia ($p=0.04$ and 0.05,). ANXA3 was lower in first onset patients ($p<0.001$) and in chronic schizophrenia patients ($p=0.052$) comparing to controls. Considering only schizophrenia patients, ANXA3 level was higher in chronic than in first onset subjects ($p=0.035$). Our results show ANXA3 is one of the proteins involved in schizophrenia besides the mechanisms already associated with PLA2 role.

P-435 Identification of biomarkers and biological mechanisms of the psychiatric disease through the study of the urine metabolome by a Systems Biology approach

PRESENTING AUTHOR: ***Nora Gutiérrez-Nájera, Instituto Nacional de Medicina Genómica, Mexico***

CO-AUTHORS: ***Ángel Polanco, Mirna Morales, José Juan Ordaz-Ortiz, Osbaldo Resendis, Claudia Erika Hernández-Patiño, Christian Diener, Juan Carlos Fernández-López, Ana Luisa Romero-Pimentel, Alma Genis, Jaime Martínez-Magaña, Humberto Nicolini***

The psychiatric disorders are complex diseases. The combination of genomic information together with a detailed molecular analysis of the samples of the patients will be important to predict, diagnose and treat psychiatric diseases.Metabolomics provides an approach to understanding the biochemical regulation of metabolism and networks in a biological system. Objective: To discover new mechanisms and biomarkers for psychiatric disease studying the composition of metabolites in urine from patients with psychiatric diseases, whose were previously genotyped with the Infinitum PsychArray Bead Chip of ILLUMINA®, integrating the metabolomic and genomic data by a Systems Biology approach.The study used samples from 37 patients with schizophrenia, 29 with bipolar disorder, 10 with obsessive compulsive disorder and 10 with autism spectrum disorders. Control samples (24 patients) from donors without psychiatric disease were used for development, feasibility and standardization of the methods. We studied the composition of the metabolites in urine of healthy individuals and patients with psychiatric disease. The patient samples were analysed by liquid chromatography- mass spectrometry. The blood samples also were genotyped with the Infinium PsychArray BeadChip Illumina® which allowed obtention of both metabolomics and genomics data. Data analysis combined genomic and metabolomic data by using a bioinformatics approach of the Systems Biology. The data led to identify metabolites as Acetyl-N-formyl-5-methoxykynurenamine as associated to psychiatric illnesses. Currently we are analyzing the relation of the levels of this metabolite with 37 single nucleotide polymorphisms of the gene indoleamine-2,3-dioxygenas..The metabolites of tryptophan, precursor of serotonin and kynurenine, are suitable for psychiatric diagnosis.

P-436 Disturbance in lipid metabolism by psychiatric disease, internet gaming disorder

PRESENTING AUTHOR: ***Chang-Wan Lee, Kookmin University, South Korea***

CO-AUTHORS: ***Jung-Eun Lee, Young-Chul Jung, Do Yup Lee***

Internet game disorder (IGD) has been recently introduced as behavioral addiction in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) as a new mental illness. IGD is a mental illness that interferes with social life by uncontrolled anxiety, depression and impulsiveness during continuous or repetitive internet gaming behavior. The mental illness is generally defined with scores through classification methods. However, more objective criteria such as biomarkers may aid pathological phenotyping and consequently clinical treatment including medication. Phospholipids (PLs) are important components of biological membranes and precursors of numerous signaling molecules. Accordingly, the aim of our current study was to investigate lipidomic signatures and explore the covarianced matrix between the illness and lipid molecular alteration reflected in biological fluid, blood plasma. Lipid profiles were acquired from 89 populations (healthy controls=28, IGD=61) using liquid-chromatography Orbitrap mass-spectrometry (LC-Orbitrap MS). Univariate statistics demonstrated 4 lipids were significantly different between healthy control and IGD group. The lipids were lysophosphatidylethanolamine (LysoPE), lysophosphatidylserine (LysoPS), phosphoserine (PS) phosphocholine (PC). Re-composited biomarker cluster based on multivariate statistics showed fairly good discrimination power between two groups. The outcome suggested putative relatedness of lipid metabolism with IGD, and applicability of the biomarker signature that can complement clinical determination.

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NEUROLOGY AND PSYCHIATRY

P-437 Untargeted metabolomics approach in the study of posttraumatic stress disorder

PRESENTING AUTHOR: ***Gordana Nedic Erjavec, Rudjer Boskovic Institute, Croatia***

CO-AUTHORS: ***Marcela Konjevod, Dubravka Svob Strac , Matea Nikolac Perkovic, Lucija Tudor, Suzana Uzun, Oliver Kozumplik, Nela Pivac, Coral Barbas Arribas***

Posttraumatic stress disorder (PTSD) is a common, prevalent, severe, disabling and debilitating mental disorder developing after traumatic exposure. The exact biological factors, associated with vulnerability or resilience to develop PTSD after traumatic exposure, are still not well known. In this research we sought to investigate a metabolic profile of patients with combat related PTSD. Study included 100 plasma samples, 50 from PTSD subjects and 50 from matching healthy control (HC) subjects. Samples were collected within the project „Genomic and glycomic biomarkers for PTSD" supported by Croatian Science Foundation. Plasma samples were analyzed by a Liquid Chromatography (Agilent 1200) coupled to Mass Spectrometry (LC -QTOF-MS (Agilent 6520) in positive and negative ionization mode. Primary analysis was followed by tandem LC-MS experiments in order to identify significant compounds. After multivariate (principal component analysis, partial least square-discriminant analysis) and univariate (Student's t-test, Mann-Whitney U test) statistical analysis, 25 significant chemical signals were found in positive and 35 in negative LC-MS mode. A trend of increased levels of several types of glycerophospholipids and decreased levels of different types of carnitines and bile acids was found in PTSD subjects when compared to HC subjects. This preliminary study could contribute to better understanding of PTSD etiology, but further research has to be carried out to validate current results.

P-439 CSF metabolomic biomarkers of LRRK2 and idiopathic Parkinson's disease.

PRESENTING AUTHOR: ***Ali Yilmaz, Research Fellow, United States***

CO-AUTHORS: ***Ali Ylilmaz, Zafer Ugur, Jan Aasly, Ray O. Bahado-Singh, David Loeffler, Stewart F. Graham***

Background:The pathogenesis and etiology of Parkinson's disease (PD) remains to be elucidated. Metabolomics has the potential to identify metabolic pathways and unique biochemical profiles associated with PD pathogenesis. In this study we aim to profile CSF from a unique group of PD sufferers. Methods: Utilizing both1H NMR and DI-LC-MS/MS we quantitatively profile CSF from patients suffering from sporadic PD (n=20) and those who are genetically predisposed (LRRK2) to the disease (n=20) and compare them with age- and gender-matched controls. Results: 1H NMR and MS based metabolomics, in combination with bioinformatic analyses, provided useful information highlighting previously unreported biochemical pathwaysand CSF based biomarkers associated with both sporadic and LRRK2 acquired PD. Conclusions:This study demonstrates that by combining two complimentary techniques we provide a much more holistic view of the CSF metabolome and inversely, the brain metabolome. .Future studies should investigate whether: a) the biochemical pathways highlighted here are recapitulated in the brain of PD sufferers and b) if the CSF panel of biomarkers as identified herein are also as effective in less invasively harvested biomatrices, for the prediction/diagnosis of those at greatest risk of developing PD.

P-440 Targeted metabolomics in multiple sclerosis post mortem brain tissue.

PRESENTING AUTHOR: ***Teodoro Bottiglieri, Baylor Scott & White Research Institute, United States***

CO-AUTHORS: ***Erland Arning, Clinton Harmon, Xuan Wang, Naveen Kumar Singhal, Jennifer McDonough***

Background: Identification of metabolites in multiple sclerosis (MS) post mortem brain tissue, compared to non-diseased control tissue, may provide further insight into the pathological mechanisms involved in this disease. Characterizing pathways in the MS diseased brain may also provide metabolomic targets that may be used to monitor disease progression through the analysis of blood plasma, urine or cerebrospinal spinal fluid. Subjects & Methods: Postmortem frozen brain tissue was obtained from the Rocky Mountain MS Center and the Brain and Spinal Cord Resource Center at University of California–Los Angeles. Tissue was matched for brain region, age, and postmortem interval (PMI) as closely as possible. Cortical gray brain tissue from 15 MS (9 males and 6 females) and 10 controls (5 male and 5 female) were stored at -80oC until time of analysis. Brain tissue (~50mg) was deproteinized in methanol containing 5 mM ammonium acetate and the supernatant analyzed using the Biocrates AbsoluteIDQ™ P180 platform by liquid chromatography on a 4000 QTrap Sciex mass spectrometer. Results: Compared to controls, MS brain tissue (males and females) showed significant decreases in 36 acyl-carnitines, 6 phosphatidylcholines and 4 lyso-phosphatidtylcholines. There were no significant differences in sphingomyelins, amino acids or biogenic amines. Conclusions: Decreases in acyl-carnitines may indicate deficits in energy metabolism and oxidation of fatty acids. Phosphatidtylcholine and lyso-phosphahtidylcholines play important roles as structural components of membranes and are also involved in neuronal signaling. Validation of these findings in plasma and cerebrospinal fluid of MS subjects is warranted.

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NEUROLOGY AND PSYCHIATRY

P-441 FAM57B Ceramide Synthase Depletion Disrupts Neuronal Lipid Membrane Homeostasis and Manifests Depressed Behavioral Responsiveness to Stimuli

PRESENTING AUTHOR: ***Danielle L. Tomasello, Whitehead Institute, Massachusetts Institute of Technology, United States***

CO-AUTHORS: ***Jasmine M. McCammon, Hazel Sive***

16p11.2 deletion syndrome is a macrodeletion of a core of 25 genes that underlies a combination of severe neurodevelopmental and mental health disorders, including autism spectrum disorder, epilepsy, attention deficit hyperactivity disorder, schizophrenia, macrocephaly, intellectual disability, and language deficits. Within these core genes, the ceramide synthase, encoded byfam57b, has emerged as a candidate gene from our previous findings indicatingfam57binteracts with a large proportion of 16p11.2 interval genes. We sought out to determine the neuronal function of the largely uncharacterized FAM57B. Usingfam57ba-/-;fam57bb-/-zebrafish larvae, we discovered disruption of integral membrane lipids by metabolite profiling of larvae brains. Strikingly, 16p11.2 deletion fibroblasts displayed a similar lipid metabolite profile, indicatingFAM57Bis a key component of lipid homeostasis. Lipid raft disorganization in the brain was observed after ventricle injection of cholera-toxin subunit B in 24 hours post fertilization (hpf) larvae compared to wild-type. Furthermore,fam57ba-/-;fam57bb-/-larvae displayed severely depressed phenotypes in baseline movement and stimulated movement after exposure to dark cycle and application of seizure inducing pentylenetetrazol (PTZ), indicating disturbed neuronal properties may affect functional behavioral output. Together, these studies uncover a previously unknown function for FAM57B to maintain lipid membrane homeostasis therein supporting neuronal function.

P-442 Diagnosis and staging of multiple sclerosis using a combined 'omics approach.

PRESENTING AUTHOR: ***Fay Probert, University of Oxford, United Kingdom***

CO-AUTHORS: ***Tianrong Yeo, Maciej Jurynczyk, George Tackley, Tim D.W. Claridge, Ana Cavey1, Mark R. Woodhall, Siddharth Arora, Torsten Winkler, Eric Schiffer, Angela Vincent, Gabriele DeLuca, Nicola R. Sibson, M. Isabel Leite, Patrick Waters, Jacqueline Palace, Daniel C Anthony***

Accurate staging for individuals with multiple sclerosis (MS) is essential for effective treatment and management, but it is difficult to deliver within the constraints of clinical practice. As there is no diagnostic biomarker or biofluid test for MS, diagnosis depends on exclusion of all other possible explanations. The existence of several demyelinating diseases of the central nervous system (CNS), all with clinical phenotypes overlapping with MS, can make this particularly challenging. We have discovered, using a range of metabolomics techniques (including nuclear magnetic resonance spectroscopy, mass spectrometry, and lipidomics) coupled with multivariate analysis, that the serum metabolite profile is able to diagnose MS with 100% accuracy compared to healthy controls. Furthermore, this metabolite profile discriminates MS from similar CNS diseases which are impossible to separate using clinical features alone; aquaporin-4 (AQP4)-antibody (Ab) and myelin oligodendrocyte glycoprotein (MOG)-Ab diseases with accuracies of 92 and 73% respectively. All three diseases exhibited a unique metabolite pattern which was independent of disease severity and serum antibody level suggesting that metabolomics of serum may be of use in diagnosing patients with CNS demyelinating diseases. Finally, we have demonstrated that metabolomics can be used to distinguish between the early relapsing remitting (RR) stage and the secondary progressive (SP) stage of MS with high accuracy up to one year earlier than in the clinic.

P-443 Metabolomic study for the evaluation of the treatment of major depression by ultrasound neurostimulation

PRESENTING AUTHOR: ***Emond Patrick, Université de Tours - Inserm 1253 iBrain, France***

CO-AUTHORS: ***Marc Legrand, Laurent Galineau, Antoine Lefevre, Catherine Belzung, Ayache Bouakaz***

Major depression is a global public health problem. Antidepressant treatment represents the classical approach, although 50% of patients do not benet from improvement. Ultrasound neurostimulation (USNS) has recently been proposed for the non-invasive stimulation of brain tissue and is attracting increasing interest. This work proposes to evaluate the efficacy of the USNS in an unpredictable mild chronic stress mouse model using behavioral tests associated with a PET imaging study and a metabolomic study. Thisstudy was carried out by LC-HRMS using an approach targeting a chemical library of 500 metabolites and on different brain regions that have been targeted by ultrasound treatment (cortical regions: cingulate, limbic and prelimbic) as well as deeper regions (amygdala and hippocampus) because of their implications for major depression and their interconnectivity. The results obtained show that treatment with USNS produces alterations in the metabolome of all brain areas studied. In particular, the alanine, aspartate and glutamate pathway is altered in all regions and we observed increased levels of glutamic acid in the PrL / IL cortex (FC = 0.37) as well as a decrease in glutamine in the Cg and hippocampus after USNS. Beyond the conclusion that the USNS represents a promising approach for the treatment of major depression; this study reinforces the idea that metabolomics is an approach of choice to evaluate the effect of new therapeutics by dissecting mechanisms of action.

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NEUROLOGY AND PSYCHIATRY

P-444 Highly Effective Preprocessing for LCMS-Based Plasma Metabolite Biomarker Analysis of Depression

PRESENTING AUTHOR: ***Daiki Setoyama, Kyushu University Hospital, Japan***

CO-AUTHORS: ***Dongchon Kang***

Conventional plasma metabolite biomarker analysis using mass spectrometry has been intensively investigated without any consideration of their mode of transport in the blood. In this presentation, we propose a highly effective preprocessing methodology for LCMS-based plasma metabolite biomarker analysis, which allows to separate plasma metabolites into free- and protein-bound form, and then verify the effectiveness of them in the free-form on biomarker investigation using clinical samples. It is generally known that hydrophobic fatty acids are mostly bound with transport proteins such as albumin, whereas hydrophilic amino acids except tryptophan are present in the free form. Interestingly, 10-20% of tryptophan and the structurally-related metabolites are found to be present as the free form in the blood. Since these compounds have been shown to be depression-related biomarkers1, we addressed whether the free form of the metabolites could be useful information for characterizing depressive patients and/or severity of depression. As a result, plasma kynurenic acid in the free form, but not whole amount of one, is identified to be the best biomarker that significantly correlated with the severity of depression and shows highest predictive performance using plasma from drug-free depressive patients(n=16). Therefore, these results suggest that the free form of the plasma metabolites may be a promising information for the biomarker analysis, more reflecting the pathological condition in the blood. 1Setoyama et al. Plasma Metabolites Predict Severity of Depression and Suicidal Ideation in Psychiatric Patients-A Multicenter Pilot Analysis. PLoS ONE. 2016 11(12):e0165267.

P-445 NMR based Selective Screening in urine for inborn errors of metabolism linked to the METAGENE database

PRESENTING AUTHOR: ***Manfred Spraul, Bruker BioSpin Germany, Germany***

CO-AUTHORS: ***Frauendienst-Egger G, Götz H, Cannet C, Beedgen L, Godejohann M, Schäfer H, Spraul M, Trefz F***

Introduction NMR analysis in urine is a new approach for highly quantitative and reproducible measurement of a high number of analytes with different substance classes running on one platform. Because of the huge number of information provided by the NMR report an automatic evaluation showing out of normal range results and their interpretation for possible diagnoses is desirable. Method Quantitative NMR analysis (Bruker Biospin Avance IVDr, B.I.Quant-URTM) was performed automatically for 152 metabolites of 12 substance classes. Metagene, a knowledgebase for Analysis support of inborn errors of metabolism (www.metagene.de) was adopted for direct interpretation of the NMR reports. Ranking of potential diagnoses explainable by the metabolic findings in the report is done by comparison to the disease database in Metagene containing 209 diseases and differential diagnoses. Results In 60 known metabolic diseases the diagnosis was made by conventional analysis and data based automatic ranking. Comparison to the NMR based method showed high concordance. Using click boxes to add additional information as age and clinical symptoms to the quantitative results, rational ranking was highly improved. Diseases which may be well-defined by one characteristic metabolite (e.g. L-Alloisoleucine in MSUD) show the best rankings. Conclusion NMR analysis provides an excellent tool for using automatic analysis to further enable high throughput screening of urine samples and to improve yield of genetic metabolic diseases in the metabolic laboratory.

INTERNAL MEDICINE

P-446 Assessment of induced CYP3A activity in pregnant women using 4β-hydroxycholesterol: cholesterol ratio as an appropriate metabolic marker

PRESENTING AUTHOR: ***Bora Kim, Seoul National University College of Medicine and Hospital, Korea, South***

CO-AUTHORS: ***Andrew HyoungJin Kim, Su-jin Rhee, Joo-Youn Cho***

Pregnancy is associated with marked changes in drug metabolizing enzyme activity, which can alter the therapeutic response to various drugs. Previous studies reported increase of CYP3A activity during pregnancy when evaluated with several CYP3A probe drugs. However, pharmacokinetics and pharmacodynamics information in pregnant women is lacking due to the safety issues. This study aimed to evaluate the changes of CYP3A activity in the pregnant women along trimesters using endogenous markers, which have advantage of avoiding unnecessary drug exposure and invasive sampling. Forty-eight pregnant women and 25 non-pregnant women were enrolled in this study. Plasma and urine samples were collected from the pregnant women during each trimester and from the non-pregnant women. The concentrations of six steroid markers (cholesterol and 4β-hydroxycholesterol in plasma, and cortisol, 6β-hydroxycortisol, cortisone and 6β-hydroxycortisone in urine) were quantified by gas chromatography triple quadrupole mass spectrometry. Finally, the CYP3A activity was evaluated by metabolic ratio of 4β-hydroxycholesterol/cholesterol, 6β-hydroxycortisol/cortisol, and 6β-hydroxycortisone/cortisone. Plasma concentrations of cholesterol and 4β-OH-cholesterol significantly increased as pregnancy progressed (1sttrimester < 2ndtrimester < 3rdtrimester). However, an increased 4β-OH-cholesterol/cholesterol ratio, consistent with high CYP3A activity, was observed in pregnant women compared with that in non-pregnant women; however, no differences were observed among trimesters. No significant differences were observed in urinary markers (6β-OH-cortisol/cortisol and 6β-OH-cortisone/cortisone). In conclusion, a significant change in CYP3A activity consistent with an altered 4β-hydroxycholesterol/cholesterol ratio was observed following pregnancy. Based on this result, we suggest that the plasma marker 4β-OH-cholesterol/cholesterol ratio be used to measure CYP3A activity in pregnant women.

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POSTER SESSIONS 3 AND 4 – **Wednesday and Thursday –** all even number presenters will be at their posters.

INTERNAL MEDICINE

P-447 Intelligent MSn-based untargeted metabolomics workflow for biomarker discovery in Crohn's disease

PRESENTING AUTHOR: ***Amanda Souza, Thermo Fisher Scientific, United States***

CO-AUTHORS: ***Ioanna Ntai, Ralf Tautenhahn, Gina Tan, Andreas Hühmer***

Crohn's disease is a chronic inflammation of the gastrointestinal tract where the etiology remains largely unknown. LC-MS profiling of serum metabolites is a powerful tool for biomarker detection. We explored the use of an intelligent LC-MSn untargeted metabolomics workflow to identify discriminant markers of Crohn's disease by analyzing 18 serum samples from healthy donors and disease donors with or without infliximab treatment. Here, an Orbitrap Tribrid™ mass spectrometer with modified acquisition software enabled dynamic acquisition for automatic data dependent MS/MS and multistage fragmentation (MSn). Differential analysis and compound annotation were applied using Thermo Scientific™ Compound Discoverer™ software. Accurate m/z measurements determined molecular formula and putative annotation of thousands of metabolites by searching against ChemSpider. To increase annotation confidence, MS/MS data was searched against the mzCloud library. MSn enabled structural elucidation of unknowns. The modified instrument control software automated inter-run inclusion and exclusion lists, facilitating MSn acquisition for more unique metabolites. Differential analysis detected metabolite perturbations in the urea cycle and catabolism of amino acids in serum from disease donors when compared to that of healthy donors, metabolic changes often associated with inflammation. Some of those inflammation-driven changes were minimized when disease donors were treated with infliximab. The combination of an untargeted metabolomics profiling approach with intelligent high resolution MSn acquisition described here, addresses the identification bottleneck in untargeted metabolomics. This strategy presents a promising and facile workflow for the discovery and identification of novel disease biomarkers. For Research Use Only. Not for use in diagnostic procedures.

P-448 NMR metabolomic profiling of serum and exhaled breath condensates discriminate patients with asthma COPD overlap (ACO) from asthma and COPD

PRESENTING AUTHOR: ***Nilanjana Ghosh, School of Medical Science and Technology, Indian Institute of Technology Kharagpur, India***

CO-AUTHORS: ***Priyanka Choudhury, Elavarasan Subramani, Mamata Joshi, Rintu Banerjee, Parthasarathi Bhattacharyya, Sushmita Roy Chowdhury, Koel Chaudhury***

Asthma COPD overlap (ACO) presents itself with persistent yet reversible airflow limitation, a history of asthma or COPD, atopy, allergies, exposure to noxious agents with clinical manifestation of either sputum neutrophilia or eosinophilia, and age 40 years or older. Given the nascent nature of ACO, no single accepted definition exists for the disease so far. ACO is has a highly confusing clinical identity without any clear diagnostic or therapeutic guidelines. In this study we sought to determine whether ACO is a single disease entity or simply a manifestation of the characteristic features of asthma and/or COPD at a metabolic level. We characterized paired serum and exhaled breath condensates (EBC) samples of asthma (n=32), COPD (n=32), ACO (n=40) and healthy controls (n=33) using nuclear magnetic resonance (NMR) spectrometry. Multivariate analysis identified 8 metabolites (glucose, pyruvate, valine, lysine, glutamine, phenylalanine, creatine and ornithine) to be down-regulated in serum of ACO patients when compared with asthma, COPD and healthy controls. However, the expression was not similar in EBC; here 3 metabolites (pyruvate, valine and fatty acid) were up-regulated and 3 (propionate, acetone and acetate) down-regulated in ACO cases. The findings suggest that ACO has an enhanced energy and metabolic burden associated with it when compared to asthma and COPD. Identification of unique metabolic signatures in the present study is expected to motivate researchers and direct their attention towards unravelling the intricacies of ACO pathophysiology.

P-449 Identification of biomarkers for COPD associated PH in a minimally-invasive manner: A 1H NMR based metabolomics study

PRESENTING AUTHOR: ***Priyanka Choudhury, School of Medical Science and Technology, Indian Institute of Technology Kharagpur, India***

CO-AUTHORS: ***Nilanjana Ghosh, Mamata Joshi, Parthasarathi Bhattacharyya, Koel Chaudhury***

Group III pulmonary hypertension (PH) encompasses PH associated with hypoxic lung diseases including chronic obstructive pulmonary disease (COPD). PH is considered to be a delayed complication of COPD, responsible for increased morbidity and mortality of COPD patients. COPD associated PH (COPD-PH) often gets overshadowed by the symptoms of COPD and therefore, the disease presents a diagnostic challenge to the clinicians. Right heart catheterization, the gold standard diagnostic tool, is an invasive process and is not always feasible. This study is the first of its kind and we hypothesize that 1H nuclear magnetic resonance (NMR) based metabolomics may help in the identification of novel candidate markers for accurate diagnosis of COPD-PH. Serum samples were collected from patients with pure COPD (n=33), COPD-PH (n=31) and healthy controls (n=37). NMR spectra of the samples were acquired using 800 MHz BrukerAvance III spectrometer and the data subjected to univariate and multivariate analysis. On comparing COPD-PH with COPD and controls, a distinct metabolic differentiation of COPD-PH was obtained with the OPLS-DA model. R2Y and Q2 values [COPD-PH vs COPD (R2Y=0.902 and Q2=0.755); COPD-PH vs controls (R2Y=0.94 and Q2=0.898)] validate that the model fits well and has a good predictive ability. Receiver operating characteristic curve identified the metabolites responsible for distinguishing COPD-PH which include isoleucine, valine, L-leucine, acetic acid, glutamate, glucose, betaine, glycerol and creatinine. These metabolites seem promising and may be validated as potential biomarkers of COPD-PH in a fresh patient cohort. The possibility of diagnosing these patients at an early stage is also envisioned.

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POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

INTERNAL MEDICINE

P-450 1H NMR metabolic profiling of allergic and non-allergic asthma in early childhood

PRESENTING AUTHOR: *Chiang Meng Han, Clinical Metabolomics Core Laboratory, Chang Gung Memorial Hospital, Taiwan*

CO-AUTHORS: *Chih-Yung Chiu, Gigin Lin*

Asthma is a complex disease with a diverse genetic and environmental interaction. Although there is a strong association between IgE and allergy, it can be induced without increase in allergic response. Despite the fact that there are distinct pathogenesis and clinical features between allergic and non-allergic asthma, few studies have addressed the potential molecular mechanisms between allergic and non-allergic asthma by using metabolomics, especially in children. The aim of this study was to investigate plasma metabolic profiling in children with asthma and identify IgE-mediated metabolites associated with asthma. Spot plasma samples were collected and metabolites were analyzed by 1H-nuclear magnetic resonance spectroscopy (NMR). NMR spectra were processed by a new open source software NMRProcFlow and subsequently analyzed by MetaboAnalyst. In this study, a total of 53 children with mean age of 3.6 ± 0.7 years were enrolled. Among them, there were 13 allergic asthmatics, 15 non-allergic asthmatics, and 25 healthy controls. After analysis, total 64 buckets refer to 46 known metabolites were identified by Chenomx software. Compared to healthy controls, six and three metabolites were significantly different in children with non-allergic and allergic asthma respectively. Most importantly, isobutyric acid, fumarate, glutamine and valine were significantly associated with IgE-mediated asthma ($p < 0.05$). Further metabolic pathway analysis of these metabolites revealed metabolism associated with amino acid including alanine, aspartate and glutamate. In clinics, a comprehensive analysis of molecular mechanisms and functions related to these 4 metabolites could potentially provide further clinical management and treatment in children with allergic asthma.

P-451 An untargeted metabolomics approach using label free LC-DIA-MS method to identify potential biomarkers in spontaneous pre-term birth (sp-PTB)

PRESENTING AUTHOR: *Shirish Yakkundi, University College Cork, Ireland*

CO-AUTHORS: *Dr. Lee Gethings, Ms Aude Claire Morillon, Prof James Langdrige, Prof Louise Kenny*

There is currently no clinically useful screening test, hence spontaneous pre-term birth (sp-PTB) remains the leading cause of perinatal morality. Early sp-PTB is defined as delivery before 34 weeks' gestation and is particularly associated with high rates of mortality, morbidity, hemorrhage, respiratory distress syndrome and neurological deficits. The BiomarkErs FOR Early (BEFORE birth) project is looking at untargeted metabolomics for identification of a useful early pregnancy-screening test for sp-PTB in the maternal blood. 20-week heparinised plasma samples (Auckland, SCOPE study) from women whose pregnancies reached term gestations (Control) as compared to pregnancies subsequently complicated by sp-PTB prior to 34 weeks' gestation (Case) were used. Plasma samples were extracted using IPA based extraction methods. Quality Control (QC) sample was constructed by pooling aliquots from all samples (cases and controls). Samples were stratified, randomised (before extraction and MS analysis) and data was acquired, with the pooled QC sample injected every 10 injections. Samples were treated with isopropanol, stored overnight at -20°C and centrifuged to precipitate proteins. Data were collected for all samples in triplicate using LC-MS on a hybrid quadrupole oa-TOF mass spectrometer (Synapt G2-S) for positive and negative ion respectively. The label-free data were normalised, processed and database searched [HMDB, LipidMaps, ChemSpider (KEGG-ChEBI)] using the software Progenesis QI (Nonlinear dynamics, UK). Further analysis showed around 21 features were statistical significant, some showing a differential fold change of 3-fold. Features include ceramides (Cer), steroids, phosphatidylcholines (PC), phosphatidylserine (PS), diglycerides (DG) and triglycerides (TG), hence highlighting that lipids could be the key biomarkers.

P-452 Utility of Trans-pulmonary Whole Blood Metabolomics to Distinguish Lung Metabolism in Experimental Septic and Hemorrhagic Shock

PRESENTING AUTHOR: *Kathleen A. Stringer, Michigan Center for Integrated Research in Critical Care, United States*

CO-AUTHORS: *Robert P. Dickson, Hakam Tiba,Yihan Sun, Brendan MacCracken, Brandon Cummings and Kevin R. Ward*

Assessment of the change in metabolite concentration difference between arterial and venous blood may give insight into organ specific metabolite utilization. To test feasibility, we employed swine models of hemorrhagic (HS) and septic shock (SS). HS was induced by hemorrhage to an oxygen debt of 80 mL/min (D80); SS, by direct injection of E. coli into the kidney parenchyma. Paired PA and CA WB samples were drawn at multiple time points. Metabolomics data were generated by quantitative 1H-NMR spectroscopy. Baseline PA-CA difference (DPA-CA) was zeroed and the median change for total DPA-CA at each time point was compared to baseline. In addition, the median DPA-CA at baseline and peak shock were determined for each metabolite. A total of 37 WB metabolites were quantified. Shock resulted in an overall increase in total metabolite excretion (decrease in DPA-CA) over time (HS: D80, $p<0.0001$; SS: 18h, $p<0.01$). Lactate and glucose had the greatest absolute DPA-CA in both models. At peak shock (SS, T18h: MAP 32.5+6.3; HS, D80: MAP 29+3.8 mm Hg; $p=0.3$), the DPA-CA for all metabolites except for acetate and ATP, was positive, indicating extraction by the lungs during SS. However at peak HS, the DPA-CA for all metabolites except for 3-hydroxybutyrate, 2-oxoisocaproate, and IMP was negative, indicating excretion by the lungs. These data support the concept that DPA-CA can distinguish metabolomics profiles across the lung due to different etiologies of shock. As such, this approach could be used to further understanding of lung specific metabolic changes that underlie shock-induced lung injury.

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INTERNAL MEDICINE

P-453 NMR-based metabolomics: understanding Cystic Fibrosis

PRESENTING AUTHOR: ***Abigail Leggett, Ohio State University, United States***

CO-AUTHORS: ***Lei Bruschweiler-Li, Kathrin Krause, Anup Viadya, Kaitlin Hamilton, Amal Amer, Rafael Bruschweiler***

One of the unique strengths of metabolomics is the ability to study complicated diseases through both genomic and environmental effects that result in the disease phenotype. NMR-based metabolomics can globally detect, quantify, and provide structural information of all metabolites in a complex biological mixture that are relatively concentrated. Cystic fibrosis (CF) results from a mutation causing abnormally functioning ion transport channels in most cells, including epithelial and immune cells. The lungs are most affected in CF as they elicit severe inflammation, often involving infection, ultimately leading to respiratory failure. While there have been many studies on the mutated channels, the pathobiology of CF is poorly understood. Additionally, macrophages in CF have been shown to be defective in autophagy, but the origin of this defect remains unclear. Metabolomic profiling of CF samples may provide a better understanding of the phenotype exhibited, with the potential to inspire new approaches for treatment. Furthermore, studying metabolomics in the presence of bacterial infections may reveal pathways that could be targeted to restrict infection. We will discuss metabolites identified from mouse lung tissue and bone marrow-derived macrophages in the absence and presence of bacterial infections in relationship to altered metabolism and immune response in CF.

P-454 MONITORING OF BIOLOGICAL PROCESSES IN ACTIVE PULMONARY TB PROGRESSION THROUGH A METABOLOMICS APPROACH: IMPLICATIONS FOR DRUG DISCOVERY

PRESENTING AUTHOR: ***MIGUEL FERNÁNDEZ-GARCÍA, CEU San Pablo University, Spain***

CO-AUTHORS: ***Maria Fernanda Rey-Stolle, Antonia Garcia, Vineel P Reddy, Joanna Godzien, Julien Boccard, Serge Rudaf, Adrie JC Steyn, Coral Barbas***

Introduction and Objectives: The global burden of tuberculosis (TB) is vast, 10.44 million new TB cases and 1.7 million deaths in 2016, mostly in tropical countries1. Multidrug-resistant strains of Mycobacterium tuberculosis seriously threaten TB control and prevention efforts. Metabolomics allows studying the host-bacterial co-metabolome and clarifying mechanisms of onset and prognosis of mycobacterial diseases. Therefore, we aimed to obtain a deeper knowledge of the pathophysiological processes underlying disease onset and progression and suggest potential therapeutic targets through an untargeted metabolomics approach. Methods: Uninfected and TB-infected lung tissues at 4 and 9 weeks after infection from a mouse model of active TB were analysed through a multiplatform approach with GC-QTOF-MS, CE-TOF-MS and LC-QTOF-MS, all accurate-mass platforms. Previously CEMBIO analytical protocols were optimised2, including fully robotised derivatization. Univariate and multivariate statistical analysis were applied from which consensus OPLS-DA3, a high-level data fusion approach, was employed to track global metabolic changes. Associated metabolic changes were evaluated by over-representation analysis (ORA) and pathway analysis (PA). Results and Conclusions: Samples clustered according to infection time trajectory. By the inference of data from the alterations occurring in the lung of TB-infected mice and previous studies, we suggest that our analysis is capable of monitoring highly-interconnected biological processes particularly related to the central carbon metabolism, oxidative stress, proteolysis, inflammation, immunomodulation, and lipidome regulation. Ultimately, enzymes potentially involved in such processes are proposed as candidates for anti-TB drug development. 1. WHO (http://www.who.int/mediacentre/factsheets/fs104/en/) 2. Naz, S et al. (doi: 10.1007/s00216-013-6882-5) 3. Boccard, J et al. (doi:10.1016/j.aca.2013.01.022)

P-455 Metabolomic Profiling of Glucose-6-Phosphate Dehydrogenase Deficient Erythrocytes

PRESENTING AUTHOR: ***Alexandra C. Schrimpe-Rutledge, Vanderbilt University, United States***

CO-AUTHORS: ***Alexandra C. Schrimpe, Jagrati Jain, Simona G. Codreanu, Stacy D. Sherrod, David W. Wright, Larry Walker, Babu L. Tekwani, and John A. McLean***

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathy with more than 400 million cases worldwide. G6PD deficient (G6PDd) individuals are at an increased risk for hemolytic anemia triggered due to infections, dietary supplements, and drugs known to cause oxidative stress. An understanding of the cellular and molecular basis for the drug-induced hemolytic toxicity is critical for prevention of toxicity and design of safer drugs for G6PDd individuals. A global untargeted metabolomics analysis was performed using ultra high performance liquid chromatography/high resolution tandem mass spectrometry analyses. Data were processed and analyzed using an untargeted metabolomics workflow (Progenesis QI v2.0, Waters Corporation). Nearly 1000 features were detected by hydrophilic interaction chromatography (HILIC) positive ion mode data acquisition. Unsupervised principal component analysis of the metabolomic dataset showed distinct clustering of normal and G6PDd erythrocyte sample groups as well as separation of G6PDd types Med- and A-. Annotations were filtered for endogenous small molecules present in the HMDB database and classified into confidence levels based on supporting evidence. Among the detected features, 128 were found to be statistically significant different in G6PDd erythrocytes versus normal erythrocytes (fold change ≥ 1.5 and p-value ≤ 0.05). Metaboanalyst and mummichog software were used to extract biological pathway knowledge from the prioritized list of annotated compounds with altered abundance in G6PDd erythrocytes. The GSH-methionine-glutamate pathway metabolites were greatly affected by G6PD deficiency. Perturbations to the erythrocyte metabolome in the G6PDd genotype were consistent with an alteration of amino acid metabolism as well as the antioxidant defense systems.

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INTERNAL MEDICINE

P-456 Liver metabolomics in Mice with Erythropoietic Protoporphyria

PRESENTING AUTHOR: ***Pengcheng Wang, University of Pittsburgh, United States***

CO-AUTHORS: ***Madhav Sachar, Jie Lu, Xiaochao Ma***

Erythropoietic protoporphyria (EPP) is a genetic disease resulted from defective mutation of ferrochelatase (Fech), the enzyme that converts protoporphyrin IX (PPIX) to heme. Liver disease is one of the major complication in EPP patients, and around 5% of EPP patients will develop liver damage and even liver failure. However, no ideal approach is available for the management of EPP-associated liver disease. The current study investigated the biochemical basis of EPP-associated liver injury in Fech mutant (Fech-mut) mice, a genetic mouse model of EPP, using a metabolomic approach. As expected, we observed excessive PPIX accumulation in the liver of Fech-mut mice. We also observed the accumulation of bile acids in the liver and serum, suggesting cholestasis in Fech-mut mice. Furthermore, we found the phospholipids and sphingolipids homeostasis was disrupted in the liver of the Fech-mut mice. Specifically, phosphatidylcholine (PC), lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE), and lysophosphatidylethanolamine (LPE) were significantly decreased, whereas ceramides (CM) were significantly increased in the liver of Fech-mut mice when compared with WT mice. We further illustrated that the decrease of PE and PC was due to the elevation of biliary excretion and the accumulation of CM was due to the increase of biosynthesis. In summary, this study reveals that PPIX accumulation disrupts bile acids, phospholipids, and sphingolipids homeostasis, which provide novel insights into the pathophysiological basis of EPP-associated liver injury.

P-457 Untargeted metabolomics for prediagnostic biomarkers discriminating between acute and chronic nephritis in rat model

PRESENTING AUTHOR: ***Woori Chae, Seoul National University, Korea, South***

CO-AUTHORS: ***Bora Kim, Kyoung Hee Han, Hee Gyung Kang, Il Soo Ha, Joo-Youn Cho***

Nephritis is a common condition where the kidneys become inflamed. It can be manifested by various causes in several types. Glomerulonephritis is one of the common types in nephritis and can be classified into acute and chronic nephritis according to the onset of disease. To prevent severe kidney failure, early diagnosis and appropriate treatment are essentially required. We have sought to find the novel metabolic biomarkers to prediagnose acute or chronic nephritis in anti-Thy1-induced nephritis rat model. Sham surgery was applied to the acute nephritis model and uninephrectomy was used for the chronic nephritis model. Serum and urine samples were collected and untargeted metabolomics analysis was done by ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry. We have identified three urinary metabolites, epoxyoctadecenoic acid (EpOME), dihydroxyoctadecenoic acid (DiHOME) and α-linolenic acid (ALA). EpOME is known to be mainly metabolized into DiHOME by soluble epoxide hydrolase (sEH). Compared to acute nephritis group, urinary levels of EpOME and DiHOME were higher in chronic nephritis group and those metabolites gradually increased over time, which reflect continuous inflammatory state in chronic nephritis. ALA, one of endogenous sEH inhibitors, was also higher in early-stage urine of chronic nephritis group, which may contribute to the levels of EpOME and DiHOME in vivo. In conclusion, we have found prediagnostic biomarkers that can discriminate between acute or chronic nephritis in the early state of nephritis. Furthermore, these biomarkers may contribute to understand the mechanism of pathogenesis in nephritis.

P-458 Metabolodmics study of the therapeutic mechanisms of ursodeoxycholic acid in liver function abnormal patients

PRESENTING AUTHOR: ***Da Jung KIM, Seoul National University College of Medicine and Hospital, Korea, South***

CO-AUTHORS: ***Da Jung KIM, Sang-Chun Ji, SeungHwan Lee, Kyung-Sang Yu, In-Jin Jang, Sunghae Yoon, Jae-Yong Jeong, Joo-Youn Cho***

Background & Aimes: Ursodeoxycholicacid (UDCA) is a metabolic byproduct of intestinal bacteria and has long been used as a treatment for hepatoprotective activities.Molecular mechanism governing its protection remains unclear. The purpose of this study was to evaluate the therapeutic mechanisms of UDCA on liver dysfunction using LC-QTOFMS based metabolomics analysis. Methods: In this study, we analyzed plasma and urine samples from patients with abnormality of liver function test (LFT) (ALT 200 and BMI 24 0 kg/m2). Nine of them were assigned to take UDCA 300 mg and ten subjects received Vitamin E 400 IU twice per day for 8 weeks. Results: UDCA significantly improved liver function and was also effective to reduce hydrophobic bile acid (deoxycholic acid) and serum miR-122 level. To better understand its protective mechanism, global metabolomics study was conducted and we found that UDCA regulates uremic toxins (hippuric acid, p-cresol sulfate and indole-derived metabolites), antioxidants (ascorbate sulfate, N-acetly-L-cysteine) and phenylalanine/tyrosine pathways. Further, contribution of microbiome, such as Lactobacillus and Bifidobacterium was also found through metagenome study. Vitamin E (vit E) treatment, conversely, did not reveal such mechanistic alternations except for reducing uremic toxins and LFT scores. Conclusion: UDCA was able to regulate hydrophobic bile acid, serum miR-122, levels of metabolites belonged to phenylalanine/tyrosine pathway and microbial compositions. Therefore, UDCA and vit E both were effective to improve liver function, underlying mechanisms might differ over the antioxidative effects.

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POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

INTERNAL MEDICINE

P-459 Metabolite profiles of Maternal Plasma and Uterus during the Peri-implantation Period of Early Pregnancy in Mice

PRESENTING AUTHOR: ***Ting-Li Han, The University of Auckland, New Zealand***

CO-AUTHORS: ***Yang Yang, Hua Zhang, Philip N Baker***

Background: Embryo implantation is a complex progression, which involves biochemical and physiological interactions between an implantation-competent blastocyst and receptive uterus. However, the exact biochemical changes of the uterus during implantation remains unclear. Aim: To explores the biochemical and physiological variations during the peri-implantation period of early pregnancy using mice as an animal model. Method: Gas chromatography-mass spectrometry was used to characterize the metabolite profiles of plasma and uterus between pre-implantation, implantation, and decidualization. Compound identification and quantification were performed by our in-house MassOmics software. Multivariate analysis of ANOVA and Tukey's HSD test were applied to detect significant changes in metabolites and metabolic pathways. Results: Dynamic global metabolic changes were observed in plasma, while little was shown in the uterus between pregnancy at day 1 and pregnancy at day 4. Five days after pregnancy, we found a lower level of amino acids and a higher level of TCA cycle intermediates on implantation site compared with inter-implantation as well as plasma compared with pseudopregnancy of uterus. This suggests an increasing consumption of amino acids and increasing energy production for embryo implantation. Moreover, comparisons between artificially induced decidualization (ID) and non-ID identified significant differences in 56 metabolites, including amino acids, TCA cycle intermediates, fatty acids, antioxidants, and cofactors. Metabolic pathway analysis showed that amino acid metabolism, carbohydrate metabolism, and energy metabolism were upregulated in ID. Conclusion: Our study demonstrated that central carbon metabolism and energy metabolism were metabolic remodeling in the receptive uterus, thus appearing to be important for embryo implantation.

P-460 Prospectively Assessed Plasma Metabolites and Risk of Rheumatoid Arthritis in the Nurses' Health Study Cohorts

PRESENTING AUTHOR: ***Su H. Chu, Brigham and Women's Hospital & Harvard Medical School, United States***

CO-AUTHORS: ***Kevin Blighe, Jing Cui, Jeffrey A. Sparks, Medha Barbhaiya, Sara K. Tedeschi, Cianna Leatherwood, Cameron B. Speyer, Clary B. Clish, Jessica Lasky-Su, Karen H. Costenbader, Elizabeth W. Karlson***

Background:Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease, resulting in synovitis and joint destruction. During the several year pre-clinical period, autoantibodies and elevated systemic inflammatory biomarkers are detectable, representing an important period for predicting RA risk. While several studies have previously explored global metabolomics profiling of RA with promising results, they have suffered from small sample sizes, heterogeneity of clinical features, and cross-sectional study design. Methods: We performed untargeted liquid chromatography mass-spectrometry molecular profiling of plasma samples from 256 women who subsequently developed RA and 511 matched controls in the Nurses' Health Study (NHS) I and II. After quality control, 437 unique, known metabolites were analyzed for association with RA risk, using conditional logistic regression. Metabolites were assessed for association with RA overall, and stratified by samples collected 1-5 or > 5 years prior to diagnosis. Results:Metabolites most strongly associated with increased RA risk included C18:1 lysophospholipid (OR: 1.23, p=0.012), and C22:0 lysophosphatydlserine isomer (OR: 1.22, p=0.018). Conversely, 4-acetamidobutanoic acid (OR: 0.78, p=0.006) and N-acetyltryptophan (OR=0.83, p=0.031), C5:1 carnitine (OR: 0.84, p=0.016), and C5 carnitine (OR: 0.82, p=0.043) were associated with decreased risk. In the 1-5 years pre-RA, homoarginine, which has previously been positively associated with cardiometabolic diseases such as type II diabetes, was significantly associated with RA (OR: 1.77, p=0.002). Conclusion: Several metabolites were associated with RA risk in women using a prospective, matched case-control study design. Replication in an independent cohort is currently underway to confirm findings.

P-461 Metabolic Phenotyping of Urine in Asthmatic Cohort: Preliminary data

PRESENTING AUTHOR: ***Marianne Koliana, Imperial College London, United Kingdom***

CO-AUTHORS: ***Jonathan R. Swann, Matthew Lewis, Jake T.M. Pearce, Clair Barber, Peter H. Howarth, Gary Frost, Elaine Holmes***

Asthma is a chronic inflammatory disease with high complexity and heterogeneity, with variation within patients as well as in between. The complexity has resulted in increased difficulties of diagnosing and treating asthmatics, in particular severe asthmatics. Metabolic phenotyping could contribute to the possibility of discovering biomarkers that could aid the diagnosing of asthmatics. Metabolic profiling using 1H NMR spectroscopy and UPLC-MS was performed on urine samples collected from 377 individuals, consisting of 72 healthy controls, 66 mild to moderate asthmatics and 239 severe asthmatics. Multivariate and univariate analysis were performed on the spectral data and statistical models were generated. Multivariate analysis on the 1H NMR spectroscopy data suggested metabolic correlation with YKL-40, a protein in serum which is increased with asthma severity. This finding was also observed in the UPLC-MS data. Furthermore, data suggests metabolic differences between healthy controls, mild to moderate and severe asthmatics. Differences in BMI, anxiety, depression and asthma control for all the subjects was also perceived. The same observations were also seen for only severe asthmatics. These results suggest that there is a metabolic difference between healthy patients and asthmatics as well as other factors triggered from asthma. The findings for severe asthmatics demonstrates the complexity of this group and the possibility of separating subjects within. These results opens up the possibility of finding potential biomarkers that could contribute to diagnosing asthma and asthma severity.

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INTERNAL MEDICINE

P-462 Endometriosis: a deep insight into the pathology through metabolomics

PRESENTING AUTHOR: ***Justine Leenders, University of Liège, Belgium***

CO-AUTHORS: ***Martin Manon, Nisolle Michelle, Munaut Carine, Govaerts Bernadette, de Tullio Pascal***

Endometriosis is defined as the presence of endometrial tissue outside the uterine cavity, affecting approximately 10% of woment in reproductive age and is mostly associated with infertility and/or pelvic pain. The current lack of an efficient non-invasive diagnosis method greatly affects the delay between the first symptoms and the identification of the pathology. Therefore, the discovery of combination of biomarkers measurable in biofluids is essential for a more efficient treatment of patients. Metabolomics appears to be an innovative and powerful tool to obtain a deep insight into this disease. In this suty, we applied a NMR-based metabolomics analysis on urine and sera samples obtained from 90 patients with endometriosis ("endometriotic") and from 98 healthy patients ("controls"). The analysis of sera spectral data did not allow the distinction between the metabolic profiles of endometriotic and control patients. However, the analysis of urine samples revealed a clear separation between endometriotic and control groups. This distinction is already observable at an early stage of the disease. This analysis led to the identification of relevant changes in some metabolites levels. Finally, these changes in the metabolic profiles could be linked to some biochemical pathways that seems to be affected by endometriosis. These results could greatly help understanding the occurence and the evolution of this pathology. This study demonstrates that despite the complexity of this disease, metabolomics could be a valuable tool to explore endometriosis. The results obtained by the analysis of urine samples hold the key to finally unravel endometrial clinical biomarkers.

P-463 Distinguishing Adult Asthma from Health using Untargeted NMR-based Metabolomics

PRESENTING AUTHOR: ***Steven R Van Doren, University of Missouri, United States***

CO-AUTHORS: ***Yan G. Fulcher, Geneline Mazzola, Rachel Weaver, Mario Castro, Steven R. Van Doren***

Asthma is prevalent but difficult to diagnose. We compared body fluids such as Exhaled Breath Condensate (EBC) and serum for NMR-based diagnostic metabolomics. We applied to human specimens the methodological lessons learned from untargeted NMR-based metabolomics of EBC from cats with allergen-induced asthma (Fulcher et al. 2016, PLoS One). Though serum is more widely available, EBC captures droplets emanating directly from fluids in the lung. The Severe Asthma Research Program (SARP and SARP III) provided exploratory sets of samples from adult asthma patients. Using a limited number of EBC specimens, supervised statistics (OSC-PLSDA) separated asthma from health with an area under the ROC curve (AUROC) of 0.92. Using intact serum, both the lipoprotein component and the small molecule content can be analyzed and compared. Health and asthma were largely separable by applying supervised statistics to either the macromolecular or small molecule NMR spectra, yielding AUROC of 0.85 and 0.90, respectively. Removal of most of the protein from the serum specimens improved the resolution of the NMR spectra and improved statistical separations to an AUROC of 0.99.

P-464 Exometabolome of methamphetamine treated humane primary macrophages

PRESENTING AUTHOR: ***Katarzyna Lech, University of Nebraska Medical Center, United States***

CO-AUTHORS: ***Katarzyna Lech, Katarzyna Pawlak, Akou Vei, Emma Harwood, Spencer Jaquet, Pawel Ciborowski***

HIV-1 infection combined with the use of illicit drugs such as methamphetamine (meth) has devastating effects, at various levels, on the function of the entire organism. The complexity of HIV infection and drug abuse treatment is complicated as it targets two diseases that are different in nature. The main goal of this study is to perform full, unbiased mass spectrometry based extracellular metabolomic profiling of human monocyte derived macrophages (MDM) infected with HIV and/or treated with meth. We expect that targeted profiling, computational biology, and bioinformatic analyses will uncover new and unreported mechanisms of MDM regulation and will help with modeling a functional network under pathological conditions. The effect of meth on the mechanisms regulating MDM metabolism is not known. Thus, we applied a multiple reaction monitoring (MRM) based targeted metabolomics approach coupling ultra-high performance liquid chromatography (UPLC) with a QTrap 6500 (Sciex) mass spectrometer with electrospray ionization (ESI) to study the exometabolome of methamphetamine-treated MDMs. Metabolites from culture supernatants of MDM were fractionated using two extraction techniques. The first was liquid-liquid extraction (LLE) with three different solvents (chloroform, methanol and water). The second was developed based on a solid phase extraction procedure (SPE) using a strong cation exchanger with mixed-mode sorbent characteristics and two-step elution (with mixture of organic solvents and alkaline). We have found a statistically significant increased secretion of propionyltyrosine and propionylproline. Finding differentially secreted exometabolites may provide insights into the metabolic alterations of MDM under the insult of methamphetamine.

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INTERNAL MEDICINE

P-465 GC-MS-based untargeted metabolomics workflow for biomarker discovery in Crohn's disease

PRESENTING AUTHOR: ***Xin Zheng, Thermo Fisher Scientific, United States***

CO-AUTHORS: ***Ioanna Ntai, Jason Cole***

Crohn's disease (CD) is a subtype of inflammatory bowel diseases (IBDs) often thought to be a result of genetic predisposition and environmental factors. Despite extensive research, the etiology and pathogenesis remain largely unknown. Gas chromatography-mass spectrometry (GC-MS) is one of the most commonly used analytical platforms for metabolomics studies due to its high sensitivity and low detection limits. In this study, 18 individual serum samples including six healthy donors, six CD donors not taking any medications, and six CD donors treated with infliximab were derivatized and analyzed on a GC coupled to a quadrupole-Orbitrap mass spectrometer with electron ionization interface. Compound identification was achieved using spectral deconvolution software equipped with a GC-Orbitrap™ high-resolution accurate mass (HRAM) metabolomics library. Chemical ionization was also performed to generate pseudo-molecular ion information for unknown identification. Excellent mass accuracy (< 1ppm) and ultra-high resolution (>60,000) offered on the Orbitrap™ analyzer allowed confident molecular formula elucidation for novel biomarkers. Significantly changed metabolites such as amino acids and TCA cycle metabolites were detected in CD donor serums. Differential analysis was employed on detected metabolite perturbations by using Thermo Scientific™ Compound Discoverer™ software. These metabolomic changes are often associated with inflammation. These inflammation-driven changes were minimized when CD donors were treated with infliximab, an immunosuppressive drug prescribed for CD. For Research Use Only. Not for use in diagnostic procedures.

WELLNESS AND AGING

P-467 The association of sleep with metabolic pathways and metabolites: evidence from the Dietary Approaches to Stop Hypertension (DASH) - Sodium Feeding Study

PRESENTING AUTHOR: ***Rachael Stolzenberg-Solomon, DCEG, NCI, NIH, United States***

CO-AUTHORS: ***Vanessa Gordon-Dseagu, Andriy Derkach, Qian Xiao, Ishmael Williams, Joshua Sampson***

Few epidemiologic studies have explored the associations between sleep habits and metabolomic profile. We examined the association between recorded sleep habits and 891 fasting plasma metabolites in a subgroup of 106 participants (89 with serial measures) with complete sleep data from the Dietary Approaches to Stop Hypertension (DASH)-Sodium feeding trial (1997-1999). We assessed the association between sleep (midpoint, duration, bed and wake time) and log transformed metabolites using linear random effects models. We then combined the resulting p-values using Fisher's method to calculate the association between sleep and 38 metabolic pathways. Sixteen pathways were associated ($p < 0.05$) with sleep mid-point, with the γ-glutamyl amino acid metabolism pathway reaching the Bonferroni-corrected threshold of 0.0013. Eighty-three metabolites were associated with sleep mid-point (FDR<0.20). Top metabolites (pathways given in brackets) associated with sleep were erythrulose (advanced glycation end-product) which was positive, and several γ-glutamyl pathway metabolites, CMPF (fatty acid, decarboxylate), isovalerate (fatty acid metabolism) and HWESASXX (polypeptide) which were inverse. We observed similar associations for wake time (41 metabolites). Neither bed time or duration were strongly associated. We found multiple metabolites and metabolic pathways associated with sleep midpoint and wake time. Several of the individual metabolites have previously been linked to inflammation and oxidative stress, key processes involved in diseases such as cardiovascular disease, diabetes and cancer.

P-468 The effect and possible mechanism of exercise training and diet restriction upon the age-induced elevation in cardio-metabolic risk factors

PRESENTING AUTHOR: ***Eman Elbassuoni, Minia University Faculty of Medicine, Egypt***

Metabolic syndromeis not a disease, per se. It is a cluster of factors including obesity, insulin resistance, hypertension and dyslipidemia that indicating a dysfunctional metabolism and has been identified as a multiplex risk factor for cardiovascular disease. Since metabolic syndrome and cardiovascular disorders increase with age, this study aims to determine effect and possible mechanism of both diet restriction and physical exercise training alone or combined with each other upon the age-induced elevation in cardio-metabolic risk factors. Methods: Fifty male albino rats classified equally into: young control (8 weeks) rats, 4 groups of aging (24 months) rats divided into; aging control, aging with twelve weeks diet restriction, aging with twelve weeks exercise, and aging with twelve weeks diet restriction and exercise. The following parameters were measured; blood pressure, blood glucose, insulin, lipid profile, some cardiac injury markers and some oxidative, inflammatory and apoptotic markers in blood and cardiac tissue. Results: control aging group displayed hypertension, dyslipidemia, insulin resistance, with significant increases in the blood cardiac injury markers, and the oxidative, inflammatory and apoptotic markers in blood and cardiac tissue. Both diet restriction and exercise in aging rats definitely decreased aging detrimental effect on the above measured parameters in blood and cardiac tissue, and this effect increase more when combined with each other. Conclusions: lifestyle modification based on behavior therapy as diet restriction and exercise can be considered as an effective nonpharmacological antiaging management against aging induced metabolic disorders and cardiac tissue damage through their anti-oxidative, anti-inflammatory, and anti-apoptotic effects.

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POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

WELLNESS AND AGING

P-470 NMR-Based metabolomics study of Age-related Macular Degeneration (AMD): from pre-clinic model to new target discovery

PRESENTING AUTHOR: ***Schoumacher Matthieu, Uliege_CIRM, Belgium***

CO-AUTHORS: ***V. Lambert, S. Hansen, J. Leenders, B. Govaerts, B. Pirotte, J-M. Rakic, A. Noël, P. De Tullio***

Age-related Macular Degeneration (AMD) is the leading causes of blindness among the elderly population in developed countries. 90% of all vision loss due to AMD result from the exudative form, which is characterized by choroidal neovascularization (CNV). Treatments are mainly based on regular intravitreal injection of anti-VEGF to stabilize CNV. Nevertheless, despite such important advances, some clinical issues remain to be addressed. Among those, the personalization of the therapeutic strategies and the discovery of new therapeutic approach are essential. In order to study CNV occurrence and evolution, we decided to apply a NMR-based metabolomics approach on a murine laser-induced CNV model and on patient's cohorts. Metabolomics led us to identify some metabolites linked to CNV developments in both human and murine samples. These metabolites could be considered not only as markers of the pathology, but also as putative target for a new treatment of AMD. Among those, lactate emerges as a key metabolite in both settings. Mechanistically, we demonstrated that lactate, initially produced in the eyes increases at the systemic level and plays a critical role in the onset of the inflammatory and angiogenic phases. The control of its systemic concentration by PDHK inhibition or by LDH modulation decreases significantly CNV development. Based on a metabolomics approach, our data support the innovative concept of lactate as a parainflammation- and angio-metabolite associated to AMD and CNV progression. It appears as a putative target for a new therapeutic approach as well as a useful marker for patient's follow-up during treatment.

P-471 A pilot study of untargeted metabolomics for classification of skeletal muscle ageing

PRESENTING AUTHOR: ***Daniel James Wilkinson, University of Nottingham, United Kingdom***

CO-AUTHORS: ***Warwick B. Dunn, Iain J Gallagher, Bethan E Phillips, Kenneth Smith, Philip J Atherton***

Classification using omics techniques has yielded gene clusters that associate with tissue ageing, including those exhibiting age-related declines e.g. skeletal muscle in relation to age-related sarcopenia (Sood et al., 2015. Genome Biol 16:185). Herein, we tested the proof-of-concept that: i) metabolomics could be used to classify human skeletal muscle tissue ageing, and ii) an anabolic stimulus opposing the negative effects of muscle ageing (resistance exercise training (RET)) would alter the ageing metabolome. Muscle biopsies were collected from healthy males (Young (Y): 18-30y; N=10, and Older (O): 65-75y; N=19) before and after 12-weeks RET, both in the fasted state and following a single-bout of RE. Metabolite data were generated using HILIC UHPLC-MS normalized to tissue wet-weight, and analysed using R (i.e. age-comparisons across conditions). Random Forest algorithm was applied to identify metabolite(s) classifying age (randomForest package), with the highest performing metabolites extracted based on Gini index. Univariate statistics using robust t-tests were applied to candidate metabolites to assess differences in abundance between age groups. Metabolite clusters showed robust ability to predict older age (i.e. OBB error 5-15%; high-performing metabolites included creatine and iso/leucyl-proline). However, those metabolites deemed most important to predict age changed as a result of RET (both in the fasted-state and after acute exercise), suggesting a RET-induced shift in the human muscle metabolome. This study reveals that metabolomics is a potentially useful approach to classify tissue age, and intriguingly, that RET leads to a shift in the age-related metabolome. Future work should investigate predictive links to clinical outcomes.

P-472 Gender associated differences in adult human urine NMR metabolomic profiles

PRESENTING AUTHOR: ***Ivan Vučković, Mayo Clinic, United States***

CO-AUTHORS: ***Song Zhang, Maria Irazabal, Aleksandar Denic, Petras Dzeja, Slobodan Macura***

NMR-based urine metabolomics showed considerable potential in disease diagnostics and biomarker discovery1. Urine has several advantages: it is abundant, easily obtained, requires little sample preparation and is rich in chemical diversity. However, large variations in metabolites concentrations make the use of urine in biomarker discovery challenging. Characterization of these variations is critical to avoid confounding effects in case-control studies. Recent LC-HRMS based metabolomic study of 183 adults found the significant impact of gender, age and BMI on urinary metabolome2. Another study used plasma and urine of 301 healthy adults and multi-platform (GC-MS, LC-MS, NMR) metabolomics analysis to identify metabolite patterns that successfully classified participants according to gender3. Here, we used NMR to analyze the urines from two independent cohorts (Cohort A, 94 females and 113 males; Cohort B, 242 females and 267 males). 1H-NMR spectra, collected using Bruker IVDr platform, were binned and normalized by total spectrum area. The binning data were subjected to univariate (Student T-test) and multivariate (OPLS-DA) statistical analysis. The two cohorts showed similar results and revealed significant differences in metabolomic profile between genders. The bins with lowest T-test p values and highest VIP in OPLS-DA models were attributed to following metabolites: 1) creatinine, 2-deoxythreonate, taurine, carnitine (higher in males), and 2) citrate, succinate, creatine, glycine (higher in females). Our results confirmed that gender related metabolomic differences need to be taken into account in urine biomarker discovery studies. 1. Journal of Proteome Research 2016 15(2)360-373 2. Journal of Proteome Research 2015 14(8)3322-3335 3. PLoS ONE 2017 12(8)e0183228

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WELLNESS AND AGING

P-473 Low variability metabonomic fingerprint of blood plasma from healthy Han Taiwanese revealed by CPMG NMR

PRESENTING AUTHOR: ***Chung-ke Chang, Academia Sinica, Taiwan***

CO-AUTHORS: ***on behalf of the Taiwan Biobank***

Metabonomic profiling of biofluids are potential tools for precision medicine because of its capacity to detect changes that may presage pathological states and as a diagnostic tool. However, before this potential may be realized, a key question is to define the “normal”, or “healthy”, metabonomic state of a subject or a population. Here we leveraged the large sample collection of the Taiwan Biobank to obtain the average metabonomic profile of ~300 physically fit individuals (normal BMI, normal blood pressure, non-diabetic, non-lipidemic, non-smoking, non-alcoholic, no cancer history, excellent exercise habits) of Han descent through proton nuclear magnetic resonance (NMR) spectroscopy using Carr-Purcell-Meiboom-Gill (CPMG) experiments. The spectra were processed using an in-house pipeline which implemented continuous wavelet transform, Whittaker smoothing and CluPA algorithms to obtain a well-aligned data set for downstream analysis. Our results reveal that certain spectral regions exhibit low variability (signal amplitude %CV < 20%) among the subjects regardless of gender and age. These spectral regions may serve as good references for comparing healthy vs. disease states in large-scale cohort studies. Notable metabolites in this region include glucose, histidine, and ethanol, among others. Comparison with publicly available data from other sources seem to indicate that these metabolites may be homeostatic across different populations, highlighting their importance to human health.

P-474 Long-lived humans have specific adaptions in their lipidomic profile

PRESENTING AUTHOR: ***Joaquim Sol, University of Lleida, Spain***

CO-AUTHORS: ***Irene Pradas, Kevin Huynh, Natalia Mota-Martorell, Alba Naudí, Marta Ingles, Consuelo Borras, Jose Viña, Peter Meikle, Mariona Jové, Reinald Pamplona***

Human longevity is partially explained by genetic factors, and the metabolome as the final step of cellular biochemical activity, reflexes the changes in gene expression and its interaction with the environment. Particularly, some lipid species and their characteristics have been previously described to be associated with animal longevity. Besides, human aging and longevity phenotypes are very heterogeneous and centenarians can be considered the best example of successful aging. In order to describe the plasma lipid profile of centenarians and define which lipid species are a signature of healthy aging we applied liquid chromatography-mass spectrometry techniques to plasma samples from centenarian (n=29), octogenarian (n=30) and adult (n=30) subjects. Principal component analysis and hierarchical clustering algorithm revealed the existence of a specific lipidomic signature in centenarian subjects conferring them a more oxidative stress resistance phenotype, which could contribute to their healthy aging. This resistant signature involves lipids such as ceramides, gangliosides and glycerophospholipids species. All in all, we concluded that extreme longevity phenotypes can be defined by their plasma lipid profile and some ceramides or lipid unsaturation can be used as biomarkers of longevity. Changes in centenarian plasma lipid species are focused on achieve an oxidative stress resistant phenotype.

P-475 Investigating the effects of lycopene and green tea on the metabolome of men at risk of prostate cancer: The ProDiet randomised controlled trial.

PRESENTING AUTHOR: ***Rhona Beynon, University of Bristol, United Kingdom***

CO-AUTHORS: ***Rebecca C. Richmond, Diana L. Santos Ferreira, Andrew R. Ness, Margaret May, George Davey Smith, Emma E. Vincent, Charleen Adams, Mika Ala-Korpela, Peter Würtz, Sebastian Soidinsalo, Christopher Metcalfe, Jenny L. Donovan, Athene J. Lane, Richard M. Martin***

Background Lycopene and green tea consumption have been observationally associated with reduced prostate cancer risk, but the underlying mechanisms have not been fully elucidated. Methods We investigated the effect of factorial randomisation to a 6-month lycopene and green tea dietary advice or supplementation intervention on 160 serum metabolite measures in 128 men with raised PSA levels (but prostate cancer-free), analysed by intention-to-treat. The causal effects of metabolites modified by the intervention on prostate cancer risk were then assessed by Mendelian randomization, using summary statistics from 44,825 prostate cancer cases and 27,904 controls. Results The systemic effects of lycopene and green tea supplementation on serum metabolic profile were comparable to the effects of the respective dietary advice interventions (R2= 0.65 and 0.76 for lycopene and green tea respectively). Metabolites which were altered in response to lycopene supplementation were acetate (β (standard deviation difference versus placebo): 0.69; 95% CI= 0.24, 1.15; p=0.003), valine (β: -0.62; -1.03, -0.02; p=0.004), pyruvate (β: -0.56; -0.95, -0.16; p=0.006), and docosahexaenoic acid (β: -0.50; -085, -0.14; p=0.006). Valine and diacylglycerol were lower in the lycopene dietary advice group (β: -0.65; -1.04, -0.20; p=0.004 and β: -0.59; -1.01, -0.18; p=0.006). A genetically instrumented SD increase in pyruvate increased the odds of prostate cancer by 1.29 (1.03, 1.62; p=0.027). Conclusions An intervention to increase lycopene intake altered the serum metabolome of men at risk of prostate cancer. Lycopene lowered levels of pyruvate, which our Mendelian randomization analysis suggests may be causally related to reduced prostate cancer risk.

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WELLNESS AND AGING

P-476 NIH's consortium on Molecular Transducers of Physical Activity (MoTrPAC)

PRESENTING AUTHOR: ***Padma Maruvada, National Institutes of Health, United States***

CO-AUTHORS: ***Sealfon Stuart, Michael Snyder, Charles Burant, Robert E. Gerszten, Dean P Jones, Sreekumaran Nair, Joshua N Adkins, Karyn Esser, Stephen B. Montgomery, Steven Carr, Venugopalan Nair, Kevin Smith, Ian Lanza, Charles R. Evans, Martin Walsh, Wei-Jun Qian, Facundo M. Fernández, Chris Newgard, Clary Clish, Neil M. Johannsen, Kate Early, Ashley Xia, Maren Laughlin, Lindon Joseph, John Williams***

Health benefits of regular physical activity across the lifespan are indisputable and potentially reduce disease burden. However, the molecular mechanisms by which these benefits are mediated across various organs are not known. In order to support and facilitate the identification of molecular signatures, and to build a knowledgebase on human physical activity, NIH has recently invested ~170 million dollars over 6 years in the "Molecular Transducers of Physical Activity" (MoTrPAC) program. This NIH Common Fund program supports human and rat exercise studies to comprehensively characterize biomolecular responses to physical activity, via multiomic platforms (i.e. genomic, transcriptomic, epigenomic, proteomic and metabolomic). Human clinical samples will include measurements of muscle, adipose, blood, and circulating exosomes. Rat studies will complement clinical studies and enable analyses in additional tissues such as liver and kidney. The multiomic measurements will create a comprehensive molecular knowledgebase that will support subsequent hypothesis-driven physical activity research. In pilot studies, the different omics assays were performed across the consortium, which assessed measurements in blood, muscle, and adipose tissue from resting and exercised rats, as well as in plasma from humans at rest and following exercise. The data was assessed for sensitivity, breadth, and reproducibility of various assays and platforms, suitability of uniform sample processing methodologies, QC methodologies, and assessing the feasibility of the consortia-wide collaborative interaction. This presentation will describe some of the key efforts and promising results from these pilot studies.

P-477 Effect of physical exercise and micronutrients complementation during pregnancy on serum metabolic profile at 20 and 32 weeks of gestation. A Pilot study

PRESENTING AUTHOR: ***Monica Cala, Department of Chemistry, Universidad de los Andes, Bogotá D.C., Colombia., Colombia***

CO-AUTHORS: ***Mónica Cala, Jose Guillermo Ortega, Elizabeth Jimenez, Mildrey Mosquera, Isabella Echeverri, Blanca Salazar, Wolfram Baumann, Cecilia Aguilar***

In the present study, we explored the potential of metabolomics to evaluate the effect to micronutrient supplementation and regular aerobic exercise on the metabolic profile at 20 and 32 weeks of gestation during a healthy pregnancy. Study was performed on serum samples obtained at 20 and 32 weeks from 200 pregnant women attending usual prenatal care in Cali, Colombia. Women were assigned to the following intervention groups:1. Control group:usual prenatal care (PC) and placebo (maltodextrine).2. Exercise group:PC, placebo and aerobic physical exercise. 3. Micronutrients group:PC and a micronutrients capsule consisting of zinc (30 mg), selenium (70 µg), vitamin A (400 µg), alphatocopherol (30 mg), vitamin C (200 mg), and niacin (100 mg). 4. Combined interventions Group:PC, supplementation of micronutrients, and aerobic physical exercise. Samples were analyzed by LC-MS in both positive and negative electrospray ionization (ESI) modes, followed by data alignment and filtration. Differences between profiles from different interventions at 20 and 32 weeks groups obtained by LC-MS analysis, were evaluated with univariate (UVA) and multivariate (MVA) analysis. Both UVA and MVA showed a clear discrimination between 20 and 32 weeks of gestation. The significant altered metabolites correspond mainly to lipids, in particular, fatty acylcarnitines, fatty acids, lysophosphatidylethanolamines, lysophosphatidylcholines, phosphatidic acids and phosphatidylglycerol. Interventions with exercise, micronutrients and combined interventions during a healthy pregnancy did not show significant differences in the metabolite profiles by MVA, and only few significant altered metabolites were selected based on UVA in the applied interventions.

P-478 Temporal profiling of the metabolic response to exercise: studies in rats and humans with high and low VO2max

PRESENTING AUTHOR: ***Charles R. Evans, University of Michigan, United States***

CO-AUTHORS: ***Heidi B. IglayReger, Christine A. Parker, Katherine A. Overmyer, Mary K. Treutelaar, Jeffery F. Horowitz, Charles F. Burant***

Physical activity is associated with numerous health benefits including prevention and control of cardiovascular disease and diabetes, strengthening of bones and muscle, and improvement of mental health. The molecular mediators of these and other benefits have not been fully elucidated, but may include small-molecule metabolites altered in response to exercise. Thus, developing a more comprehensive map of the changes to the metabolome induced by exercise may help lay the groundwork for novel therapies for major human diseases. In this work we present time-resolved metabolomics data from exercising rats and humans. In rats, strains bred for either high or low maximal exercise capacity underwent ascending-effort treadmill exercise. Plasma, muscle, liver and adipose were collected at one-fourth, one-half, and maximal exercise intensity. In humans, 20 male volunteers age 18-30 were divided into groups with mean VO2max of 34(+/- 4) and 56(+/-4) ml/kg/min and underwent cycle ergometry. Plasma samples were collected every 3 minutes during exercise and muscle biopsies were performed pre/post exercise. Samples were analyzed by LC-MS-based targeted and untargeted metabolomics. Complex patterns of change were observed including alterations in TCA cycle intermediates, purine derivatives, acylcarnitines, and several metabolites not previously reported as exercise-responsive. Most exercise responses were common between rats and humans, but some metabolite classes including acylcarnitines appeared distinct. Our results demonstrate the dynamics of the metabolome induced by exercise, highlight advantages and challenges of complementary studies in rodents and humans, and reveal targets for further study in the search for molecular mediators of the health benefits of exercise.

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NICHE METABOLOMICS

P-479 Impact of repeated doses of stable iodine in the rat using a metabolomic approach

PRESENTING AUTHOR: ***Rosique Clément, Aix-Marseille Université, France***

CO-AUTHORS: ***Lebsir Dalila, Souidi Maâmar, Martin Jean Charles***

The Fukushima nuclear power plant blast resulted in the release of 131Iodine for several weeks. This unexpected issue challenged the iodin doctrine, in which the counter-measure is to provide a unique iodine tablet to saturate thyroid during the radioactive contamination not expected to last more than several hours. A new doctrine must be implemented to take into account such case based on repeated iodine administration with adapted dosage. But repeated administration of iodine can block the thyroid and bring about deleterious effects, especially in the mother-offspring pairs. Our goal was to evaluate the potential undesirable effects of such repeated iodine administration using an untargeted metabolomic approach on a rat reproductive model. Pregnant rats received repeated doses of potassium iodine (KI group: 1mg/kg/24h) or water for injection (control group) for 8 days. The potential metabolic disruption was investigated in the offspring 30 days after weaning. Using LC-MS, we compared the blood's metabolite composition between KI and control rats; using a high throughput annotation procedure with an in-house databank, 264 metabolites were annotated (based on retention time and exact mass), combined in 52 functional biological modules/pathways, and converted into corresponding scores using a PLS multiblock algorithm. Running a random forests test, we found 17 modules significantly impacted by the KI treatment (VIP values > 0.05) including pathways of redox status, aminoacids, TCA cycle or oxidative stress. These findings indicated a prenatal effect of KI administration that lasted over the long term (adolescence). Whether or not these outcomes are pathological is unknown.

P-480 Lipidomics analysis of cultured human fibroblasts from individuals with autism spectrum disorders

PRESENTING AUTHOR: ***Amy Li, University of Washington, United States***

CO-AUTHORS: ***Anne Arnett, Micah Pepper, Raphael Bernier, Libin Xu***

Autism is a complex spectrum disorder that is thought to have a strong basis in genetics. However, the underlying mechanisms of how genes or environmental factors contribute to autism spectrum disorders (ASD) are still poorly understood. Some of the genes associated with ASD are related to lipid metabolism. Thus, we hypothesize that some commonly affected lipid metabolic pathways contribute to the molecular mechanisms underlying ASD progression. To test this hypothesis, we collected human fibroblasts derived from ASD individuals with de novo mutations in CHD8, ADNP, and DYRK1A genes, along with two healthy Control fibroblast cell lines acquired from Coriell Institute, and cultured them in lipid-deficient media to promote de novo lipid biosynthesis. Subsequently, untargeted lipidomics analysis was performed on the fibroblasts in both positive and negative ionization modes on a Waters Synapt G2-Si, using a multi-dimensional HILIC-ion mobility-mass spectrometry method developed in our lab, to separate glycolipid, phospholipid and sphingolipid classes. Principal component analysis (PCA) of preliminary lipidomics data shows clustering of Control samples on the PCA plot, separated from the autism samples which are roughly grouped together by mutations. Relative to Control samples, the autism samples displayed an overall decrease in some glycerophospholipids, including phosphatidylethanolamines (PEs), phosphatidylglycerols (PGs), phosphatidylcholines (PCs), and phosphatidylinositols (PIs), and an overall increase in glucosylceramides (GlcCers). Within the ASD samples, correlations are being explored between various metabolic changes and quantitative clinical patient data, such as verbal and non-verbal IQ test scores, vocabulary tests, and behavioral assessments.

P-481 The Harmonization of Lipidomics: A Comprehensive Review of the NIST Interlaboratory Comparison Exercise and a Path Forward

PRESENTING AUTHOR: ***John A. Bowden, National Institute of Standards and Technology, United States***

As the lipidomics field progresses, efforts aimed to dissect the finer details of lipid measurement and improve community-wide harmonization are concomitantly needed. Recently, we introduced consensus means (with associated uncertainties) for 339 lipids in human plasma Standard Reference Material (SRM) 1950, representing a significant step toward community-wide harmonization and quality control. This was the first result of the National Institute of Standards and Technology (NIST) interlaboratory comparison exercise for lipidomics. Here, we present a further interrogation of the comparison exercise. Specifically, we highlight observed method-data trends and potential pitfalls in lipidomic workflows and provide a more in-depth comparison between the findings of the comparison exercise and a previous LIPID MAPS study of SRM 1950. Differences in summed lipid class concentrations between this exercise and the previous LIPID MAPS study were examined using a third mass spectrometry-independent approach, employing colorimetric, fluorometric, and enzyme-linked immunosorbent assay (ELISA) kits. We also engaged the lipidomics community (125 respondents) with a follow-up survey to learn more about lipid measurement within the community. The survey covered current laboratory demographics, lipidomic methodologies and SOPs, analytical platforms, quantitation, reference materials, quality control procedures, and opinions regarding challenges existing within the community. This presentation will also discuss the community-wide analysis of other SRMs (e.g., SRM 2378, Fatty Acids in Frozen Human Serum), the creation of new omics-based reference materials (including system suitability standards), and proposed activities to continue advancing lipid measurement.

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NICHE METABOLOMICS

P-482 Rapid and sensitive characterization of FAHFA lipids using an untargeted lipidomics approach

PRESENTING AUTHOR: ***Tong Shen, NIH West Coast Metabolomics Center, University of California, Davis, United States***

CO-AUTHORS: ***Bryan Roberts, Oliver Fiehn***

Novel metabolically beneficial lipids—branched fatty acid esters of hydroxyl fatty acids (FAHFAs) were first revealed in mouse adipocytes when overexpressing Glucose Transporter Glut4. FAHFA levels positively correlate with glucose tolerance and also with insulin sensitivity in humans. Therefore, increasing attention has been drawn to detect these physiologically important, yet low abundant lipids. Previous efforts tried to enrich FAHFAs by solid phase extraction and derivatization, in combination with targeted MS/MS. Identifying FAHFAs at the molecular level is crucial to scrutinize their biochemical pathways. Utilizing an untargeted lipidomics method on a UPLC-Sciex TripleTOF MS/MS platform, we identified 49 FAHFA lipids in human serum and stool samples. Underivatized lipids were extracted from 40 µl of serum, and analyzed on a Sciex TripleToF system using data-dependent acquisition after a 15 min reversed-phased UPLC separation. 49 FAHFA molecular species were annotated by matching accurate mass (< 2 ppm) and matching MS/MS against an in silico FAHFA MS/MS library that was constructed similar to LipidBLAST based on MS/MS spectra of authentic standard compounds. The library is publicly available at MassBank of North America (http://massbank.us). In human serum, FAHFA 18:1-(O-18:0) (OAHSA) was found as the most abundant FAHFA, followed by 20:4-(O-18:0) and 18:2-(O-18:0). In general, FAHFAs containing O-18:0 (hydroxyl stearic acid, HAS) were detected as most abundant species, followed by O-18:1 (hydroxyl oleic acid, HOA), O-20:0, O-22:0, and O-16:0. Compared to hydroxyl fatty acids, fatty acyl chains in FAHFAs were found to exhibit more variety, including various kinds of short, middle, and long chain fatty acids.

P-483 Make Copper Great Again - Toxicity and Metabolism of Copper Nanoparticles

PRESENTING AUTHOR: ***MATTHEW J. WINANS, West Virginia University, United States***

CO-AUTHORS: ***J. E. Gallagher, J. Cumming, G. Oporto***

Copper has been used since antiquity as a broad spectrum antimicrobial agent to prevent water born diseases, protect agricultural food securities, and treat medical ailments. Novel carboxymethyl cellulose (CMC) nanoparticles engineered with copper (c-CuNPs) enhance the toxicity of copper to S. cerevisiae via a unique mode of action when compared to copper sulfate. C-CuNPs are a promising emerging nanotechnology composed from timber industry byproduct; These can be imbedded into poly vinyl alcohol plastics for use in medical devices and food packaging. Reactive oxygen species, such as the hydroxyl radicals are generated through Fenton reactions with transition metals in the presence of aqueous solutions; this is the general reaction scheme that provides toxicity to copper. In this study we found the antioxidants N-acetylcysteine (NAC), a glutathione (GSH) precursor, could not rescue c-CuNP toxicity, but GSH rescued both copper forms by serial dilutions and spotting. Atomic absorption spectroscopy revealed that less copper is found associated with the cells via c-CuNP when controlled for toxicity. Utilizing flow cytometry and confocal microscopy we have been able to support our hypothesis that the copper nanoparticle is dispersed close to the cell wall. Future studies are being designed to assess the metabolic differences between copper treatments and determine which cellular compounds become oxidized. We hypothesize that lipids associated with the phospholipid bilayer are damaged. Mitophagy is suspected to play a role in the differing mechanism between copper sulfate and c-CuNPs. This value-added product is poised for studies on biocompatibility and nosocomial infection prevention studies.

P-484 Investigating the Exposome of Reproductive Age Women Using Targeted and Untargeted Metabolomics

PRESENTING AUTHOR: ***Clayton S. Bloszies, UC Davis West Coast Metabolomics Center, United States***

CO-AUTHORS: ***Ulrike Luderer, Oliver Fiehn***

While the genetic contribution to chronic disease has been heavily researched, the environmental contribution remains poorly understood, highlighting the need for better characterization of the exposome. Here, we propose using a combination of targeted and untargeted mass spectrometry to elucidate relationships between low abundance environmental exposures, food and drug compounds, and endogenous metabolites in the same urine sample. Hydroxylated polynuclear aromatic hydrocarbon (OH-PAH) levels have been linked to many diseases including cancer, cardiovascular disease, and developmental disorders. We have developed a method to separate and detect nine OH-PAH isomers using a Sciex QTRAP 6500+ mass spectrometer, with an LOD between 5pg/mL and 30 pg/mL in urine. Spike-recovery experiments have been performed to validate the efficiency of the extraction protocol. We have analyzed urine samples from 50 women over three time points to measure OH-PAH levels, and then used the exact same samples to extend the search for other exposure compounds using a combined targeted / untargeted screen on PRM and full scan DDA on a ThermoScientific QExactive HF mass spectrometer. We will examine correlations between the compounds identified using the untargeted approach with the targeted OH-PAHs. With this data set, we hope to show that untargeted exposomics can be integrated with both traditional targeted toxicology and metabolomics to better understand the environmental contribution to chronic disease.

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NICHE METABOLOMICS

P-485 Mass Isotopomer Distribution Analysis of lipid-derived fatty acid acylium ions circumvents the need for sample saponification in metabolic labeling studies

PRESENTING AUTHOR: ***Jaewoo Choi, Oregon State University, United States***

CO-AUTHORS: ***Cristobal L. Miranda, Claudia S. Maier, Jan F. Stevens***

Metabolic labeling in combination with Mass Isotopomer Distribution Analysis (MIDA) is a well-established technique for determining fractional synthesis (f) of endogenous metabolites, such as lipids, carbohydrates, and steroids. The disadvantage of current protocols determining the f of fatty acids (FAs) as an index of de novo lipogenesis is that the biological sample requires saponification and subsequent measurement of FAs by GC- or LC-MS. We explored an alternative approach in which we measured metabolic deuterium labeling of FAs, without saponification of the biological sample, by data-independent MS/MS of lipids which produces FA acylium ions of all isotopologues for any given lipid species. Male C57BL/6J mice were administered an investigational inhibitor of de novo lipogenesis, xanthohumol (XN), in the diet for 2 weeks. Five days prior to trial termination, mice (n=5) were given an intraperitoneal injection of 2H2O (2% of body weight) and then maintained on 5% 2H2-labeled drinking water, while the other mice (n=5) were maintained on H2O drinking water. At the end of trial, collected liver tissues were homogenized and extracted with methylene chloride:methanol:isopropanol =25:65:10. Data-independent MS/MS analysis of triacylglycerides (TAGs) revealed incorporation of deuterium into its FA acylium ions. Using MIDA, we calculated f=0.73 for mice given labeled drinking water and determined the maximum number of deuterium atoms incorporated into hepatic TAG palmitate to be N=17. After oral treatment of mice with XN, the f and N values decreased to 0.62 and 10, which demonstrates that dietary XN treatment reduces de novo lipogenesis and synthesis of TAGs.

P-486 Lipidomics Profiling of Mouse Plasma and Liver and Herat Tissues

PRESENTING AUTHOR: ***Jinchun Sun, National Center for Toxicological Research, United States***

CO-AUTHORS: ***Zhijun Cao, Tom Schmitt, Vijayalakshmi Varma, Richard Beger***

Lipids play important roles in mammalian health as signaling, energy storage, and as major structuring components of cell membranes. Alterations in lipid levels and profiles are related to diseases including metabolic syndrome, cardiovascular, neurological diseases, diabetes, cancer and toxicity. However, little work has been conducted to show the extent of tissue specificity of lipid profiles. Here, we employ the Lipidyzer platform to reveal gender difference and biological variance in lipidome, and to discover tissue-specific lipids. Liver tissues and plasma were obtained from B6C3F1 female (n=3) and male (n=3) mice at 17 wk of age to study the lipidome gender difference and evaluate biological variance of the lipidome. Heart tissues and plasma were collected from B6C3F1 (n=4 female, 1 male) at 20-21 wk of age. The pooled pulverized liver, heart and the pooled plasma samples were used to evaluate the lipidome difference across tissues, and to examine the reproducibility of the Lipidyzer platform. In total, 762 lipid species were measured in mouse blood, liver and heart. The PCA plots from the lipidomics data showed the separations between blood, heart and liver tissues. Female and male mice showed significant difference in the class of Lysophosphatidylcholine (91.9±11.2 vs 128.7±11.3, $p<0.05$). Most importantly, several tissue-specific lipids were identified but absent in the plasma under normal condition which could be potential biomarkers to indicate the organ health status.

P-487 Understanding Adolescent Exposures of Tobacco Products by Urine Metabolomics

PRESENTING AUTHOR: ***Ping-Ching Hsu, University of Arkansas for Medical Sciences, United States***

CO-AUTHORS: ***Min-Ae Song, Kenneth Riedl, Morgan Cichon, Quentin A. Nickerson, Brittney L. Keller-Hamilton, Amy K. Ferketich, Peter G. Shields***

Most smokers and smokeless tobacco (ST) users begin before the age of 18. In adolescents, the prevalence of ST and e-cigarette (e-cig) use has been increasing and the prevalence of dual use is high, and even higher in Ohio than many other states. A cohort of both rural and urban adolescent males in Ohio was established to determine the differences in exposure to tobacco toxicants for smokers, ST users, e-cig users, and dual/poly users among adolescents using untargeted metabolomics controlling for urinary cotinine levels. Among 94 adolescent who reported ever use of any tobacco product, 36.2% used more than one product and 23% had used all three products (cigarette, ST, and e-cig). For single users, ever use of e-cig was the highest (16%) compared to ever cigarette use (12.8%) and ever ST use (11.7%). Significant differences were observed in global metabolomic profiles between active tobacco users and those with background cotinine levels (< 100ng/ml), as well as e-cig users vs. cigarette and ST users. Among active tobacco users, the nicotine metabolic ratio (NMR) was used to determine their metabolic capacity for nicotine. 15 urinary metabolites were significantly higher among fast than slow metabolizers ($p<0.05$, |fold change|>2), including metabolites of flavorings, cigarette, and known biomarker with hepatotoxic effects. Metabolomic profiling is distinguishing adolescents who are choosing different types of tobacco products, including by their ability to metabolize nicotine. Such profiles may be useful as biomarkers of exposure, and identify disease mechanisms and pathways that are differentially affected by product choice.

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POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

NICHE METABOLOMICS

P-488 The NIH West Coast Metabolomics Center: How to run a service core for thousands of samples, diverse matrices and complex requests.

PRESENTING AUTHOR: *Kelly Paglia, University of California, Davis, United States*

CO-AUTHORS: *Kelly Paglia, Luis Valdiviez, Oliver Fiehn*

The NIH West Coast Metabolomics Center offers fee-based services for targeted and untargeted metabolomics, isotope-tracer studies, compound identification and training courses. We exclusively rely on UPLC-MS/MS and GC-TOF MS assays for primary metabolites, complex lipids, biogenic amines, steroids/bile acids, oxylipins, Vitamin D epimers, short-chain fatty acids, absolute amino acid quantifications, and p180 and p400 Biocrates kits. Based on demand, we develop new core services for target metabolites. In 2017, we serviced over 500 projects with 30,000 samples from 65 different sample matrices using 17 mass spectrometers and 13 full-time laboratory staff, in addition to statisticians, administrative and informatics staff. This throughput is possible by utilizing specialized software and database developments, namely BinBase, MassBank of North America (MoNA), MS-DIAL, and MS-FINDER. Due to the complexity of tasks, the problem is to provide an adequate turnaround time while maintaining the highest standards of quality control. The primary bottleneck is funding for new instruments and laboratory space, as sample preparation is increasingly moved to microtiter-well plate formats and data processing becomes more automated. We help investigators during sample submissions, specifically adhering to our guidelines for detailing the study designs. In addition to communication by phone and email, we created submission forms to help investigators and our laboratory staff to provide all necessary information that is automatically linked to our sample LIMS system. We will presents the statistics and trends in metabolomics services over the past years, highlight example studies, and detail the tools and SOPs that help the core perform at outstanding quality.

P-489 Quantitative anaylsis of vitamin D metabolites based MALDI-TOF MS

PRESENTING AUTHOR: *Da-Hee Ahn, Soonsil University, Korea, South*

CO-AUTHORS: *Han-Gyu Park, Won-Suk Song, Yun-Gon Kim*

Vitamin D is fat-soluble secosteroid prohormone and a crucial role in various disease including osteomalacia, cardiovascular disorders, some cancers and autoimmune disease. Therefore, a measurement of vitamin D status is important for diagnosis of vitamin D deficiency. Though 1a,25-dihydroxyvitamin D (1,25(OH)$_2$D) metabolites is bioactive form, 25-Hydroxyvitamin D (25(OH)D) is biomarker for monitoring vitamin D status because 25(OH)D levels are longer half-time than the other metabolites. Analysis of vitamin D metabolites are used liquid chromatography tandem-mass spectrometry (LC-MS/MS) because the method is high-sensitivity, repeatability. However, it is time consuming, labor intense and poor ionization of 25(OH)D because hydrophobic character and lack of chargeable group. Therefore, derivatization of 25(OH)D has been used because the method improves their sensitivity and selectivity through the modification of the chemical and physical properties. Especially, Cookson-type reagents are the powerful dienophile so react with conjugated diene group like as vitamin D metabolites. We use matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) because it is simple procedure, rapid analysis and extremely sensitive so it's an appropriate alternative tool of analysis of 25(OH)D. Here, we demonstrate a quantitation of 25(OH)D using MALDI-TOF MS. We introduced a permanently-charged Cookson-type reagent, secoSET is suitable for mass analysis and enhance sensitivity. The reliability of the quantitative analysis was validated using 25(OH)D. The derivatized 25(OH)D are higher the sensitivity, reproducibility and linearity (R2>0.99) than non-derivatized 25(OH)D. Moreover, we analyze the change of the absolute amount of 1,25(OH))$_2$D3 in MCF-7 cells resulting from treatment with inhibitor using deuterated 25(OH)D3.

P-490 Development of Undergraduate Metabolomics Research Strategy Utilizing Team-based Vertically Integrated Projects (VIP)

PRESENTING AUTHOR: *Tyler Ray Carter, University of Georgia, United States*

CO-AUTHORS: *Sung Alexander, Oudhay Sohal, Ivy Lin, William Pearson, Julia Roth, Nana Awuku, Rachel Xu, Jane Guo, Justin Anthony, Kieanna Doctrine, Jaqueline Anthony, Noah Floyd, Taha Rahmatullah, Jack Doll, Sixie Liu, Arthur S. Edison*

The Vertically Integrated Project (VIP) program is a type of undergraduate research experience (URE) developed by Ed Coyle at Purdue University in 2001 as scalable way to involve more students by structuring them into teams to conduct research unified by an ultimate research question or goal. VIP is currently in place at seventeen U.S. institutions, the United Kingdom, South Korea and China, programs are also being developed in Latvia and Columbia. Our team is divided into 4 cores; CRISPR, sample preparation, bioinformatics, and nuclear magnetic resonance (NMR), collaboratively working to characterize the metabolome of selected Caenorhabditis elegans mutants via analysis using NMR Spectroscopy and Liquid Chromatography Mass Spectrometry (LC-MS). Understanding this connection will provide novel insight into human metabolism and genetics that could lay the foundation for training the future researchers and practitioners of precision medicine. Quality control and reproducibility are critical to the advancement of metabolomics research. The VIP project offers the opportunity for students to learn the importance of replication, reproducibility, statistical power, and the trial and error that is the nature of scientific research. The team must demonstrate a high level of skill and aptitude to prepare and analyze robust replicates of C. elegans. The focus of this study is to quantifiably measure the quality and reproducibility of the NMR data as a metric to measure the scientific effectiveness of the VIP structure. This fusion of metabolomics and education research seeks to provide novel insight of how to optimize both undergraduate research self-efficacy and quality metabolomics research.

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NICHE METABOLOMICS

P-491 MEtabolomics standaRds Initiative in Toxicology (MERIT)

PRESENTING AUTHOR: ***Richard D Beger, NCTR, United States***

CO-AUTHORS: ***Jean-Lou Dorne, Timothy M. D. Ebbels, Drew Ekman, Claude Guillou, Amber Goetz, Hennicke Kamp, Pim Leonards, George Loizou, Bernard van Ravenzwaay, Saskia Sperber, Mark R. Viant, Tilman Walk***

The MEtabolomics standaRds Initiative in Toxicology (MERIT) is a European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) supported project to provide guidance on best practice, quality standards and the reporting of analytical and computational metabolomics methods used in regulatory toxicology. ECETOC relies on world-wide expert collaboration "to develop an agreed understanding on how the state of the science can be used to improve [chemical] risk assessment by developing novel tools". Although there is strong interest in using metabolomics from industry and regulatory scientists, the lack of best practice guidelines is a concern that has hindered its use in regulation. Fourteen metabolomics scientists from academia, industry, regulatory and government agencies were recruited as the MERIT core team, with a much larger number acting as a wider consultative group. The primary objective of MERIT is to develop best practice guidelines and minimal reporting standards in the context of metabolomics in regulatory toxicology. Several case studies representing near-future applications of metabolomics - including for the discovery of molecular key events and biological read-across - are being used to ensure the relevance of the guidelines to regulatory toxicology. MERIT will publish its recommendations for best practice and reporting standards and is liaising with other groups, such as the Metabolomics Quality Assurance and quality Control Consortium (mQACC) and the Metabolomics Society, to ensure harmonization of the recommendations.

P-492 1-Butanol production improvement in transgenic Escherichia coli by regression model-based metabolomics

PRESENTING AUTHOR: ***Katsuaki Nitta, Osaka University, Japan***

CO-AUTHORS: ***Sastia P. Putri, Sammy Pontreli, James C.Liao, Eiichirou Fukusaki***

Rational selection of gene targets plays an integral part in the success of strain improvement in metabolic engineering strategies. Although conventional methods such as deduction from biochemical information are more straightforward, these approaches are time-consuming. Here, metabolomics can be useful to provide clues on which pathways are important in relation to a particular phenotype. Usually in metabolic engineering studies, several strains are constructed, thereby producing a range of values for a certain quantitative phenotype. Therefore, these strains possess huge amounts of biological information that can offer various insights. Regression analysis, which can correlate quantitative values with a phenomenon is a useful tool for offering insights to understand the state of a biological sample. In this study, OPLSR (Orthogonal Partial Least Squares/Projections to Latent Structures Regression) model was applied to 1-butanol producing Escherichia coli. Here, obtained metabolite data was used as explanatory variables to correlate with 1-butanol production. However, information obtained by OPLSR model show only correlation trends not causal relationship in a biological context. Hence, it is important to understand the meaning of correlation by using other supporting information. In this study, we interpret the result of OPLSR model through validation experiments such as addition of metabolites and several genetic modifications. Consequently, the metabolome information that acetyl-CoA accumulation and free CoA limitation has causal relationship with acetate accumulation was identified. By resolving the problems, the highest 1-butanol productivity for E. coli (11.3 g/L in 24h) was achieved. Through study, the usefulness of OPLSR-based metabolomics in metabolic engineering field was demonstrated.

P-493 MetaboLights Labs - Online metabolomics data analysis and deposition platform

PRESENTING AUTHOR: ***Venkata Chandrasekhar Nainala, EMBL-EBI, United Kingdom***

CO-AUTHORS: ***Keeva Cochrane, Kenneth Haug, Kalai Vanii Jayaseelan, Jose Ramon Macias, Pablo Moreno, Rachel Spicer, Mark Williams, Namrata Kale, Claire O'Donovan***

Over the last few years, MetaboLights has grown very rapidly and is hosting a rich variety of raw and processed data from NMR and Mass Spectrometry metabolomics experiments. With the global adoption of the FAIR principles and the general demand for open data deposition, in addition to significant improvements in data capture and deposition systems, analysis and reuse is now at the forefront of the metabolomics community. New workflow projects like MetaboFlow, established projects such as PhenoMeNal, W4M, Galaxy-M and the UK Phenome Centers, facilitate large-scale data analysis through metabolomics analysis pipelines based on Galaxy workflows. However, moving data between these infrastructures needs to be more efficient. MetaboLights Labs is a new online workspace that enables metabolomics data analysis, workflow data integration and data deposition to MetaboLights. Data can be pushed seamlessly to Galaxy workflows for processing. Metadata can be automatically extracted from community standard formats such as mzML and nmrML. This all enables easy and direct deposition to MetaboLights. Latest technologies for data upload and download ensure high-speed data transfer and an API driven architecture also facilitate third-party integrations, including Laboratory Information Management System (LIMS). Plans are now in place to include a more comprehensive set of validation and analysis tools both within the Labs and also in the integrated Galaxy workflow infrastructure. We welcome interactions with the community to drive this forward.

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NICHE METABOLOMICS

P-494 Analysis of folate cycle metabolites in cytoplasm and nucleus

PRESENTING AUTHOR: ***Bettina Guertl, CeMM - Research Center for Molecular Medicine, Austria***

CO-AUTHORS: ***Sara Sdelci, Gerald Hofstaetter, Stefan Kubicek, Kristaps Klavins***

The folate pathway plays an important role in cell division, protein synthesis, DNA/RNA synthesis and repair. To resolve the mechanisms of these biologically processes at their deepest molecular level, the understanding of the metabolic composition of different cellular compartments are needed. Despite the common belief that metabolites simply diffuse into the nucleus through nucleopores, there are evidences that subcellular metabolite concentration plays a fundamental role orchestrating important biological processes. The major aim of this study was to establish a method for the differential detection of folates cycle metabolites in the nuclear and cytoplasmic compartment. Additionally, the effect of methotrexate treatment, a well-known antifolate compound, on folate cycle metabolite levels was investigated. For this purpose a quantitative method based on high performance liquid chromatography-tandem mass spectrometry has been developed for the simultaneous analysis of seven metabolites from the folate cycle. The major challenges for the analysis of folate metabolites are low abundance and instability. Therefore, the influence of light, solvents, anti-oxidants and storage stability was investigated. The optimized sampling and sample preparation procedure ensured robustness for the quantitative analysis of all metabolites of interest. First, the folate cycle metabolite levels in nucleus and cytoplasm fractions were compared. The obtained results showed a difference in metabolite abundances and several folate metabolites were not detected in cytoplasm. This indicates organelle specific localisation of folate cycle metabolites. Furthermore, methotrexate treatment induced changes in folate cycle metabolism were investigated. Significant differences in cellular levels of dihydrofolic acid, 5-methyltetrahydro folic acid, and 5,10-methenyl-tetrahydrofolic acid were observed.

P-495 Lipidomic analysis of microalgae using integrated SFE-SFC-MS

PRESENTING AUTHOR: ***Unnikrishnan Kuzhiumparambil, University of Technology Sydney, Australia***

CO-AUTHORS: ***Jens Altvater, Peter Ralph***

Microalgae are unicellular photosynthetic organisms, cultured and harvested for numerous high value biochemicals. They represent an important group of organisms for biotechnological exploitation. With a high phospholipid content, microalgae have raised large expectations within the bioeconomy as a untapped source of products with commercial applications for the chemical, pharmaceutical and cosmetic industries. Establishing a comprehensive profile of lipids within and across taxonomic groups of algae, will provide a better understanding of cellular functionality as phospholipids play a vital role in algal cellular signalling and cell-cell interactions. In this study, we compare the phospholipid profile of various strains of chlorophytes and diatoms using an integrated Supercritical Fluid Extraction- Supercritical Fluid Chromatography- Mass Spectrometry (SFE-SFC-QqQ MS) system. A comprehensive profile including phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylserines, sphingomyelin and phosphatidylinositols were obtained using the technique. Chemometric and statistical analysis of the fingerprint from each taxa revealed variations in the phospholipid composition between the algal species. Information on differentially abundant phospholipid groups was revealed, giving perspectives to potential chemotaxonomic markers. SFE-SFC-MS technique allowed for a reliable, accurate and fast characterisation of the phospholipid profile and has the potential to yield great insights into the biochemical diversity of microalgae species.

P-496 Profiling and identification of neuroprostane like metabolites of docosahexaenoic acid in vivo and in vitro by mass spectrometry

PRESENTING AUTHOR: ***Jeevan K. Prasain, University of Alabama at Birmingham, United States***

CO-AUTHORS: ***Jeevan K. Prasain, Ekta Tiwary, Shara Legg, Landon Wilson, Taylor Berryhill, John Parant, Michael M. Miller***

Docosahexaenoic acid (DHA)- the major 22 carbon n-3 fatty acid is an important precursor for bioactive resolvins and neuroprostanes. Neuroprostanes are prostaglandin (PG) like molecules produced by free radical mediated peroxidation of DHA. We previously identified specific F-series PGs produced from C20-PUFA precursors dihomo-γ-linolenic acid (DGLA, C20:3), arachidonic acid (AA, C20:4), eicosapentaenoic acid (EPA, C20:5) in vivo in C. elegans via Cox-independent pathway. Based on these observations, we next asked whether PG like compounds are formed from DHA independent of previously reported free radical mechanism. When worm lysate was incubated with DHA at room temperature for 10 min, a number of neuroprostane or PG like compounds were detected in significantly higher levels, compared to DHA reaction without worm lysate. Our studies provide the first evidence based on LC-MS/MS analysis of reaction products of C. elegans lysates and DHA and triple Cox-knockout zebrafish lipid extract that an alternative pathway other than Cox catalytic or free radical mechanism may be involved in the formation of these novel products. Further investigations on target enzyme(s), major metabolic pathways and biological consequences of these products presently underway in our laboratory.

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POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number presenters will be at their posters.

NICHE METABOLOMICS

P-497 **Urine metabolomics profile in patients with reversible cerebral vasoconstriction syndrome**

PRESENTING AUTHOR: ***Wei-Hsiang Hsu, China Medical University, Taiwan***

CO-AUTHORS: ***Shih-Pin Chen, Shuu-Jiun Wang, Yun-Lian Lin***

Background: The pathophysiology and molecular mechanisms of Reversible Cerebral Vasoconstriction Syndrome (RCVS) are unknown. Objective of the study was to identify putative biomarker metabolites for RCVS. Methods: Patients were recruited from our institution's prospective RCVS registry. Urine samples were collected from 47 RCVS patients and 51 non- RCVS controls. We performed metabolome profiling of urine samples to identify potential biomarkers for diagnosing RCVS. Advances in 1H-Nuclear magnetic resonance (1H-NMR) and liquid chromatography tandem mass spectrometry (LC-MS/MS) have led to the application of metabolomics in RCVS, toward identifying metabolic alterations in RCVS. Results: Metabolites are considered as a putative biomarker, the relative abundance needed to be at the t-test derived p-value < 0.05 and VIP score >1. Gruanidoacetate, hippurate, 1,3,7-trimethyluric acid, ascorbic acid, D-glucurono-6,3-lactone, and D-threo-isocitric acid in the RCVS subjects were significantly different from the control cases. Conclusion: Results of this study show metabolites that might be potential biomarkers for RCVS. Furthermore, these data suggest that panels of analytes may be valuable to translate metabolomic findings to clinically useful diagnostic tests. Potentially, the present study provides promising diagnosis tool for RCVS.

BIG DATA, STATISTICS, INFORMATICS

P-498 **Northwest Metabolomics Research Center, University of Washington, Seattle**

PRESENTING AUTHOR: ***Nguyet Nguyen, Northwest Metabolomics Research Center, United States***

CO-AUTHORS: ***Lisa F. Betcher, Robert Pepin, G. A. Nagana Gowda, Daniel Raftery.***

The Northwest Metabolomics Research Center (NW-MRC) aims to bring together, coordinate, and integrate significant capabilities in metabolomics at the University of Washington, local institutions including the Fred Hutchinson Cancer Research Center and the Institute for Systems Biology, and beyond. The Center provides an extensive array of metabolomics assays using its MS and NMR platforms. Some unique capabilities include a 300 aqueous metabolite targeted MS assay with absolute quantitation of over 30 metabolites, an NMR based assay with absolute quantitation of >70 aqueous metabolites including the simultaneous analysis of redox coenzymes, energy coenzymes and antioxidants, and a 55 bile acid assay with absolute quantitation. Recently, the Center has developed GOT-MS, globally optimized, targeted-MS, which uses a QQQ-MS instrument to optimize all the detectable signals from a particular sample type. In addition, the Center can quantify up to 1100 lipids across 13 different lipid classes using the Lipidyzer platform, has developed a number of assays to detect acylcarnitines, cardiolipins, and metabolic flux, along with global metabolite profiling and other capabilities. The complementary platforms of NMR and GS-MS, help reinforcing the results of flux measurements. Specific metabolic pathway-focused assays are routinely developed to suit research interests of our collaborators. NW-MRC is currently working and collaborating with over 100 investigators at the local, regional or national level, involving a variety of metabolomics studies. Some of the unique features of the Center will be highlighted through several experimental examples.

NICHE METABOLOMICS

P-499 **13C isotopically non-stationary metabolic flux analysis of Methylomicrobium buryatense 5GB1 under methanol-limiting conditions**

PRESENTING AUTHOR: ***Lian He, University of Washington, United States***

CO-AUTHORS: ***Yanfen Fu, Mary E. Lidstrom***

Methylomicrobium buryatense 5GB1 is a promising methylotroph for industrial applications. To facilitate a better understanding of its metabolism, flux balance analysis and steady-state 13C metabolic flux analysis have been performed. However, these studies give either predicted enzymatic reaction rates or a qualitative description of the central metabolism. To experimentally quantify the in vivo fluxes, we performed 13C pulse-chase experiments and isotopically nonstationary metabolic flux analysis on M. buryatense 5GB1 under methanol-liming conditions. Metabolite pool sizes were measured by using 13C-labeled E. coli metabolites as internal standards. Dynamic changes of metabolite labeling patterns were analyzed by LC/MS-MS. These experimental data, together with substrate uptake rates and product formation rates, were fitted for flux calculations. The results show that, under methanol-limiting conditions, M. buryatense 5GB1 features a strong flux through the ribulose monophosphate (RuMP) cycle and a small flux through the TCA cycle that is branched at the oxaloacetate (OAA) node. The carboxylation reaction in the anaplerotic pathway is strong and contributes to the majority of OAA synthesis. Additionally, the Embden–Meyerhof–Parnas (EMP) pathway proves to be the dominating glycolytic pathway in M. buryatense 5GB1 under methanol-limiting conditions, while the Entner–Doudoroff (ED) pathway shows a minimal flux. This finding is corroborated by the measurements showing that 6-phosphogluconate, an upstream metabolite in the ED pathway, has a smaller pool size yet shows a slower 13C enrichment rate than metabolites in the EMP pathway. Overall, our study gives the first quantitative measurement of in vivo flux distributions in an obligate methylotroph.

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NICHE METABOLOMICS

P-501 Systematic Metabolic profiling for bioconversion of Aceraceae with enhancing bio-activity

PRESENTING AUTHOR: ***Jinyong park, Konkuk University, Korea, South***

CO-AUTHORS: ***Dong Ho Suh, Digar Singh, Jong Seok Lee, Sarah Lee, Choong Hwan Lee***

Plants are an important and inexhaustible source of bioactive compounds in food, medicine, agriculture, and industry. In this study, we performed systematic liquid chromatography–mass spectrometry (LC-MS)-based metabolic profiling along with antioxidant assays for indigenous plant family extracts. Partial least-squares discriminant analysis (PLS-DA) of LC-MS datasets for the extracts of 34 plant species showed that these species were clustered according to their respective phylogenies, Aceraceae, Asteraceae, and Rosaceae. In particular, seven Aceraceae species were clearly delimited with higher average antioxidant activities, rationalizing their application for bioconversion studies. On the basis of further evaluation of the interspecies disparity of metabolic profiles and antioxidant activities among Aceraceae family plants, we found that Acer tataricum (TA) extracts were clearly distinguished from other Aceraceae species, with a relative higher abundance of tannin derivatives. Further, we discovered a strong positive correlation between most tannin derivatives and the observed higher antioxidant activities. Following Aspergillus oryzae-mediated bioconversion of Acer plant extracts, we identified a time-correlated (0–8 days) linear increase in antioxidant phenotypes for all Acer species, with TA having the highest activity. Temporal analysis of the MS data revealed that tannin bioconversion mechanisms about a relative higher accumulation of gallic acid (m/z 169) at the end of bioconversion process, particularly in TA. Similarly, quercetin glycoside metabolites were also converted to quercetin aglycones (m/z 301) in most Acer plant extracts. The present study underscores the efficacy of fermentative bioconversion strategies aimed at improving the availability and quality of bioactive metabolites from plant extracts.

P-502 Optimization of universal SWATH-MS conditions for quantitative proteomics and metabolomics using Skyline predicted parameters

PRESENTING AUTHOR: ***Yuanyuan Shi, University of Washington, United States***

CO-AUTHORS: ***Bhagwat Prasad***

Proteomic and metabolic analyses using the sequential window acquisition of all theoretical fragment ion spectra (SWATH) has enormously expanded the capacity of quantitative mass spectrometry (MS). In SWATH-MS method, the initial MS conditions and optimum use of software tools are important. We evaluated the impact of major acquisition parameters such as declustering potential (DP), collision energy (CE), and accumulation time (AT) on the signal quality and intensity. To do so, we used either SWATH default settings or Skyline predicted parameters for iRT standard peptides (Biognosis). In order to fully consider the effect of the three main parameters, we adopted central composite design (CCD), which was validated by single factor optimization. Our analysis concluded that AT should be less than 50 ms to ensure the cycles >1000 and data points >7. We recommend AT of 25 ms to be optimum for the 11 iRT peptides. Skyline predicted DP and CE for the iRT peptides (mass range, m/z 225-800) range from 49-87 V (DP) and 11-37 eV (CE), respectively. Increasing Skyline predicted CE increased the intensity of the peptides and the optimization point of CE was predicted to be +7. Effect of change in DP from Skyline predicted +10 to -10 was minimal; but we observe that DP+30 could reach a peak point for some iRT peptides. By using this robust methodology, we developed the optimized SWATH parameters for iRT peptides, which will be further applied to drug transporters expressed in dog kidney cortex.

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ECOLOGY AND ENVIRONMENT

P-503 **Measuring oceanic concentrations of dissolved organic acids with a unique high-efficiency, low-energy EI approach**

PRESENTING AUTHOR: ***Stephan Andreas Baumann, Agilent Technologies, United States***

CO-AUTHORS: ***John R. Casey***

We are applying a novel Low Energy EI approach to better understand marine microbial metabolism. These multi-step cycles are an important driver in oceanic productivity and respiration, fueling the microbial loop (Azam et al., 1983). Existing protocols for measuring oceanic concentrations of low molecular weight carboxylic and hydroxy acids have been plagued by low recoveries, high analytical variance, as well as chromatographic interferences. We expect that applying a novel high efficiency EI source will allow high sensitivity measurement of microbial metabolites at biologically relevant concentrations while concurrently reducing chemical interferences. Moreover, using a standardized metabolic workflow allows for greater coverage of known biological pathways and gives deeper insight into the complexity of marine microbial community metabolism. Carboxylic and hydroxy acids are found in seawater at the 1 nM to 100 nM range (10-9 mol/L). While not inherently difficult to detect at these levels, extraction out of seawater matrix has proven difficult for the analysis of polar acids. The seawater samples are stored frozen to hinder metabolism. Once thawed, they are partitioned by liquid-liquid extraction, keeping the pH below the dissociation constant of the target analytes to aid in recovery. The samples are then derivatized before analysis by GCMS. The results are first de-convoluted, then identified with a metabolomic library, and then processed by a multi-variant software package where metabolic flux data can identify active biological pathways.

METABOLITE ID

P-504 **Structure Elucidation of Unknown Metabolites in Metabolomics by Combined NMR and MS/MS Prediction**

PRESENTING AUTHOR: ***Kerem Bingol, Pacific Northwest National Laboratory, United States***

CO-AUTHORS: ***Rene M. Boiteau, David W. Hoyt, Carrie D. Nicora, Hannah A. Kinmonth-Schultz, Joy K. Ward, Kerem Bingol***

We introduce a cheminformatics approach that combines highly selective and orthogonal structure elucidation parameters; accurate mass, MS/MS (MS2), and NMR into a single analysis platform to accurately identify unknown metabolites in untargeted studies. The approach starts with an unknown LC-MS feature, and then combines the experimental MS/MS and NMR information of the unknown to effectively filter out the false positive candidate structures based on their predicted MS/MS and NMR spectra. We demonstrate the approach on a model mixture, and then we identify an uncatalogued secondary metabolite inArabidopsis thaliana. The NMR/MS2approach is well suited to the discovery of new metabolites in plant extracts, microbes, soils, dissolved organic matter, food extracts, biofuels, and biomedical samples, facilitating the identification of metabolites that are not present in experimental NMR and MS metabolomics databases. [REF: Bingol, et al,Metabolites2018,8(1), 8; doi:10.3390/metabo8010008]

NEUROLOGY AND PSYCHIATRY

P-505 **Alterations of eicosanoids and related mediators in patients with schizophrenia**

PRESENTING AUTHOR: ***Dongfang Wang, Peking University, China***

CO-AUTHORS: ***Bing Cao, Lailai Yan, Qingbin Lu, Biao Ren, Haiwei Gu, Jingyu Wang***

Schizophrenia (SCZ) is a multifactorial psychiatric disorder. Currently, its molecular pathogenesis remains largely unknown, and no reliable test for diagnosis and therapy monitoring is available. Polyunsaturated fatty acids (PUFAs) and their derived eicosanoid signaling abnormalities are relevant to the pathophysiology of schizophrenia. However, comprehensive analysis of eicosanoids and related mediators for schizophrenia is very rare. In this study, we applied a targeted liquid chromatography-mass spectrometry based method to monitor 158 PUFAs, eicosanoids and related mediators from enzyme-dependent or independent pathways, in the serum samples of 109 healthy controls, and 115 schizophrenia patients at baseline and after an 8-week period of antipsychotic therapy. Twenty-three metabolites were identified to be significantly altered in SCZ patients at baseline compared to healthy controls, especially arachidonic acid (AA) derived eicosanoids. These disturbances may be related to altered immunological reactions and neurotransmitter signaling. After 8-week antipsychotic treatment, there were 22 metabolites, especially AA and linoleic acid derived eicosanoids, significantly altered in posttreatment patients. Some metabolites, such as several AA derived prostaglandins, thromboxanes, and di-hydroxy-eicosatrienoic acids were reversed toward normal levels after treatment. Based on univariate analysis and orthogonal partial least-squares discriminant analysis, anandamide, oleoylethanolamine, and AA were selected as a panel of potential biomarkers for differentiating baseline SCZ patients from controls, which showed a high sensitivity (0.907), good specificity (0.843) and excellent area under the receiver operating characteristic curve (0.940). This study provided a new perspective to understand the pathophysiological mechanism and identify potential biomarkers of SCZ.

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ECOLOGY AND ENVIRONMENT

P-506 **Metabolomics application in immunotoxicological studies of mussel haemocytes exposed to copper**

PRESENTING AUTHOR: ***Thao V. Nguyen, Auckland University of Technology, New Zealand***
CO-AUTHORS: ***Andrea C. Alfaro, Fabrice Merien, Ronnie Lulijwa, Tim Young***

Copper is a common contaminant in aquatic environments which may cause immune dysfunction in marine organisms when it is accumulated in high concentration. However, the toxicity mechanisms of copper at a molecular level in marine bivalves is not fully understood. In this study, we applied flow cytometry and GC-MS-based metabolomics to characterize cellular and molecular mechanisms of copper immunotoxicology in mussel (Perna canaliculus) haemolymph exposed to copper. The results from the flow cytometric assays showed significant increases in mortality, production of reactive oxygen species and apoptosis of haemocytes in haemolymph exposed to increasing high concentrations of copper (250, 500 and 750 µM) compared to a low copper concentration (100 µM) and the control (0 µM). These results suggest that copper induces oxidative stress and apoptosis for mussel haemocytes at concentrations that may be commonly found within wild and aquaculture settings. In addition to flow cytometric data, our metabolomics results showed alterations of 25 metabolites within the metabolite profile of copper-exposed haemolymph (500 µM) compared to those of control samples. The changes in these metabolites are important signatures of oxidative stress and apoptosis process in copper-exposed haemolymp. Among the altered metabolites, the increase in glutathione may be an important signature of oxidative stress, while accumulation of alanine and reduction of glutamic acid are indicators of apoptosis in copper-exposed haemolymph. This study provides insights into the cellular and molecular mechanisms of oxidative stress and apoptosis in marine bivalves and highlights the reliability of metabolomics techniques for immunotoxicological studies in marine organisms.

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