Session #	Abstract Number : Title	Presenter	Organization	Abstract Text	Theme
1 Keynote	360: Genome-wide association study of the human lipidome provides new insight to lipid metabolism and metabolic disease	**Peter Meikle**	Baker Heart And Diabetes Institute	Dysregulation of lipid metabolism is as an important –and modifiable– risk factor for multiple diseases, including type 2 diabetes, cardiovascular disease and age-related dementia. While the metabolic pathways of lipids are well characterised, dysregulation of these pathways due to environmental and genetic influences is not well understood. To address this issue, we have applied an integrative approach to link genetic variants with altered lipid metabolism and metabolic disease. Using lipidomic profiling data from 4,492 genotyped individuals from the Busselton Family Health Study, we performed genome-wide association analysis of 596 lipid species and 33 lipid classes using linear-mixed models, correcting for age, sex, their interactions. To account for lipoprotein mediated associations, the analysis was repeated with HDL-C, triglycerides and total cholesterol as covariates. Additionally, collider bias was avoided by conditioning using multi-trait-based conditional and joint analysis. Validation of genome-wide significant associations is supported by replication and meta-analyses in the Australian Imaging, Biomarker & Lifestyle Study of Ageing (n=1,112) and Alzheimer's Disease Neuroimaging Initiative (n=757) cohorts. Over 70,000 genome-wide significant (p<5e-08) associations were identified, with 543 lipid species in 733 independent genomic regions, of which 520 have not previously been reported in metabolic-QTL analyses. Using the ProGeM framework, biologically plausible genes were identified in 524 genomic regions. Approximately 70% of the observed associations were independent of lipoprotein measures. Pleiotropic associations of genetic variants were determined by integration of results from expression-QTL and protein-QTL studies. New insights into the function and specificity of established lipid metabolic pathways was revealed by associations with novel lipid species. Genetic correlations between lipid species and CVD in the UK Biobank and CARDIoGRAMplusC4D consortium highlight the shared genetic effects. Colocalisation analyses then identified 47 shared causal variants for coronary artery disease and lipid species, demonstrating the potential to identify targets for monitoring, prevention and treatment of cardiometabolic disease.	Technology, Systems Biology & Advancing the Field
1.1	78: Single cell analysis of lipids using chip-based nanoelectrospray ionisation mass spectrometry	**Sarah Hancock**	UNSW Sydney	As single-cell transcriptomics technologies have evolved we have begun to appreciate the wide degree of heterogeneity inherent within seemingly identical cells (i.e., those from the same clonal population/genetic background). This insight has provided us with a new understanding of the fundamental biological processes of growth, development, and ageing, as well as new information on cancer initiation and metastasis. Despite these advances, however, our ability to fully probe this cellular diversity is limited with the development of methods to assay metabolites from single cells (i.e., single-cell metabolomics) being hindered by technological challenges. Mass spectrometry-based methods hold the promise of being able to overcome many of these challenges and deliver the ability to perform single-cell metabolomics, but currently available methodological approaches have distinct limitations. To overcome some of the limitations of single-cell metabolomics methods presently in use we have developed a workflow that combines fluorescence-activated (FACS) single-cell sorting with chip-based nanoelectrospray ionisation to deliver high-throughput detection of membrane lipids from single cells. Using a shotgun lipidomics approach we have been able to detect up to 60 phosphatidylcholine and sphingomyelin species from singly sorted HepG2 and C2C12 cells. Future work will focus on expanding the coverage of membrane lipid classes and polar metabolites able to be detected using this workflow through the use of charge-switching derivatisation to improve metabolite signal.	Technology, Systems Biology & Advancing the Field
1.2	165: Multi-omic signatures of chronic metal tolerance	**Katie Hillyer**	CSIRO	The Derwent Estuary (Hobart, Tasmania) is subject to heavy metal contamination, elevated nutrients and low dissolved oxygen, from a combination of industrial, urban, aquaculture and agricultural uses. Despite these human influences, the estuary continues to support important natural habitats and species; a major management challenge therefore is translating discrete environmental monitoring data into understanding of contaminant bioavailability, impact and system condition. Here we apply high throughput systems biology tools (i.e., genomics, proteomics, lipidomics and metabolomics), to provide detailed and holistic insight into the functional status of benthic microbial and macroinfauna communities in this human impacted estuary. Coupled with traditional environmental monitoring data (water and sediment quality), we characterise estuary wide patterns of biological composition and metabolic function, to explore the effects of co-occurring environmental drivers of change. Our data provide much needed insight of functional outputs in a highly complex, non-model system. These data are critical if we are to effectively monitor, prioritise recovery efforts and restore valuable goods and services in increasingly human influenced environments.	Environmental, Plant, Animal, Agriculture, Food and Model Organisms
1.3	121: A reference map of sphingolipids in murine tissues	**Federico Torta**	National University Of Singapore	Sphingolipids (SP) have both a structural role in the cell membranes and a signalling function that regulate many cellular processes. In mammals, their structural differences in specialised cell types and tissues far superseded that of other lipids. This enormous structural diversity and low abundance of many SP pose a challenge for their identification and quantification. Here we used LC-MS to compile a ‘murine sphingolipid atlas’, containing the qualitative and quantitative distribution of 114 sphingolipids in 21 tissues of a widely utilised wild-type laboratory mouse strain (C57BL/6). We report tissue-specific sphingolipid fingerprints, as well as sex-specific differences in the same tissue. This is a comprehensive, quantitative sphingolipidomic map for mammalian tissues collected in a systematic fashion. It will complement other tissue compendia for interrogation into the role of sphingolipids in mammalian health and disease.	Environmental, Plant, Animal, Agriculture, Food and Model Organisms
2 Keynote		**Yulan Wang**	Singapore Phenome Center, Lee Kong Chian School of Medicine		

Session #	Abstract Number : Title	Presenter	Organization	Abstract Text	Theme
2.1	52: Discovery and validation of serum metabolites associated with wholegrain consumption using nontargeted metabolic profiling	**Stefania Noerman**	University of Eastern Finland	Background and aim: Wholegrain (WG) consumption has been associated with lower risk factors of metabolic diseases. However, the interplay between WG biochemicals and endogenous metabolites in conveying the potential health benefit of WG intake remains unclear. Here, we present the application of nontargeted metabolic profiling to identify fasting serum metabolites associated with WG intake. Methods: From a subset (n=364) of population-based cohort Kuopio Ischaemic Heart Disease Risk Factor study (KIHD), we analyzed the fasting serum samples collected at baseline using nontargeted LC-MS metabolomics technique. Association with WG intake was investigated using either Spearman correlation analyses or random forest, followed by linear regression. Several risk factors of metabolic health were modeled as covariates: age, BMI, smoking, physical activity, energy and alcohol consumption. Features selected by any of these analyses were shortlisted for annotation. Annotated metabolites were then validated in an independent subset from the same cohort (n=200). Results: We found an association between WG and several phytochemicals, such as pipecolic acid betaine, sinapyl alcohol, and alkylresorcinols. After validation, pipecolic acid betaine, tetradecanedioic acid, alkylresorcinols C19:1-glucuronide and C21:1-glucuronide, and an unknown metabolite showed significant association even after adjustment for selected covariates. Conclusions: The metabolites associated with WG intake, even after adjustment for potential covariates in both discovery and validation subsets, show the strongest association with WG intake. They deserve a closer examination for their potential role in the maintenance of metabolic health.	Environmental, Plant, Animal, Agriculture, Food and Model Organisms
2.2	51: Processing of Proton Transfer Reaction Time-of Flight Mass Spectrometry (PTR-TOF-MS) data for untargeted biomarker discovery in exhaled breath: application to COVID-19 intubated ventilated patient	**Camille Roquencourt**	CEA	The analysis of Volatile Organic Compound (VOCs) in exhaled breath is a promising non-invasive method for early diagnosis and therapeutic monitoring. Proton Transfer Reaction Time-Of-Flight Mass Spectrometry (PTR-TOF-MS) has recently emerged as an innovative technology for the real time analysis of exhaled VOCs. However, there is currently a lack of methods and software tools for the processing of such breath data from cohorts. We therefore developed a suite of algorithms that process the raw data and build the table of feature intensities in all samples, through expiration and peak detection, quantification, alignment between samples, missing value imputation, and feature annotation. Notably, we developed an innovative 2D peak deconvolution method based on penalized splines signal regression. Our software tool is publicly available as the ptairMS R package on GitHub (and submitted to Bioconductor). We applied our methodology to the characterization of exhaled breath from mechanically ventilated adults with COVID-19 infection. Analyzes of exhaled breath from 28 patients with COVID-19 ARDS and 12 patients with non-COVID-19 ARDS were performed daily from the hospital's entry to the discharge. First, we performed classification model to predict the status of the infection, using the closest available acquisition of the hospital entry. Then, we used all data for univariate longitudinal analysis of the VOCs evolution in function of the hospitalization time. After feature ranking and selection, four biomarkers of COVID-19 infection could be identified. Altogether, these results highlight the value of the PTR-TOF-MS data and ptairMS software for the biomarker discovery in exhaled breath.	Technology, Systems Biology & Advancing the Field
3 Keynote	362: Metabolomics in the Early Detection of Cardiovascular Disease: Taking the road less traveled	**Carina Mels**	Hypertension In Africa Research Team	Despite extensive knowledge on the prevention and treatment of hypertension global incidence, prevalence, and associated complications are increasing. Globally, this may be due to population growth and ageing, but in low- and middle-income countries (including in sub Saharan Africa) inadequate prevention, diagnosis, and control of blood pressure are also important contributing factors. Since hypertension is largely preventable and in the context of an ageing population, attention is shifting towards lifetime exposure to cardiovascular disease (CVD) risk factors. For centuries, the measurement of blood pressure was considered as the ideal biomarker for hypertension. However, due to its multifactorial nature, the potential for a new definition of hypertension based on its molecular phenotype as characterized by complex biomarkers, such as the metabolome, was acknowledged by the Lancet Commission on Hypertension. The application of metabolomics in CVDs is not novel but focused mainly on advanced stages of CVD. Since the pathogenesis of hypertension is dynamic it is important to investigate metabolic changes during the early stages when intervention will be most effective. To this end, we are using metabolomics to profile children and young adults in relation to early cardiovascular deterioration. Some of our findings include an inverse link between more abundant levels of non-essential amino acids with central blood pressure and left ventricular mass in apparently healthy black adults. In the context of their lower dietary protein intake these findings may suggest that the biosynthesis of these amino acids may be upregulated to protect the cardiovascular system against the onset of early deterioration. In a subsequent targeted metabolomics approach, we profiled amino acids and acylcarnitines according to CVD risk factors and identified three distinct metabolic patterns associated with an increasing amount of CVD risk factors. These findings may suggest that different metabolic pathways are affected as the risk for CVD development increase.	Metabolomics in Health and Disease
3.1	192: FlyMet.org: an online metabolomics tissue atlas and multi-omics resource using Drosophila as a model organism	**Karen McLuskey**	University of Glasgow, UK	In multicellular organisms, the metabolomes of different tissues are likely to differ significantly, reflecting the different specialized jobs they perform. Studying the composition of tissues in humans can be difficult to achieve; however the tiny fruit fly Drosophila melanogaster has proved itself to be an excellent model for many human processes: The Drosophila genome is 60% homologous with the human genome, and 75% genes involved in human disease are also found in the fruit fly. By using Drosophila as a model, metabolomes for the whole organ/tissue can be collected from several animals allowing an average metabolome for each tissue can be obtained. To make use of this system, we have produced an atlas of 19 reference tissue metabolomes have been obtained by separate micro-dissection of adult (male and female) and larval Drosophila. In addition to the tissue metabolomes, metabolomes for different aged male and female adult Drosophila have also been analysed to allow investigations into aging processes. To present this data, we have developed FlyMet (www.flymet.org): a database and Web application that provides user-friendly visualization of metabolite profiles across Drosophila tissues and aged flies. Users can investigate Flymet data as annotated chromatographic peaks, metabolites, or pathways; with the Pathway Explorer ranking differentially-expressed metabolic pathways. To assist with correct data interpretation, FlyMet uses a traffic light system of buttons to show confidence levels for metabolites identified using standard compound libraries and fragmentation spectra and reveals all the peaks associated with less certain identifications. FlyMet is also being developed to incorporate multi-omics data by linking genomic data from our Drosophila gene expression tissue atlas (http://flyatlas.gla.ac.uk/FlyAtlas2) to the compounds, reactions and pathways found in the FlyMet data. It is hoped that Flymet that will provide an invaluable resource to investigate tissue functionality and ageing using Drosophila as a model system.	Environmental, Plant, Animal, Agriculture, Food and Model Organisms

Session #	Abstract Number : Title	Presenter	Organization	Abstract Text	Theme
3.2	18: Finger Sweat Analysis Enables Metabolic Biomonitoring in Humans	**Julia Brunmair**	Department Of Analytical Chemistry, Faculty Of Chemistry, University Of Vienna	Metabolic biomonitoring in humans is typically based on sampling of blood, plasma or urine. Although established in the clinical routine, these sampling procedures are often associated with a variety of compliance issues and are impractical for performing time-course studies. The analysis of minute amounts of sweat collected with a filter placed between two fingers provides a solution to this challenge. Sweat sampling is non-invasive, robust and can be accomplished repeatedly by untrained personnel. Sample preparation is straight-forward and takes approximately 5 minutes per sample. High-resolution orbitrap mass spectrometry hyphenated with liquid-chromatography showed that sweat represents a rich source for metabolomic phenotyping, providing relevant information on ingested substances from diet or individual medication as well as helping to sketch the lifestyle of a person. The feasibility of short interval sampling of sweat from the fingertips to kinetic analyses was confirmed in three time-course studies after coffee consumption or ingestion of a 200 mg caffeine capsule, successfully monitoring all known caffeine metabolites in 40 individuals. Fluctuations in the rate of sweat production were corrected for by mathematical modelling to reveal individual rates of caffeine uptake, metabolism and clearance. A 30-day sampling study revealed bioactive compounds such as natural polyphenols and flavonoids as well as potential hazardous substances such as pesticides in sweat of volunteers in correlation to food intake. Furthermore, the identification of metabolites from nicotine after cigarette smoking and metabolites from orally ingested medications such as metamizole indicated the applicability of this assay to observe specific enzymatic processes. Remarkably, we have found that histamine was significantly increased in sweat of individuals experiencing allergic reactions. In summary, we show that sweat analysis provides accurate, reproducible, and quantitative means to individualized biomonitoring in humans, which offers numerous practical applications for personalized medical diagnostics, including the detection of xenobiotics, metabolic traits or biomarker discovery.	Metabolomics in Health and Disease
3.3 Sponsor	272: Trends and technology in metabolomics-based microbiomics for gut-liver-brain research in oncology and neurology	**Therese Koal**	Biocrates Life Sciences	Microbiome research has dramatically reshaped our understanding of how microbes impact a multitude of (patho-)physiological processes in the host and effect metabolic disease developments in diabetes, cancer, cardiovascular disease, and neurological disorders. Metabolomics allows the investigation of microbial metabolic activities and thus is the ideal technology to assess functional nutrition-microbiota-host crosstalk. Here, we discuss the application of a developed standardized targeted metabolomics platform (MxP® Quant 500 kit) fulfilling uniquely the FAIR principle compliance (i.e. Findability, Accessibility, Interoperability, Reusability) for the quantification of up to 860 endogenous and microbiota-derived metabolites and metabolism indicator sums and ratios. Recent advancements of metabolomics for disease commonalities, specificities and trends in microbiome research will be discussed. You will be presented with an overview of key metabolic pathways and bioactive molecules e.g. enterosynes relevant for the gut-liver-brain axis linking intestinal bacteria to distant organs, including choline metabolism, tryptophan metabolism, and bile acid metabolism. Based on several high-impact literature-based examples, you will learn how the gut microbiome impacts pathophysiological processes, disease development, and response to drug treatment with a focus on cancer and neurological disorders and how to use the value of data quality, comparability, and translation across studies.	Metabolomics in Health and Disease
4 Keynote		**Theodore Alexandrov**	European Molecular Biology Laboratory (EMBL)		
4.1	62: MetaboAtlas21: A metabolome atlas of 21 mouse tissues and biofluids in response to the metabolic challenge	**Tomas Cajka**	Institute of Physiology of the Czech Academy of Sciences	Genome, transcriptome, and proteome atlases that comprehensively characterize various tissues, biofluids, or cells have become available over the last decade. However, similar resources mapping metabolites are very rare and only a fraction of the metabolome is captured by analytical platforms used. This shows a lack of sufficient data on the metabolome characterizing different tissues and biofluids. Here, we present a specific atlas of mouse metabolome and lipidome (MetaboAtlas21) in the context of systemic energy balance (chow diet) and under chronic nutrient stress (high-fat diet). Male mice were fed a control (chow) diet for 2 months or a high-fat diet for 2 months and 10 months. Urine, plasma, feces, and 18 different tissues were collected from each animal for metabolomics and lipidomics analysis. These matrices cover digestive, excretory, respiratory, reproductive, endocrine, muscular, cardiovascular, and nervous systems. Also, chow and high-fat diet feeds were analyzed along with quality control human plasma/serum materials (NIST SRM 1950 plasma, Merck S1-100ML serum, Sigma–Aldrich S7023 serum). In total, 408 samples were included in this study. An 'all-in-one' extraction protocol LIMeX using methyl tert-butyl ether, methanol, and water was used to isolate metabolite fractions and analyzed using a multiplatform LC-MS-based approach (7 platforms for non-fat tissues and biofluids; 8 platforms for adipose tissues). Ultimately, we annotated over 3,000 unique polar metabolites and complex lipids. To better understand the structure of generated data, we provide a user-friendly data visualization tool (metaboatlas21.metabolomics.fgu.cas.cz) to easily access and analyze the different combinations of tissues and biofluids in response to the metabolic challenge.	Metabolomics in Health and Disease
4.2 Sponsor	253: New research perspectives using accurate mass technology for the advancement for metabolomics in systems biology.	**Jose Castro-Perez**	Sciex	Most MS/MS-based metabolomics experiments rely on collision induced dissociation (CID) for inducing fragmentation. This presentation will showcase a new ion fragmentation paradigm with enhanced sensitivity for enabling richer, more comprehensive data and structural metabolite information with the new innovative, accurate mass spectrometry. Dr. Hankemeier will share his perspective on how this technology will deliver more complete libraries for more accurate metabolomic identification, offering researchers the capability to make more informed decisions and unambiguous conclusions.	Metabolomics in Health and Disease

Session #	Abstract Number : Title	Presenter	Organization	Abstract Text	Theme
5 Keynote	363: Exploring the Potential of Metabo-Endotypes and MultiOmic-Endotypes in the Improvement of Disease Classification	**Rachel Kelly**	Harvard Medical School	The classification of complex disease has historically relied largely on symptoms and clinical manifestation. However, the outward presentation of a disease is not necessarily representative of its underlying mechanisms. Therefore, subgrouping individuals based on phenotype can lead to misclassification and suboptimal therapeutic management in certain groups. Instead, it is likely to be more informative to look upstream on the central biological dogma; to classify individuals by the functional or pathobiological mechanisms of their disease and tailor therapies appropriately. Metabolomics provides a particularly compelling approach to the derivation of such disease endotypes (ie. subtypes defined by mechanism). We have previously demonstrated that we can derive and validate metabolomic driven endotypes, or “metabo-endotypes”, of common complex disorders, including asthma. We have further shown that metabo-typing healthy individuals before the clinical presentation of a disease can identify those subgroups who are most at risk. Intriguingly, our work in asthma shows that these at-risk groups demonstrate similar metabolomic profiles to those in the most severe disease endotypes. This suggests that understanding the mechanistic basis of a disease through endotyping may also have utility in prediction and prevention. However, disease dysregulation often occurs at multiple levels and therefore the most accurate endotypes may be defined by incorporating multiple levels of omic data. Accordingly, we have extended upon our metabo-endotype work to consider whether individuals are classified into the same disease endotypes when using their transcriptomic, epigenetic, and mRNA profiles, and when using a combination of omics. By determining the clinical utility of these respective endotypes, we can explore what they can tell us about the pathophysiology of disease, how they can help us to identify therapeutic targets and ultimately what role omic-endotyping may play in the future of precision medicine.	Technology, Systems Biology & Advancing the Field
5.1	93: Metabolite Profiling of Fourteen By-Products from the Coffee Production Chain	**Mariana da Silva**	São Paulo State University	Coffee beans are one of the most important food commodities worldwide, with an estimated production of 10.5 million tonnes for the 2020/2021 harvest (ICO, 2021). Several by-products are generated during coffee fruit processing to obtain the commodity (endosperm) while other by-products are generated inside coffee ground factories. These by-products are currently of low value and some are potentially harmful to the environment. This work presents the most wide-ranging chemical investigation of coffee by-products collected from farms to factories, including eight never previously investigated. A comprehensive two-liquid phase extraction (EtOH-H2O 7:3 and n-heptane) was developed and the hydroethanolic and n-heptane extracts were analysed by UHPLC-PAD/UV-HRMS/MS and GC-MS, respectively. Forty-two compounds were found for the first time in coffee by-products including bioactive neomangiferin, kaempferol-3-O-rutinoside, lup-20(29)-en-3-one and 3,4-dimethoxy cinnamic acid. Squalene, a component of COVID-19 vaccines which is extracted from non-renewable resources, was found in eight by-products. Five by-products generated inside a factory showed caffeine (53.0-17.0 mg/g) and/or chlorogenic acid (72.9-11.7 mg/g) content comparable to coffee beans, and three by-product samples collected in farms contained 16.5-6.8 and 38.9-0.8 mg/g, respectively. Mature leaf from plant pruning presented mangiferine levels of 19.4 mg/g. Such by-products are therefore a potential source of a range of bioactive compounds and could be explored with potential economic and certainly environmental benefits. This is because caffeine, tannins and polyphenols are persistent chemicals that exhibit toxic effects in various biological systems and should not be discharged into the environment. International Coffee Organization (2021). Crop year production by country. Janissen, B., & Huynh, T. (2018). Resources, Conservation and Recycling, 128, 110–117.	Environmental, Plant, Animal, Agriculture, Food and Model Organisms
5.2	41: Application of chemoresistive gas sensors and chemometric analysis to differentiate the fingerprints of global volatile organic compounds from diseases. Preliminary results of COPD, lung cancer and breast cancer.	**Rogelio Flores Ramírez**	Coordinación para la Innovación y Aplicación de la Ciencia y la Tecnología	Background: Analysis of volatile organic compounds (VOCs) in exhaled breath has been proposed as a screening method that discriminates between disease and healthy subjects, few studies evaluate whether these chemical fingerprints are specific when compared between diseases. The objective of this study was the evaluation of global VOCs and their discrimination capacity in chronic obstructive pulmonary disease (COPD), lung cancer, breast cancer and healthy subjects by chemoresistive sensors and chemometric analysis. Methods. A cross-sectional study was conducted, 30 patients with lung cancer, 50 with breast cancer, 50 with COPD and 50 control subjects was conducted. Each participant's exhaled breath was analyzed with the electronic nose. A multivariate analysis was carried: Principal Component Analysis (PCA) and, Canonical analysis of principal coordinates (CAP). Twenty single-blind samples from the four study groups were evaluated by CAP. Results. A separation between the groups of patients to the controls was achieved through PCA with explanations of over 90% of the data and with a correct classification of 100%. In the CAP of the four study groups, discrimination between the diseases was obtained using two canonical axes with a correct general classification of 91.35%. This model was used for the prediction of the single-blind samples resulting in correct classification of 100%. Conclusion. The application of chemoresistive gas sensors and chemometric analysis can be used as a useful tool for a screening test for lung cancer, breast cancer and COPD since this equipment detects the set of VOCs present in the exhaled breath to generate a characteristic chemical fingerprint of each disease.	Metabolomics in Health and Disease
5.3 Sponsor	453: Metabolomics Sample Collection in the Wild	**Annie Evans**	Metabolon	The study of disease in human subjects often requires the analysis of a large numbers of samples due to the diversity of the human population including our genetic profiles, diets, and lifestyles. Investigators must therefore recruit individuals who are willing and able to visit a clinic for sample collection. This itself can reduce recruitment but also limits investigations to those individuals who are mobile and within proximity to the sampling locations. In addition, certain sample types have associated challenges and limitations such as the increased sampling, processing and cold storage requirements for blood and feces, of interest in microbiome research, being not amenable to on-demand collection. Given the increased demand for large human population studies and the desire to expand the population base that can be reached, sample collection methods that permit collection of samples away from the clinic would address many of these challenges and reduce costs. Furthermore, collection of samples away from the clinic requires the stabilization technology to preserve the integrity of samples during transit from collection location to the lab. The underlying biological information must be maintained in the absence of a cold-storage chain of custody. Over the last several years, Metabolon has been investigating and validating the use of various “in the wild” blood and fecal collection strategies for use in global metabolomics studies. While many different collection options were piloted, full validation was conducted on dried blood spots using Whatman 903 cards and feces using DNA Genotek’s OMNImet-GUTTM fecal collection tube. Both sample collection strategies were fully validated and included precision, stability, fidelity and biological testing. The results and findings from the validation activities will be presented.	Technology, Systems Biology & Advancing the Field

Session #	Abstract Number : Title	Presenter	Organization	Abstract Text	Theme
6 Keynote	372: Untargeted Metabolomics Approach to Investigate Systematically the Mechanistic Action of Prospective Drugs for Neglected Diseases	**Marina F.M. Tavares**	University Of Sao Paulo	Neglected diseases, endemic to low-income urban and rural populations of developing countries, are a group of medically diverse infections afflicting millions of people worldwide. Clinical practices resorts to long and costly treatments with a limited number of drugs often exhibiting high toxicity, severe adverse effects, offering little or no effectiveness to resistant strains. This work proposes that the mechanistic action of candidate drugs for neglected diseases be contrasted by untargeted multiplatform metabolomics approaches. Prospective drugs are classified systematically into five categories: reference drugs in use in clinical practice, repositioning drugs, compounds extracted from natural products, synthetic Selenium and Tellurium chemical libraries, and compounds derived from in silico methods. Preliminary results for Leishmaniasis and Chagas Disease will be presented demonstrating the impact of the listed drug classes into the parasite metabolism. The results generated so far can therefore contribute in a concerted manner to elucidate the broad action of several candidate drugs against neglected diseases, paving the way into the search of novel alternative therapies.	Metabolomics in Health and Disease
6.1	225: In vivo estimation of ketogenesis using metabolic flux analysis framework and double tracer method - technical challenges and model interpretation	**Stanislaw Deja**	UT Southwestern Medical Center	Ketogenesis occurs in liver mitochondria, where 2 acetyl-CoA derived from lipid oxidation are condensed into acetoacetate (AcAc) and reduced to β-hydroxybutyrate (BHB). During carbohydrate scarcity, these two ketones are released into circulation at high rates and used as oxidative fuels in peripheral tissues. Despite their physiological relevance and emerging roles in a variety of diseases, endogenous ketone production is rarely measured in vivo using tracer approaches, since it requires simultaneous BHB and AcAc tracers. Here we describe the novel implementation of a 2-pool model of ketogenesis using a metabolic flux analysis (MFA) approach. The method simultaneously regresses LC-MS/MS ketone isotopologues and tracer infusion rates to estimate relative ketone pool sizes, and 8 fluxes including individual ketone rate of appearance, disappearance and exchange. We conducted in vivo dual-tracer infusions in conscious and unrestrained mice under different physiological conditions that should either stimulate or attenuate ketogenesis. A mixture of [U-13C4]BHB and [3,4,-13C2]AcAc provided unambiguous labeling of plasma ketones. To capture AcAc which can be unstable under some conditions, blood samples were immediately treated with sodium borodeuteride (NaB2H4) to reduce AcAc to its stable BHB analog with an M+1 mass shift. Hence, both AcAc and BHB labeling patterns were encoded in the combined [2H]BHB mass isotopomer distribution (MID), but any BHB detected as an odd mass (M+1, M+3, M+5) must have originated from an AcAc. Additionally, 1H NMR real-time reaction monitoring was used to evaluate AcAc tracer and analyte stability during infusion and sample analysis, which were critical for accurate flux calculations. The MFA regression analysis provided confidence intervals and was capable of detecting errors in experimental data. Using this approach, we observed relationships between individual ketone metabolic fluxes and ketone pools, including the variable interconversion rate between AcAc and BHB as ketosis progress.	Metabolomics in Health and Disease
6.2	222: Detecting dynamic shifts in time-series metabolic data using machine learning	**BJ Stubbs**	Tufts University	In time-series metabolomics data, metabolites are measured through targeted or untargeted metabolomics at many time points. Analyzing the longitudinal aspect of this data sheds light on dynamic metabolic shifts and can elucidate disease progression and assess the impact of nutritional and drug interventions. Further, pairing this data with the sample's underlying network model allows for identifying important time dynamics in metabolic pathways and. While techniques such as principal component analysis and metabolite enrichment set analysis can be adapted to analyze time-series data, they may be limited in uncovering metabolic dynamic shifts. Here, we adapt a promising machine learning technique called dynamic stochastic block models to uncover dynamic shifts in metabolomics data. Stochastic block models (SBM) are used for detecting groups (or blocks or clusters) within a network. Dynamic SBMs infer the evolution of such blocks through time while aiming to stabilize within-group connectivity. To adapt Dynamic SBM to analyze metabolomics data, we represent relationships among measured metabolites by their correlation similarity to other metabolites. Dynamic SMB thus identifies groups of measured metabolites that have similar correlation patterns. Using the underlying biological network for the sample under study, we weight correlation similarity by the corresponding distance in the biological network. We adopt dynSMB, the Dynamic Stochastic Block Model R package https://cran.r-project.org/web/packages/dynsbm/, to analyze metabolomics data. We demonstrate this method on metabolomics data from Chinese hamster ovary cells culture (Sumit et al, 2019). A custom visualization tool allows the exploration of model parameters that yield different groups and the migration of metabolites among groups. Results reveal that dynamic SBM can detect metabolic temporal shifts associated within several metabolic pathways. We compare our findings against PCA-based methods and metabolite enrichment set analysis that were reported earlier for this dataset. Our technique is general and can be applied to multi-omics data.	Technology, Systems Biology & Advancing the Field
7 Keynote	361: Targeted and untargeted metabolomics in grape and wine research	**Farhana Pinu**	New Zealand Institute For Plant And Food Research Ltd	Wine fermentation is a complex process where microorganisms, particularly wine yeasts, convert perishable grape juice into a non-perishable aromatic beverage, thus avoiding spoilage and allowing prolonged storage. Traditional grape and wine research mostly relied on the analysis of major group of metabolites that were present in high concentrations. Therefore, the role of metabolites present in very small amount has been largely unknown. With the recent improvements of analytical instrumentations and data analysis approaches, we are now able to determine thousands of metabolites present in grape and wine matrices. This is providing us a unique opportunity to explore a wide range of metabolites and how they impact overall wine quality, thus aiding innovation in wine research. Using metabolomics approaches (including lipidomics and flavoromics), large amount data have been generated over the last two decades that shed lights on various problems related to different stages of winemaking process and grape growing condition. Metabolomics has also been used as a data driven hypothesis generating tool by wine researchers. In this presentation, application of different metabolomics approaches in grape and wine research will be highlighted while showcasing the key findings from recent research carried out in New Zealand on Sauvignon blanc grape and wine. These include our approaches to better characterization of Sauvignon blanc juices and wines and research outcomes from the studies that explored the role of juice composition and wine yeast metabolism on final wine quality. A brief outline about how metabolomics based grape and wine research is assisting us to develop the platforms for personalized wine production will also be provided.	Environmental, Plant, Animal, Agriculture, Food and Model Organisms

Session #	Abstract Number : Title	Presenter	Organization	Abstract Text	Theme
7.1	187: NP-MRD: The World's Largest NMR Database for Natural Products	**Zinat Sayeeda**	University of Alberta	NP-MRD or the Natural Products Magnetic Resonance Database is a new, open-access resource developed in collaboration with NIH's National Center for Complementary and Integrative Health (NCCIH). It was officially launched in the second quarter of 2021. The NP-MRD is designed to contain all available NMR data on all known natural products, including 1D and 2D NMR spectra, chemical shift assignments and chemical structures. The NP-MRD is intended to assist with the identification, characterization and dereplication of natural products and to complement other natural product databases such the GNPS mass spectrometry database. The NP-MRD uses a very broad definition of natural products and welcomes the submission of any compounds produced or synthesized by animals, plants, marine organisms or microbes. This includes both primary and secondary metabolites. In this regard, the NP-MRD will soon house the largest collection of metabolite NMR spectra in the world and should be particularly useful to the metabolomics community. The NP-MRD has been designed to be a freely available cloud-based, FAIR electronic database. It accepts NMR data (spectra and assignments) and associated metadata from newly undertaken natural product studies. It also accepts, converts and stores all major NMR vendor formats and several common NMR data exchange formats from nearly all standard NMR experiments. Data deposition for NP-MRD has been designed to be fast and easy using the NPN-Dep system. Furthermore, all deposited structures and spectral files are run through a series of validation checks to inform submitters of any potential problems. A number of utilities are also available through NP-MRD to assist users with NMR spectral assignments and NMR-based structure elucidation. Currently NP-MRD has >10,000 structures with experimental NMR data and >200,000 structures with accurately predicted NMR data.	Technology, Systems Biology & Advancing the Field
7.2	76: Metabolomics of Switchgrass-Diazotroph Interactions	**Darian Smercina**	Pacific Northwest National Laboratory	Plants and soil microorganisms interact through exchange of organic molecules including metabolites like carbohydrates, organic acids, and amino acids. These exchanges lead to both detrimental (e.g., colonization by pathogens) and beneficial (e.g., recruiting nitrogen fixing bacteria (diazotrophs)) plant-microbial associations. Exchanged metabolites could also potentially act as biosignatures for plant or microbial function. Predicting and manipulating plant-microbe interactions requires qualitative and quantitative analysis of this metabolic exchange under differing environmental conditions. To better understand how plant-microbe interactions contribute to plant productivity and the potential for metabolite biosignatures, we examined the metabolite profiles of switchgrass (Panicum virgatum), a bioenergy crop and several diazotrophs under changing nitrogen availability. We measured rhizosphere metabolites of hydroponically grown switchgrass under high or low nitrogen and with or without Azotobacter vinelandii, a diazotroph. Metabolite profiles measured via NMR were predominately driven by nitrogen (R^2 = 0.311; $p < 0.001$) with some impact of diazotroph presence (R^2 = 0.168; p = 0.019). Analysis of metabolites collected in situ from field switchgrass revealed similar profiles to low nitrogen hydroponic rhizospheres (p = 0.987). Recently, we assessed switchgrass rhizosphere metabolomics in response to different diazotrophs (Azotobacter, Azospirillum, Paenibacillus or Sphingomonas sp. or a community of all four) and their nitrogen fixation potential. We have also characterized metabolomics of nitrogen fixation for two diazotrophs under nitrogen free and replete conditions, respectively inducing or inhibiting nitrogen fixation. This work demonstrates how plant-microbe interactions are mediated by the environment and the potential for metabolites to act as biosignatures of plant and microbial function.	Environmental, Plant, Animal, Agriculture, Food and Model Organisms
8 Keynote	365: Metabolic dysregulation in women with dormant genital tuberculosis	**Koel Chaudhury**	Indian Institute Of Technology Kharagpur	Although women constitute half the global population and directly influence health of the fetus, women's health remains one of the relatively less explored areas, especially using the omics platform. We use metabolomics to acquire mechanistic insight into the pathogenesis of complex female reproductive system disorders and discover early predictive biomarkers with clinical utility. One of the areas well explored by our group is genital tuberculosis (GTB). Dormant but viable tubercular bacilli capable of reactivating themselves to cause the disease may reside in the female genital tract for a considerable period of time without any signs or symptoms. Diagnosis and management of dormant GTB is often challenging. We acquired metabolomic signatures of dormant GTB affected endometrium during window of implantation. Endometrial tissue metabolites associated with energy metabolism and amino acid biosynthesis were found to be significant altered and could be correlated with the reduced expression of endometrial receptivity markers. Furthermore, till now there is no non/minimally-invasive marker for detection of GTB in the sub-clinical stage. A panel of serum markers with sensitivity and specificity > 90% could be identified and seem promising, more so because a positive correlation between their expression in endometrial tissue and serum could be established. The process of decidualization involves significant morphological and biochemical changes of the endometrial stromal cells (ESCs) and is essential for successful implantation and establishment of pregnancy. It is likely that the presence of dormant yet viable mycobacterial infection in the endometrium adversely affects the decidualization process. To test this hypothesis, metabolic profile of in vitro decidualized human ESCs treated with 65 kDa mycobacterial heat shock protein was generated. Compromised decidualization due to reduced LIF mediated STAT3 signaling and altered metabolism was observed. A possible molecular mechanism of implantation failure in women with dormant GTB is hypothesized.	Metabolomics in Health and Disease
8.1	22: NMR metabolic profiling of bronchoalveolar lavage fluid to understand the pathogenesis of hypersensitivity pneumonitis	**Sanjukta Dasgupta**	IIT Kharagpur	Hypersensitivity pneumonitis (HP) is an immune-mediated diffuse parenchymal lung disease (DPLD) that results from repeated inhalation of certain antigens. Despite major advances in research, pathogenesis of the disease still remains poorly understood. This study, the first of its kind, uses nuclear magnetic resonance (NMR) metabolomics approach to explore the metabolic fingerprints present in bronchoalveolar lavage fluid (BALF) of HP patients. BALF samples were collected from patients with HP (n=15) and non-HP controls (n=15) and characterized using NMR spectrometry. An 800 MHz Bruker Avance III spectrometer equipped with a cryoprobe was used for this purpose. The data were subjected to multivariate and univariate analysis. A distinct metabolic differentiation was observed on comparing the two groups. R2Y and Q2 values [HP vs non-HP control (R2Y=0.902 and Q2=0.755)] of the OPLS-DA model validate that the model is a good fit and has a good predictive ability. Univariate analysis revealed lactate, glutamate, acetone, valine, proline to be significantly elevated and pyruvate reduced in HP as compared with controls. Also, dysregulated pathways associated with HP could be identified through metabolic networks, using Markov clustering-based algorithm. Cytoscape based Metscape plugin (http://www.metscape.ncibi.org/tryplugin. html) was utilized for analyzing and visualizing the compound networks. The generated model demonstrated the involvement of several disrupted energy metabolism pathways in HP subjects including glycine, serine, alanine, and threonine metabolism, glycolysis, and gluconeogenesis. The findings of the present study are a step towards better understanding of HP pathogenesis and identification of potential markers of the disease.	Metabolomics in Health and Disease

Session #	Abstract Number : Title	Presenter	Organization	Abstract Text	Theme
8.2	59: Disease or medication? Delineating the metabolic effects of asthma vs. treatment – a case study for challenges in clinical metabolomics.	**Stacey Reinke**	Edith Cowan University	Introduction: Asthma is a heterogeneous disease with poorly defined phenotypes. Individuals with asthma often receive multiple treatments including oral corticosteroids (OCS). Treatment may exert a confounding effect upon the observed metabotype, rendering it challenging to use metabolomics to investigate underlying disease mechanisms. Aim: To identify dysregulated metabolic processes in asthma severity and evaluate the effects of asthma medication upon observed metabolic profiles. Methods: Baseline urine was collected from healthy controls (HC, n=108), mild-to-moderate asthmatics (MMA, n=87) and severe asthmatics (SA, n=418) from the U-BIOPRED cohort; 12-18 month longitudinal samples were collected from SA (n=305). Metabolomics data were acquired using LC-HRMS, identified with an in-house library, and analysed using univariate and multivariate methods. Results: Ninety metabolites were identified, with 40 significantly altered ($p<0.05$, FDR<0.1) in severe asthma and 23 by OCS use. Multivariate modeling showed that HC and MMA differed significantly from SA ($p=1.4 \times 10\text{-}14$), OCS-treated asthmatics differed significantly from non-treated ($p=9.52 \times 10\text{-}4$), and longitudinal samples were metabolically stable relative to baseline. Carnitines decreased in severe asthma in an OCS-independent manner. Polyamine synthesis was altered in SA in association with OCS treatment, while xanthine metabolism and methyltransfer reactions evidenced OCS-dependent changes that were asthma-independent. Conclusions: This is the first metabolomics study to delineate between the disease and OCS-associated metabolic effects in asthma. Altered carnitine metabolism is independent of OCS treatment, presenting a potential therapeutic target. The widespread treatment effects observed elicit broader implications for the clinical metabolomics community due to highlighting the importance of investigating therapeutic effects in association with disease.	Metabolomics in Health and Disease
8.3	94: Rapid Development of Improved Data-Dependent Acquisition Strategies	**Joe Wandy**	Glasgow Polyomics, University of Glasgow	Data-dependent acquisition (DDA) is a popular method for producing fragmentation (LC-MS/MS) data in metabolomics. However, a typical Top-N DDA method suffers from inefficiencies where up to the N-th most intense precursor ions in a survey scan are selected, leaving slightly less abundant ions unfragmented. Developing alternative methods that improve upon Top-N is challenging due to the high experimental cost necessary during testing and optimising of a particular method. Here, we address this problem by introducing a framework on top of the previously developed Virtual Metabolomics Mass Spectrometer (ViMMS) that allows for the development and optimisation of novel fragmentation strategies using both the simulator and also a real mass spectrometer. We demonstrate this framework using two DDA methods that prioritise ions for fragmentation in novel ways. In SmartROI, regions-of-interests are tracked in real time and fragmented according to a specific set of rules. In WeightedDEW, we generalised the dynamic exclusion window in Top-N from a binary indicator to a linear weight. Both strategies were evaluated on complex mixtures of beer and serum samples. Evaluation was performed using coverage (the number of fragmented picked peaks) and efficiency (the ratio of fragmented picked peaks to total MS2 scans). The results showed that WeightedDEW improved coverage by more than 70% over Top-N in both datasets, fragmenting more unique ions that were missed before. SmartROI produces a lower increase in coverage (50% more than Top-N in both mixtures), but its higher efficiency as compared to Top-N (125% and 186% increase in beer and serum, respectively) means that far fewer fragmentation events are needed to target more picked peaks. Our results show theoretical maximum coverage is far above current acquisition strategies and we believe that this work will contribute to closing this gap. This will benefit many untargeted metabolomics workflows including molecular networking and substructure discovery.	Technology, Systems Biology & Advancing the Field
9 Keynote	249: TB or not TB: New metabolomics biomarkers better characterizing and diagnosing tuberculosis.	**Du Toit Loots**	North-West University, Human Metabolomics	Problem Statement: Despite the fervent genomic and proteomic based research efforts to date, since the discovery of the infectious organism Mycobacterium tuberculosis in 1882, TB is still considered a major global health problem, hence new research methodologies are urgently needed in order to investigate TB from a different perspective. One such a strategy is to investigate TB from a metabolomics research perspective, in order to identify new metabolite markers which can be used to better characterize the disease and current treatment strategies, investigate alternative treatment approaches, and develop improved diagnostics. Methodology & Theoretical Orientation: A standardized metabolomics workflow, including: 1. Application of various accredited semi-targeted and untargeted extraction procedures on cell cultures and patient collected sputum, urine, blood and cerebrospinal fluid, 2. Analysis on various LC-MS, GC-MS and NMR based analytical platforms, 3. Data clean-up and biomarker identification using various univariate and multivariate statistical approaches. Findings: The new TB biomarkers identified in the different patient collected sample material, not only allowed for the better characterization of new metabolic pathways in the host and in M. tuberculosis, and subsequently an improved understanding of the mechanisms related to Mycobacterium growth, virulence, drug resistance, and host and microbe interactions/adaptations, but the information also contributed to the development of improved diagnostics, treatment and predicting treatment outcomes. Conclusion & Significance: Over the past 15 years, metabolomics has led to an exponentially increased number of new biomarkers identified, and subsequently rapid expansion of new knowledge and our understanding of TB, which is now being utilized towards improved diagnostics and treatment strategies.	Metabolomics in Health and Disease

Session #	Abstract Number : Title	Presenter	Organization	Abstract Text	Theme
9.1	27: Novel Machine Learning based Mass Spectral Similarity Scores substantially improve Metabolite Annotation Accuracy	**Justin JJ van der Hooft**	Wageningen University	Untargeted metabolomics workflows increasingly rely on mass spectrometry fragmentation approaches for structural annotation. Mass fragmentation (MS/MS) spectra aid in annotation via comparison with reference spectra in libraries (e.g. MassBank, MetLin, GNPS). The automated comparison of MS/MS spectra by assigning a score to each comparison is essential in computational metabolomics workflows to perform large-scale library matching, analogue search, and network analysis. Unfortunately, the currently widely-used cosine-based metrics suffer from substantial drawbacks when applied at large-scale due to their high false positive rates thus lowering annotation accuracy and hampering biochemical interpretations. Here, we present two novel machine learning (ML)-based spectral similarity scores: Spec2Vec (unsupervised) and MS2DeepScore (supervised). We show that - on average - both scores result in a substantially improved correlation between structural and spectral similarity when compared to cosine-based metrics. This means that a high similarity score more often represents the matching of structurally similar metabolites. The added value of the novel metrics is demonstrated in the key metabolomics task of library matching. With a dataset comprising ~90,000 spectra of ~12,000 unique structures obtained from GNPS (holding out 1000 spectra), the ML-based scores increased the accuracy of library matching substantially from 80% to 88%, returning more correct matches at higher retrieval rates. Furthermore, ML-based similarity metrics also offer fast and scalable analogue search without parent mass selection - especially at larger masses >400 Da returning structurally very similar analogues from ~50,000 spectra in a matter of seconds. We will also show the complementarity of existing and the ML-based scoring metrics with the novel library matching application MS2Query. We believe that unsupervised and supervised ML-based mass spectral similarity metrics will impact metabolomics analyses across all disciplines including clinical, food, and microbial metabolomics as well as natural product research, by improving spectral annotation, but also by fast analogue search and networking analysis.	Technology, Systems Biology & Advancing the Field
9.2	162: Diet and other determinants of blood acylcarnitine concentrations in healthy individuals of the European Prospective Investigation into Cancer and nutrition study	**Roland Wedekind**	International Agency For Research On Cancer	Introduction: Acylcarnitines (ACs) play a key role in energy metabolism by enabling the transport of fatty acids into mitochondria. Their concentrations in blood have been associated with risk of diseases such as cancer and type 2 diabetes. Diet and lifestyle factors have been shown to influence AC concentrations but a better understanding of their determinants is needed. Methods: Fifteen and forty-two circulating ACs were measured in blood by targeted and untargeted metabolomics in 7791 and 395 healthy participants of the European Prospective Investigation into Cancer and nutrition (EPIC) study, respectively. Associations with participant characteristics, dietary patterns, intake of individual foods, carnitine and fatty acids, and circulating fatty acids and amino acids were assessed. Results: Fasting status, age, sex and diet were associated with the largest proportion of AC variability. Some AC species of medium or long-chain fatty acid moiety were associated with the corresponding circulating fatty acids or with intake of foods such as dairy containing the same fatty acid. ACs of short chain fatty acid moiety (propionylcarnitine and valerylcarnitine) were highly associated with concentrations of branched chain amino acids. Intake of most other foods and of carnitine, physical activity and smoking showed little association with AC levels. Conclusions: Our results show that AC determinants vary according to fatty acid moiety, and that their concentrations are related to participant characteristics and to fasting status. Knowledge of these determinants will help interpret associations of ACs with disease risk.	Metabolomics in Health and Disease
10 Keynote	294: Discovering Pesticides, Pharmaceuticals and their Transformation Products in Luxembourg Waters using Open Cheminformatics Approaches	**Emma Schymanski**	LCSB, University Of Luxembourg	The multitude of chemicals to which we are exposed is ever increasing, with over 110 million chemicals in the largest open chemical databases and over 70,000 estimated to be in household use alone. Detectable molecules in exposomics can be captured using high resolution mass spectrometry (HRMS), which provides a “snapshot” of all chemicals present in a sample and allows for retrospective data analysis through digital archiving. However, there is no “one size fits all” data or analytical method, and scientists cannot yet identify most of the tens of thousands of features in each sample, let alone associate them with health or disease, leading to critical bottlenecks in identification and data interpretation. Despite the size of the open chemicals databases, critical knowledge gaps still exist in them – environmental transformation products and metabolites forming a key part of these missing puzzle pieces. Defining the chemical space to search, the analytical methods to use, prioritizing efforts to find significant environmental chemicals, metabolites or biomarkers as well as filling the knowledge gaps are the key to help solving the overall exposomics challenge, which involves reconciling complex samples with expert knowledge and careful validation. This talk will cover European and worldwide community initiatives and resources to help connect environmental expert knowledge and observations, highlighting this with examples from Luxembourg looking at pesticides and pharmaceuticals and their transformation products using open cheminformatics approaches including Shinyscreen and MetFrag. We will show how FAIRifying Transformation information in the NORMAN Suspect List Exchange and PubChem helps close database gaps and create new possibilities for dynamic suspect screening efforts. This talk will show how interdisciplinary efforts and data sharing can facilitate research in environmental sciences, metabolomics, exposomics and beyond. Various other contributors to these collaborative efforts will be acknowledged throughout the talk.	Technology, Systems Biology & Advancing the Field

Session #	Abstract Number : Title	Presenter	Organization	Abstract Text	Theme
10.1	115: Exposure to environmental contaminants is associated with sex-specific alterations of hepatic metabolism in non-alcoholic fatty liver disease	**Tuulia Hyötyläinen**	Örebro University	Background and Aims: Endocrine disrupting chemicals may act as a 'second hit' in the progression of non-alcoholic fatty liver disease (NAFLD). Particularly, perfluorinated alkyl substances (PFAS), a class of commonly used industrial chemicals that humans are widely exposed to, have been associated with NAFLD. Due to the their structural similarity with fatty acids, PFAS may disrupt hepatic lipid metabolism. Furthermore, functionally, PFAS share some features with bile acids, including similar enterohepatic circulation. Nevertheless, human data linking PFAS exposure and lipid metabolism in the liver are currently lacking. The aim of our study was to define the impact of PFAS exposure on hepatic metabolism, with specific focus on bile acid and lipid metabolism. Methods: In a well-characterized human NAFLD cohort (n=105), we studied the impact of PFAS exposure on liver metabolism and compared the results with a mice model. We comprehensively characterized both hepatic (liver biopsy) and serum metabolome using four analytical platforms, and measured PFAS in serum and investigated the association between the exposure, NAFLD (liver fat, NASH grade, fibrosis stage, insulin resistance) and metabolome. Results: PFAS exposure was associated with NAFLD as well as with changes in hepatic lipid and bile acid metabolism in a sex-specific manner. Specifically, we noticed differences in the impact of the exposure between females and males, as characterized both with the impact on metabolome as well as on clinical parameters. Conclusion: Our results implicate that females may be more sensitive to the harmful impacts of PFAS. The results also suggest that the changes reported in the lipid metabolism due to PFAS exposure may be secondary to the interplay of PFAS and bile acids.	Metabolomics in Health and Disease
10.2	32: Flipping the metabolomics workflow: Assigning confidence to structural annotations from mass spectra with COSMIC	**Martin Hoffmann**	Friedrich Schiller Universität Jena	The structural elucidation of metabolites in a biological sample remains one of the most challenging problems in metabolomics. One of the predominant experimental platforms for untargeted metabolomics is Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). While spectral library search is a popular option, these libraries are usually very small and vastly incomplete.To alleviate this constraint, in silico methods like CSI:FingerID emerged, enabling the search in much larger structure databases. Being able to distinguish between correct and incorrect annotations of these tools is highly important, as follow up experiments can be very time and cost consuming. This ability to assign confidence to structural annotations is still a key outstanding problem in computational metabolomics. Here, we introduce COSMIC, a workflow combining structure database generation, in silico annotation and ultimately the assignment of a confidence score to these annotations. To avoid overfitting and make the confidence assignment work in praxis, we train a linear Support Vector Machine (SVM) and enforce feature directionality to reflect intuitive expectations. We show that COSMIC confidence scores outperform hit scores of popular in silico methods for the task of separating correct and incorrect annotations. We then applied the complete COSMIC workflow to annotate spectra of a mice fecal dataset with structures from a combinatorially generated database of hypothetical bile acid conjugates. We manually evaluated the top 12 most confident annotations and were able to confirm 11 of them. In comparison to our automated analysis here, Quinn et al. identified 3 bile acid conjugates by elaborate manual analysis (Nature, 2020). To demonstrate COSMIC's ability to process data on a large scale, we processed over 17,000 LC-MS/MS runs from 123 diverse MassIVE datasets, resulting in 1,715 high-confidence annotations for which no MS/MS data was available. Furthermore, we annotated 315 structures in human samples, not contained in HMDB.	Technology, Systems Biology & Advancing the Field
11 Keynote	364: Unravelling the biochemistry of bipolar disorder: a multi-omics approach	**Alessandra Sussulini**	University Of Campinas	With the advent of systems biology, which proposes to explore how interactions between biological components (biomolecules) affect the functionality (biological processes) of an organism in a holistic manner, several experimental methods have been proposed or improved. Single-omic strategies are widely used within this context, providing a large amount of information regarding specific sets of biological components (proteins, metabolites, etc.). Most recently, omics data integration employing the multi-omics approach has been developed, which consists in an outstanding opportunity to design a reliable picture of the biochemistry and dynamics of biological systems, as well as prioritize strategies for biomarker discovery to support diagnosis, treatment choice, and follow-up. Bipolar disorder (BD) is a severe and potentially debilitating mental illness, whose molecular bases are not completely elucidated and is diagnosed based on clinical criteria only. Therefore, multi-omics can contribute to the understanding of this complex disease. In this lecture, research works from our group will be discussed. Serum samples from healthy individuals and treated BD patients were initially compared using single-omic strategies: metabolomics, proteomics, and metallomics. For metabolomics (including lipidomics), multiplatform analytical techniques were employed (^{1}H NMR, UHPLC-MS, and GC-MS). For proteomics and metallomics, nanoLC-MS and ICP-MS were the techniques of choice, respectively, and samples from schizophrenia patients were also included in these studies. After chemometric analyses of single-omics data, specific potential biomarker panels were proposed. Nevertheless, in order to improve the biological information regarding BD and unravel the interactions between altered proteins and metabolites in a pathway-based level, proteomics and metabolomics data were integrated using partial correlation and network analysis. Hemostasis and energy production appeared to be the most affected pathways in BD patients. The hypothesis that BD is a multi-system disease and involves different aspects of the metabolism must be further studied in order to understand how these systems are correlated.	Metabolomics in Health and Disease

Session #	Abstract Number : Title	Presenter	Organization	Abstract Text	Theme
11.1	183: Systemic and Lung Tissue Specific Metabolic Effects of Allergic Sensitization in Mice	**Kedir Turi**	Vanderbilt University	Background: Allergy development is associated with metabolic alteration. Comprehensive studies on the effect of allergen sensitization on the cellular metabolism are lacking. The objective of this study is to comprehensively profile the metabolic effects of house dust mite (HDM) sensitization in mice. Methods: Seven-week-old male BALB/cJ (BALB) mice were sensitized to filtered HDM extract or saline through internasal administration 5 days/week for 1 week (n=9 per group). During weeks 2-4, mice received the same dose of HDM 3 days/week. Bronchoalveolar lavage fluid (BALF), histopathological analysis, untargeted metabolomics, and targeted lipidomic analyses in plasma and lung tissue were conducted. Univariate Statistical comparisons were performed adjusting for multiple-testing and pathway analysis was performed. Results: Analysis of BALF demonstrated significant increases in eosinophils, neutrophils (PMNs), and lymphocytes as well as increased total protein and histopathological changes, all supporting an allergic response. In untargeted metabolomics analysis, 146 annotated metabolites in lung tissue were significantly different between HDM group compared to control (adjusted $p<0.05$). Pathway analysis showed the majority of these metabolites mapped to glycerophospholipid and sphingolipid pathways (subclass 1) and which were down regulated. These metabolites mapped to sub pathway (sub class 2) of glycerophosphocholines, which were the most down regulated in the HDM group compared to control. In targeted analysis, 12,13-EpOME and its downstream metabolites 9,10-DiHOME, 12,13-DiHOME, 9-HODE, and 13-HODE in plasma and 13-(S)-HOTrE, 19,20-DiHDPA, 6α-PGI1, and PGE2 in lung tissue were significantly higher in the HDM group compared to control ($p<0.05$) but were insignificant after adjusting for multiple-testing. Conclusions: Results are consistent with literature linking decreased glycerophospholipid and sphingolipid metabolites with increased broncho-reactivity may be related to its anti-inflammatory effect. Conversly, increased 12,13-EpOME and its downstream metabolites and prostaglandins with allergic sensitization. This study provides additional insight into metabolic signaling pathways triggered in HDM sensitization.	Environmental, Plant, Animal, Agriculture, Food and Model Organisms
11.2 Sponsor	343: Robust metabolic profiling for routine quantitation and confident unknown identification	**Amanda Souza**	Thermo Fisher Scientific	Metabolomics, the investigation of small molecules present in a biological system, finds application in diverse biomedical and industrial research. In large-scale applications, targeted profiling of metabolites is employed as it provides confident measurements for known compounds. However, sometimes the observed phenotype cannot be explained by only monitoring a subset of metabolites. Instead, comprehensive metabolome coverage is needed to provide knowledge about underlying biochemical mechanisms. Chemical diversity of the metabolome necessitates data collection with multiple ionization modes for a complete picture. High resolution accurate mass Orbitrap technology with polarity switching enables unbiased compound detection for the accurate analysis of target metabolites and the retro-mining of data to discover unexpected biochemical mechanisms. Here, we apply the targeted profiling workflow utilizing a Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer for the nutrient evaluation of fetal bovine serum (FBS) as supplemental material in cell culture applications. Routinely used as growth supplement in in vitro cell culture, FBS contains vital components like amino acids, sugars, and lipids essential for cell growth and proliferation. To assess potential variation of metabolite levels detected in this supplemental material, serum was commercially sourced from multiple vendors across several geographical regions using different processing protocols. High resolution full scan acquisition with fast polarity switching enabled quantitation in both positive and negative polarities for target metabolites in the Metabolomics QReSS™ standard kit and simultaneous detection of unknown compounds for increased metabolome coverage. Metabolic differences for target analytes and putative compounds were distinctively observed in serum extracts between processing protocols, but also regional sourcing. Metabolite associations further revealed differences for pathway-related metabolites. In summary, comprehensive metabolic profiling was achieved using the Orbitrap Exploris™ 120 mass spectrometer with enabled polarity switching for confident measurements of target metabolites while simultaneously allowing for the confident identification of other relevant compounds in commercially sourced FBS samples.	Technology, Systems Biology & Advancing the Field
12 Keynote		**Bing Yu**	University of Texas Health Science Center at Houston		

Session #	Abstract Number : Title	Presenter	Organization	Abstract Text	Theme
12.1	95: Plasma Metabolomic Profiles of Age-related Macular Degeneration Progression	**Ines Lains**	Massachusetts Eye And Ear, Harvard Medical School	Purpose: Age-related macular degeneration (AMD) is a leading cause of blindness worldwide. We and others have shown that plasma metabolomic profiles vary between patients with AMD and controls and across stages of disease. However, to our knowledge, no longitudinal studies have assessed how metabolomic profiles relate to AMD progression. This work aimed to analyze the association between plasma metabolomic profiles and progression of AMD over a three-year period. Methods: Prospective longitudinal study including patients with AMD and a control group (>50 years old). At baseline and three years later (± 3 months), all participants (both eyes) were imaged with color fundus photographs for AMD grading and fasting plasma samples were collected. Eyes were considered to have progressed if at three-years their AMD stage was more advanced than at baseline. Metabolomic profiling was performed using Ultrahigh Performance Liquid chromatography – Mass Spectrometry. Multilevel mixed models, accounting for age, body mass index, smoking and gender were used for analysis, using as outcome progression of AMD. Results: We included data on 196 eyes (n= 98 patients), 13% (n= 26) of them with progression at the three-years follow-up. A total of 645 plasma metabolites were considered for analysis. Among the baseline metabolites, 3 (N-palmitoyl-sphingosine, methylsuccinate and N,N-dimethylalanine) were significantly associated with three-year AMD progression (p<= 0.009). When evaluating the association between changes in plasma metabolites (between baseline and 3 years) with progression of AMD, 10 significant associations were seen (p<= 0.009). Conclusion: Baseline metabolites and changes in metabolomic profiles were associated with clinical progression of AMD at 3 years. In particular, lipid and amino acid metabolites appear to be among the relevant metabolites linked to AMD progression. This work contributes to our understanding of AMD progression and to the development of future biomarkers for this blinding disease.	Metabolomics in Health and Disease
12.2	220: Metabolomics on a Chip: Development of an Impedance-Based Metabolite Biosensor for Early Diagnosis of Colon Cancer	**Ya-Chun Chan**	University Of Alberta	Colon cancer is the third most common cancer and one of the most lethal. However, if diagnosed early, it can be readily treated. We have recently identified a set of metabolites in urine that can be used to diagnose colon cancer, namely: hippuric acid, diacetylspermine, and creatinine. We are now developing a fast, inexpensive point-of-care metabolomic device for detecting these three urinary metabolites. This hand-held, metabolomics-on-a-chip device utilizes specially prepared nanomaterials (gold nanoparticles and liposomes), which not only recognize the metabolite biomarkers but also enhance the sensitivity of detection. The metabolite biosensor, as it currently exists, consists of an impedance reader, a solution handling system, and a sensor chip with interdigitated electrodes that are functionalized with the antibodies for detecting the target metabolites. The principle of detection is based on using electrical impedance to measure the competitive binding between nanoparticle-metabolite conjugates and free metabolites found in urine. Because the free metabolites in the urine samples have higher binding affinity to the antibodies, the free metabolites will compete and remove the nanoparticle-metabolite conjugates. As a consequence, the impedance signal will change, which can be used to quantify metabolite levels. The use of signal-enhancing nanomaterials in our sensor design is vital to detect the specific metabolites at lower concentrations for accurate and sensitive diagnosis for colon cancer. Designs, preliminary results and a general assessment of this sensor system will be presented. We believe this work could open the door to measuring even more metabolites and making “metabolomics-on-a-chip” a reality.	Metabolomics in Health and Disease