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EDITORIAL AND REVIEW

Editorial and Review: 33rd Asilomar Conference on Mass Spectrometry—Impact of Metabolomics in Translational and Clinical Research

The 33rd ASMS Asilomar Conference on Mass Spectrometry focused on the Impact of Metabolomics in Translational and Clinical Research and was organized and chaired by Tim Garrett (University of Florida) and Chris Petucci (Cardiovascular Institute, Perelman School of Medicine, University of Pennsylvania). It was held on September 29–October 3, 2017 at the Asilomar Conference Center in Pacific Grove, CA. A total of 115 scientists attended, including graduate students, post-docs, faculty, and research scientists from industrial, government, and academic labs (Figure 1). Travel awards (14) were awarded to graduate students and post-docs. The conference, highlighted by contributions from leaders in metabolomics, aimed at obtaining a greater understanding of disease and translating discoveries from the laboratory into the clinic.

The opening lectures on Friday evening were given by Sunia Trauger (Harvard University) and Facundo Fernandez (Georgia Institute of Technology). Sunia presented on an LC-MS/MS-based approach to help identify a new mechanism for multiple sclerosis in a mouse model. Increased levels of LacCer in MS lesions, with corresponding findings in a mouse model, were determined by LC-MS/MS. This method, along with gene expression analysis, provided for the sensitive detection of molecular changes underlying biological mechanisms of action for a drug in multiple sclerosis. Facundo presented on the early detection of cystic fibrosis (CF) acute pulmonary exacerbations by global metabolomics of exhaled breath condensate to support proactive interventions. His group identified metabolite panels in exhaled breath condensate that enabled them to distinguish stable CF patients from those who will have an acute exacerbation in the following months. They concluded that metabolomics of exhaled breath condensate is a promising approach for monitoring CF patient health status and disease progression. This session was followed by a poster session and informal mixer sponsored by Sciex (Figure 2).

The first session on Saturday morning was on clinical applications of global metabolomics. Gary Patti from Washington University presented on tracking electrons with metabolomics to reveal therapeutic vulnerabilities in cancer. The aim of his work was to determine how consumption of NADPH for the synthesis of 2-hydroxyglutarate (2-HG), an oncometabolite, altered metabolism in cancer cells through metabolic flux experiments. Synthesis of 2-HG by mutant IDH1 increased pentose phosphate pathway activity to generate NADPH. It was determined that even when NADPH was limiting, IDH1 mutants continued to synthesize 2-HG at the expense of other NADPH-dependent pathways that were essential for cell viability. Next, Nichole Reisdorph (University of Colorado, Anschutz Medical Campus) spoke about the power of metabolomics to predict treatment response in pediatric patients with asthma. The goal of this work was to determine small-molecule biomarkers that predict a patient's response to asthma medication. Unbiased LC/MS metabolomics profiling was performed on urine and plasma from 230 pediatric asthma patients who were placed on 3 different medications for 16 weeks. A series of models were developed that supported the use of small molecules to predict responses to medication. To complete the session, Aalim Weljie (University of Pennsylvania) spoke about translational metabolomics studies in chronobiology and cancer. The rhythmicity of metabolism at a systems level was investigated as a function of chronobiology (e.g., the impact of factors such as circadian timing and loss of sleep). His work with mouse liver suggested that 50% of detected metabolites, measured by an LC-MS metabolomics method, significantly cycle. Perturbation of the clock by disease states, such as overexpression of MYC in cancer, impinged upon clock function for both polar metabolites and lipids (unpublished). Other types of lifestyle disruptions, such as sleep loss, also altered metabolic function measured by the circulating metabolome, with two specific markers, oxalic acid and 32:3-diacylglycerol, found to be common between humans and rats. These sleep and circadian studies demonstrated that metabolites have a marked and clear rhythmic character.

Hot topic talks have been an Asilomar tradition over the past several years. Justin Cross from the Memorial Sloan Kettering Cancer Center gave a talk on dynamic changes in the microbiome and fecal metabolome in patients undergoing allogeneic hematopoietic stem cell transplantation (alloHSCT). To investigate the high incidence of antibiotic-resistant infections in these patients, clinical protocols were established for the serial collection of fecal samples, 16S sequencing of the microbial communities present and comprehensive GC/MS and LC/MS metabolomics profiling. An example patient time course was shown where an intestinal domination by vancomycin-resistant enterococci resulted in a severely impaired ability to produce secondary bile salts and to ferment dietary starches into the major SCFAs. The study has implications for patient recovery, understanding the emergence of antibiotic-resistant infections and ultimately how a newly transplanted donor immune system becomes educated to

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Figure 1. Attendees of the 33rd ASMS Asilomar Conference

commensal microbes. This was followed by a presentation by Alexey Melnik (University of California, San Diego) on a 3D volume cartography of cystic fibrosis human lungs to visualize chemical and microbial distributions. Three-dimensional images were reconstructed from CT scans followed by mapping of metabolomics and microbial features onto reconstructed 3D models. Overall, microbial and metabolite mapping of diseased lungs provided an increased understanding of individual phenotypes and disease pathology and insight into the development of improved treatments.

The Saturday afternoon session was on clinical applications of targeted metabolomics. John Seal from the Ochsner Multi-Organ Transplant Institute gave a talk on metabolomics of human livers used for liver transplant. Metabolomics was used to characterize liver donation after circulatory death (DCD) and donation after brain death (standard donor) to assess the suitability of graft quality prior to transplant. Targeted metabolomics revealed several amino acids were increased in DCD livers with a corresponding decrease in adenosine phosphates, indicating energetically compromised livers. This study

Figure 2. From left to right: Picturesque seashore, group kayaking, evening reception and poster session, boardwalk trail at the beach, and aquarium

illustrated the power of metabolomics to provide insight into the suitability of donor livers. Then, Dan Raftery (University of Washington) presented on the opportunities and challenges of translating metabolomics findings into cancer related tests. One of the aims was to address various environmental and clinical factors that confound biomarker discovery. His experimental approach combined globally optimized targeted (GOT) MS with seemingly unrelated regression (SEM) to model metabolite levels based on the effects of these confounding factors. This approach has resulted in reduction of confounding effects to reveal unperturbed metabolite levels. Overall, this method has provided for improved biomarker identification and validation efforts. Finally, Clary Clish (Broad Institute of MIT and Harvard) spoke about metabolomics methods used to identify early metabolic indicators of subclinical disease in human cohorts. His aim was to identify additional metabolites associated with liver fat in the Framingham Heart Study (FHS) as well as predictors of coronary heart disease (CHD) in the Women's Health Initiative (WHI). A high-resolution, accurate-mass metabolomics platform was used to analyze 1066 plasma samples from participants in the FHS Generation III cohort, of whom 470 underwent liver CT scanning. An unknown compound represented by an ion at *m/z* 202.1185 was identified as α-keto-δ-(N(G),N(G)-dimethylguanidino)-valeric acid (DMGV) that was highly associated with liver fat in FHS and predicted incident type 2 diabetes in the Malmo Diet and Cancer Study and Jackson Heart Study participants. His group also found a novel set of lipids consisting of monohydroxyeicosatetraenoic acids and hydroxylated phosphatidylcholines that predicted CHD in the WHI. The significance of this work was that a novel metabolite associated with liver fat was identified along with being the first study showing a link between either plasma hydroxy-PCs or mono-HETEs and CHD prospectively. Before the Saturday evening poster reception and informal mixer, graduate students and post-docs gave their traditional 3-min poster highlight talks to summarize important aspects of their research.

Sunday morning began with a session on clinical applications of new technologies, including ambient desorption ionization, MS imaging, and ion mobility. Abraham Badu-Tawiah from Ohio State University spoke about ionic probes for MS signal amplification and application in paper-based on-demand diagnostics. This was followed by James Kinross (Imperial College), who presented on precision surgery involving translational applications of ambient mass spectrometry in the operating room. The focus of his work was to improve the precision of surgery using real time MS of diathermy plumes with "iKnife" technology. This method uses rapid evaporative ionization mass spectrometry of the lipid phase of diathermy plumes without the need for sample preparation. In particular, analysis of lipids is important because most cancers express high concentrations of phospholipids and triglycerides in their cell membranes. As a result, the iKnife produced data with a very high level of diagnostic accuracy for many types of cancers including detailed information on tumor phenotype and its molecular features. Erin Baker (Pacific Northwest National Laboratory) presented on utilizing ion mobility spectrometry (IMS) in omics studies to better understand biological and environmental changes. Specifically, IMS was used to separate endogenous and exogenous small-molecule isomers for better quantification and identifications in complex samples. IMS separated both polar and nonpolar molecules, and when combined with LC and ozonolysis, it could separate and distinguish different lipid isomers. The IMS separation in conjunction with MS and other analytical techniques added specificity to each molecular measurement for more informative identifications. Pieter Dorrestein (University of California, San Diego) concluded the session by presenting on his rapid response precision microbiome—multiomics analysis at clinical relevant time scales. This session was followed by a free afternoon where many participated in group activities to explore the local area (Figure 2).

The Sunday evening session began with poster highlight talks from graduate students and post-docs to highlight their research. A poster session and informal mixer sponsored by Waters followed.

The final day of the conference on Monday began with a session on translational and clinical applications of metabolic flux by mass spectrometry. Petras Dzeja from the Mayo Clinic gave a talk on the experimental and clinical applications of stable isotope ^{18}O-assisted dynamic metabolomics. He used ^{18}O-based metabolite tagging to provide quantitative measurements of metabolites and turnover rates of several metabolites that include water as a reactant in their metabolism. This ^{18}O-mass spectrometry and ^{31}P NMR technology enabled the elucidation of cellular energetics and ATP-sensing metabolic mechanisms related to genetic deficiencies, myocardial ischemia and heart failure, aging, and neurodegenerative disorders. This was followed by Richard Kibbey (Yale School of Medicine), who spoke about drug discovery guided MIMOSA mass isotopomer flux analysis, a stable isotope technique that tracks time-dependent carbon positional isotope (isotopomers) labeling to analyze the flow of metabolism. He described how stable isotopomer MS can be used to evaluate normal physiology and diabetes for drug discovery. MIMOSA identified a surprising cataplerotic pathway in beta-cells. Rather than oxidative phosphorylation, cataplerosis of phosphoenolpyruvate via PEPCK-M was a glucose-dependent metabolic signal directly coupled to insulin release. Rather than targeting oxidation, which injures islets and causes hypoglycemia, small-molecule targeting of this cataplerotic pathway in human pancreatic islets amplified insulin secretion and restored function in islets from type-2 diabetic donors. By incorporating mass isotopomers into the analysis, MIMOSA avoided pitfalls, provided precise metabolic flux measurements, and identified novel pathways to gain unique paradigm-shifting mechanistic insights into a disease such as diabetes.

Monday morning ended with presentations on metabolomics applications in the pharmaceutical industry. Alla Kloss from Genzyme discussed the development and implementation of a UPLC-HRMS metabolomics and lipidomics platform to support biomarker-based drug discovery and development. This platform provided for trend-generating study designs that allowed for fast automated data processing and discovery of phenotype-linked biomarkers. Overall, this has enabled

phenotypic cell–based screening hits with confirmed activity in cells for informed decision-making in drug development. Yutai Li (Merck) spoke about metabolomics applications in the discovery of safety biomarkers in preclinical development. A robust panel of biomarkers for drug-induced liver injury was identified to aid preclinical and clinical drug development. In vivo toxicity studies were conducted in rat urine, plasma, and liver samples followed by analysis with an LC/MS metabolomics platform. The results from urine and plasma samples of drug treated groups revealed increased dicarboxylic acids, associated with impaired fatty acid β-oxidation. Hence, this method identified toxicity biomarkers and provided a greater understanding of the mechanisms of liver toxicity. Michelle Clasquin (Pfizer) presented her work on understanding disease through unlabeled and isotope tracer–based metabolomics and lipidomics. Metabolomics was used for target identification and validation, understanding mechanisms of disease, and in vitro and in vivo model characterization. A mixed-mode HPLC/Q-Exactive HF was used to analyze several central carbon metabolites and an UPLC/QTRAP 5500 was used for lipidomics. Using isotope tracer techniques, a model system of mitochondrial branched chain keto-acid overproduction was discovered. A separate application revealed differential lipase activity between wild-type and mutant PNPLA3 I148M variant mice.

The final session after lunch was on innovations and clinical applications of the NIH regional comprehensive metabolomics research cores. Rick Yost from the University of Florida presented on mass spectrometric innovations for translational and clinical research. He first pointed out that the NIH Common Fund investments in metabolomics over the past 5 years have helped drive many of the advances in clinical metabolomics. Then, he presented on imaging MS and direct tissue analysis (both MALDI imaging and surface microextraction using the FlowProbe), showing quantitation on low microgram samples, identifying biomarkers directly in tissue rather than in blood or urine, and direct analysis of living tissue, including measurements during electrical stimulation to model deep brain stimulation as a treatment for Parkinson's disease. His work using ion mobility MS for the rapid clinical analysis of vitamin D and its epimers, or for anabolic steroids, eliminated the need for sample preparation and chromatographic separation. His work illustrated that technological advances will help move metabolomics into the clinical lab, chemical imaging and direct analysis by mass spectrometry is creating new opportunities in metabolomics, and ion mobility has tremendous potential to improve metabolomics and clinical analyses. Next, Rick Higashi (University of Kentucky) spoke about challenges of metabolic reprogramming elucidation in cancers directly from human subjects via stable isotope-resolved metabolomics (SIRM) mass spectrometry. Finally, Oliver Fiehn (University of California, Davis) presented on standardizing non-targeted metabolomics for use in clinical research. His lab aims to standardize untargeted metabolomics to enable high coverage of metabolites with the necessary precision and accuracy to conduct human cohort trials and compare data between studies and laboratories. To this end, they developed 18 different cheminformatic tools that enable such standardization. Most importantly, the data processing software MS-DIAL has now been integrated with the mass spectral database MassBank of North America and the compound identification software MS-Finder into a new tool, BinVestigate. BinVestigate enables researchers to query spectra across over 2000 studies and more than 120,000 samples to investigate the presence and abundance of compounds across a wide variety of species and organs. Dr. Fiehn has exemplified this concept with the discovery of two compounds, a new oncometabolite *N*-methyl-UMP and the microbial compound *N*-methylalanine that is excreted into plasma. For large cohort studies, precision is now improved with a new normalization software SERRF. The group proposes using chemical enrichment statistics instead of metabolic pathway enrichment studies to find significantly dysregulated metabolic modules.

After the banquet dinner, Gary Siuzdak (The Scripps Research Institute) gave his keynote talk on Activity Metabolomics to identify active metabolites that can modulate phenotype. In his talk, he highlighted the supporting discoveries made by former students including Caroline Johnson (Yale University), Sunia Trauger (Harvard), Julijana Ivanisevic (University of Lausanne), Brittney Beyer (Scripps), Erica Forsberg (San Diego State University), and Elizabeth Want (Imperial College). These discoveries include the identification of a single metabolite that promotes neuronal regeneration and metabolites that amplify protein expression. After his talk, the conference ended with a dessert and poster session, topped off with Ava Winery providing samples of their metabolomics-based synthetic wine.

The Asilomar Conference was highly successful and we achieved our goal of highlighting major innovative translational and clinical applications of metabolomics from top experts in the field. Speakers gave excellent talks that were balanced presentations of fundamentals and practical applications. Moreover, there was a high degree of inquiry, excitement about science, and social and scientific interactions all throughout the conference. Overall, this conference was a testimony of the monumental advances in mass spectrometry to drive research programs in metabolomics forward and enable major discoveries in health and human disease.

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