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LIPID MAPS: Serving the next generation of lipid researchers with tools, resources, data, and training

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Abstract

Lipids are increasingly recognized as dynamic, critical metabolites affecting human physiology and pathophysiology. LIPID MAPS is a free resource dedicated to serving the lipid research community.

Lipids play diverse roles in biology and are essential for life. They support energy metabolism, keep cells intact by forming impermeable membrane barriers, play roles in migration, apoptosis, autophagy, and cell division, and act as essential communicators in inflammation and immunity. Lipids are highly dynamic and are influenced by circadian rhythms, diet, age, lifestyle, drugs, disease, inflammation, and development.

In the emerging era of precision medicine, the biomedical world has now turned its attention to lipids in a major way. Lipids account for a large proportion of all metabolites, and their dynamic nature and complexity are fueling the promise that understanding their synthetic and degradative pathways, including fluxes, on a "global" or "big data" omics scale could revolutionize our ability to diagnose, stratify, and treat numerous acute and chronic conditions, including diabetes, infection, atherosclerosis, cancer, and autoimmunity. Lipidomics, in conjunction with transcriptomics, regulatory genomics, and proteomics, will provide deep insights into cellular and tissue function in health and pathophysiology. Lipids serve as endpoints of complex cellular and organ functions and represent key markers of normal and disease states. Because they are accessible from plasma and urine and can be fully quantified, lipids can be interrogated in a relatively noninvasive way, providing an opportunity for their integration with precision medicine methodologies that will emerge over the next 5 to 10 years (1-3). In addition, yet to be adequately addressed is the need to determine in which cellular membrane the measured changes in lipids occur; hopefully, developments in mass spectrometry imaging will progress in parallel.

The number of researchers analyzing lipids in humans and animal models has massively increased. This burgeoning interest in lipid biology is leading to challenges in education and capacity, driving the need for new analytical, statistical, and informatic tools to study lipids. In the last 20 years, our knowledge of lipid biology and biochemistry has greatly expanded, at least in part due to the emergence of "user-friendly," bench-top mass spectrometers. The combination of electrospray ionization (ESI) with high-pressure liquid chromatography (HPLC) or ultra-performance liquid chromatography (UPLC) made lipid analysis accessible to a new generation

of researchers. This has facilitated lipid research in new and exciting ways, with a high degree of sensitivity and selectivity that was previously impossible. Previously, the lipid research community consisted of a relatively small number of chemists and biochemists, who were highly collaborative, but had not yet been integrated into the much larger revolution in molecular biology and genomics that has evolved in recent years.

In 2003, a $73M investment from the U.S. National Institutes of Health (NIH) supported the establishment of the LIPID MAPS effort (www.lipidmaps.org) led by Edward Dennis (UCSD), together with academic colleagues Alex Brown (Vanderbilt), Christian Glass (UCSD), Alfred Merrill (Georgia Tech), Robert Murphy (University of Colorado), Christian Raetz (Duke), David Russell (University of Texas, Southwestern), Michael van Nieuwenhze, (UCSD), Shankar Subramaniam (UCSD), Steven White (University of California, Irvine), and Joseph Witztum (UCSD), and industrial partner Walter Shaw, President of Avanti Polar Lipids. This initiative led directly to the coining of the term “lipidomics.” During the next ten years, LIPID MAPS, which stands for LIPID Metabolites And Pathways Strategy, played a major global role in driving the development of techniques and resources, including nomenclature standardization and supporting lipidomics to be ready for the emergence of the bioinformatics age.

LIPID MAPS was focused at that time on developing mass spectrometry (MS) methods, applying them to the analysis of macrophages, generating isotope-labelled internal standards, and depositing data in a database for the global research community (4). The result was a manually curated database of around 40,000 structures, all classified and drawn using a standardized nomenclature and a structural drawing representation scheme developed by the consortium. The

legacy of this work is a lipid classification and structural representation system used by virtually all lipid researchers and journals (5-7). In developing these tools, Dennis and colleagues purposefully reached out to the international community, inviting coauthors from Europe and Japan to join and provide input, which enabled them to succeed in developing a universally accepted system that enabled lipid researchers from around the world to communicate optimally and without controversy.

In the last five years, increasing interest has attracted a new generation of scientists who view lipids in a very different way. Rather than working on individual species or classes of lipids, many younger scientists view lipids as part of the overall "big data" picture in a more holistic manner, in line with the developing era of systems biology. However, with this change, the need for biologists to have a basic grounding and knowledge in lipid biology and biochemistry is becoming even more essential if we are to make sense of the new data and harness the power of lipidomics fully to further our understanding of the roles of these diverse molecules in human health and disease.

The LIPID MAPS NIH grant ended in 2014, and for the following three years, the database and website were maintained by Subramaniam with a legacy grant from NIH, which he coordinated with a sister NIH grant from the Metabolomics Workbench (http://www.metabolomicsworkbench.org/). Without dedicated funding, the curation of new lipids would have ceased, and the continuation of the nomenclature work would have been stalled. In 2016, Valerie O'Donnell heard about this situation from her long-time collaborator Robert Murphy and realized that to lose LIPID MAPS would be devastating for lipidomics

researchers around the world. Together with the original UCSD-led team and Michael Wakelam (Babraham Institute, Cambridge), Valerie led a successful bid to the Wellcome Trust for a £1.3M, 5-year Biomedical Resources Grant, which enabled the continuation of further development work on the database and website starting in 2017. This grant now funds LIPID MAPS as a multi-center international initiative, with curation, administration, and programming at Cardiff University (O'Donnell) and at the Babraham Institute in Cambridge (Wakelam) but retaining the original operational web and chemi-informatics team (led by Subramaniam) along with Dennis at UCSD.

In early 2018, the database moved to Cambridge, a new web site was launched, which took into account feedback from the user community, curation was established in Cardiff, and the nomenclature and classification work continued. Working Groups, drawing on additional expertise, and the original members of the LIPID MAPS core groups were established and meet annually. To reflect the changes in global lipidomics needs, the Working Groups also include membership from two areas: pathways (with WikiPathways) and a new effort to harmonize and standardize lipidomics analysis and reporting (Lipidomics Standards Initiative). New nomenclature efforts are ongoing, reflecting the need to classify recently discovered lipid classes into the LIPID MAPS taxonomy.

The research needs for lipidomics have evolved since 2003; however, the original challenges remain. For example, new nomenclature and curation is still needed because new lipids are constantly being discovered. The big questions remain: How many lipids do our cells contain? Can we come close to mapping them all? As MS instruments have become ever more powerful,

our ability to survey the lipidome has increased; lipids that were undetectable 10 years ago are now discoverable. Of course, for new structures, there are major challenges in structural identification, which cannot be achieved by MS alone, and lipids present at picogram quantities in biological samples are undetectable by nuclear magnetic resonance (NMR) techniques.

There is also an increasing need for data deposition, software tools, statistical pipelines, and network and pathway analysis, all of which reflect the changing nature of lipidomics. LIPID MAPS has implemented several initiatives in these areas, both directly on the site and with other resources. Data deposition has been established in partnership with the Metabolomics Workbench. Through LIPID MAPS, lipidomics researchers can either browse more than 500 deposited datasets or deposit their own studies for data reuse by the global community. Direct links from our database entries to information held at complimentary resources, such as SwissLipids and MassBank of North America (MONA), support researchers who need as much information as possible about particular lipids to be discoverable in the same place.

Software and statistical tools are increasingly needed for the analysis of big datasets. LIPID MAPS incorporated a suite of tools for generating volcano plots and undertaking multivariate analysis, as well as a software tool, LipidFinder, that removes artifactual signals from processed datasets generated by high-resolution MS (8). To aid re-use and improve discovery of resources, we provide a list of external lipidomics software that is continually expanding. Our previous VANTED pathways were migrated to WikiPathways (www.wikipathways.org) and a new network/pathway analysis tool is in development (9). This area is one of huge current growth and, working with Metabolomics Workbench, we plan to develop and implement additional tools

to support the community. Structure drawing tools have long been supported by LIPID MAPS. We provide tools for researchers to generate all types of lipids, including a new suite for oxidized phospholipids.

As more researchers study lipids, more analytical training and educational support are needed. To this end, we host instructional videos on lipid analysis methodologies introduced by Robert Murphy (Denver), and provide a series of curated protocols from the literature covering the analysis of lipid classes using LC/MS/MS. Bill Christie's excellent LipidWeb, a veritable encyclopedia of lipids, is in the process of being migrated to LIPID MAPS for long-term hosting, and we are delighted to host Christie's excellent weekly blog. Educational workshops are held, including at Keystone Symposia and a corresponding European Lipidomics Meeting in Leipzig in 2018.

Where is the field of lipidomics headed in the next ten years? In terms of precision medicine approaches, two modalities are beginning to dominate cohort studies: high-resolution, "global" untargeted profiling and targeted quantitative methods, using tandem MS. Both have their pros and cons, and lipids present a specific challenge (unlike other metabolites) because an individual mass value can represent several different molecular species (isobars). This issue can be confusing to researchers new to lipid analytics and is a source of inaccuracy when reporting results; thus, some common guidelines for the community are needed. Global profiling is a powerful approach for screening and comparing large numbers of samples. However, it is less sensitive and not fully quantitative (low-abundance species are often missed), and validation is required. It can only identify "bulk" lipids, and isobars cannot be resolved (10). Its advantage

over targeted methods is that it can be used to discover new lipids and can cover several different lipid classes in a single analysis. However, to know the fatty acyl composition, tandem MS, considered the "gold standard" approach, is needed. Tandem MS is the only modality that can approach truly quantitative lipidomics and is the most sensitive, enabling the detection of even 1 pg of some lipids. However, targeted methods are generally optimized for single lipid classes only, making them relatively narrow and inflexible.

Clinical-grade assays to quantify molecular species of lipids do not yet exist but will become increasingly required. These will need internationally agreed standardization and accreditation (for example, ISO standards) and will most likely be based on tandem MS. To support the standardization of lipidomics, LIPID MAPS has partnered with the Lipidomics Standards Initiative, a community-wide initiative led by Kim Ekroos (Lipidomics Consulting Ltd, Finland) and Gerhard Liebisch (University Hospital, Regensberg), which aims to generate guidelines for the major lipidomic workflows, including sample collection, storage, as well as data deconvolution and reporting (https://lipidomics-standards-initiative.org/). Through this collaboration, we will ensure that our database functions are fully aligned with the needs of the community, for example in line with providing "bulk" search interfaces and addressing the issue of isobars in a user-friendly manner. To accurately measure lipids, high-quality, isotope-labelled and primary lipid standards are essential. LIPID MAPS signposts have direct links to standards available from Avanti Polar Lipids and Cayman Chemical. The availability of standards tailored for specialized workflows is key and becoming more important as standardized/recommended approaches (for example, for plasma-based studies) begin to emerge. In addition, new lipids are

being discovered all the time, and the need to identify and prioritize specific lipids most needed by the research community through our links with partner companies is an ongoing effort.

References and Notes

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