



Metabolic Profiling: Meet the Latest 'Omics'

Data on metabolites and biochemical changes provide important insights into the toxicity and mechanisms of action of chemicals

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The study of metabolic profiles in biological samples such as urine and plasma has risen in prominence over the past few years. Sophisticated, million-dollar mass spectrometers and nuclear magnetic resonance (NMR) instruments and software are already helping to identify and analyze metabolites, revealing potential toxic effects as well as mechanisms of action of chemicals. Also appealing is the non-invasive nature of the discipline, which uses easy-to-obtain biofluids like urine. That allows scientists to test animals and humans more frequently, get more detailed information to decide which compounds to exclude or advance, and save time and money.

Because of its large potential, metabolite research is moving rapidly beyond university laboratories and into the mainstream in pharmaceutical companies. The high cost of drug discovery and development, let alone the devastating consequences of withdrawing a high-profile drug from the market, underscore the need to use metabolic profiling to weed out or alter potentially damaging chemical entities earlier than has been previously possible.

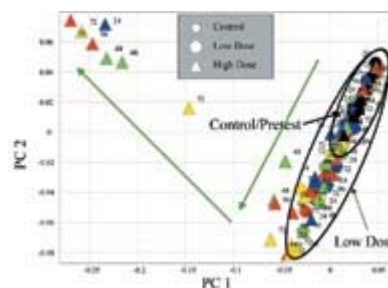
The lab many researchers credit with sparking interest in metabolic profiles is at Imperial College in London. The college has completed a three-year effort called COMET, the Consortium on Metabonomic Toxicology, which resulted in a database of NMR spectra covering 150 compounds or biofluid fingerprints that can be used to check for toxicity. The database has already proven successful in tests of liver and kidney toxins. The second phase of the project, which aims to perform more mechanistic toxicology studies and to supplement the current database with mass spectrometry data, is scheduled to begin in January.

Elaine Holmes, PhD, a principal in the COMET project, says NMR is very good at detecting possible toxicity and that such metabolic data could have made a difference in some drugs that have been recalled and would have kept others off the market. "You can use NMR to profile a person pre-dose or pre-administration and predict which people are going to react in a certain way to certain drugs," she says.

Defining nomenclature

The field of metabolic profiling has evolved to the stage where arguments over nomenclature are diminishing. The terms metabonomics, metabolic profiling and metabolomics are becoming synonymous among many researchers. Some, however, still prefer to segment the terms. Metabonomics, for example, is used by some to refer to a systems approach to metabolite profiling, to drug interactions with metabolites, to metabolite data from NMR instruments, or to results from the COMET consortium. Metabolomics is used to refer to cell-based metabolite profiling, to plant-based research, or as a general reference to the study and analysis of metabolites. For the purposes of this article, we will use the nomenclature cited by individual researchers.

The promise of metabolic profiling is not lost on US government laboratories and agencies. The Food and Drug



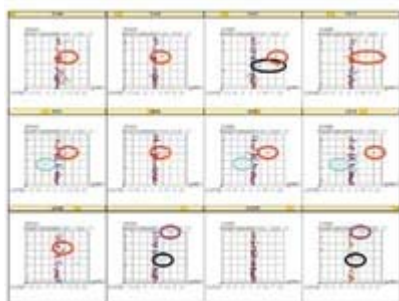
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Principal component plot of urine NMR spectral data collected from rats treated for four days with a compound that causes mesenteric vascular pathology (vasculitis). The figure demonstrates that there is a dose-related shift in urinary spectral patterns that progresses with duration of treatment (following the trajectories indicated by the green arrows). Importantly, animals that do not develop lesions do not have urinary spectral shifts, indicating that the spectral changes are not simply a dose-related reflection of the efficacy or off-target effects of the compound. (Source: Pfizer Metabonomics Evaluation Group.)

Administration (FDA) is already looking at extra, but not required, metabolic profiling data being submitted by pharmaceutical companies. The information has shown so much potential that last year the FDA's Center for Drug Evaluation and Research (CDER) developed draft pharmacogenomic guidelines for future submissions of genomic and microarray data by pharmaceutical companies. The proposed guidelines eventually will include metabolic profiling data as well.

The draft could become a guideline within six to nine months, says Daniel Casciano, PhD, director of the FDA's National Center for Toxicological Research (NCTR) in Jefferson, Ark. The NCTR uses a combination of genomic, proteomic, and metabolic tools to check whether data from model systems such as mice can be used to predict safety in humans.

The National Institutes of Health has also added metabolite studies to its fiscal 2004 budget for what it is calling "New Pathways to Discovery." This roadmap is designed to advance the understanding of biological systems and to build better tools for medical research in the 21st century. The initiative, announced this March, includes promoting development of novel technologies to study metabolites such as lipids, carbohydrates, and amino acids. It also includes developing quality and data standards for proteomics and the comparatively new study of metabolic profiles.



click the image to enlarge

Metabolon examined approximately 30 potential antibiotic leads, many structurally related, to determine their safety and efficacy profiles. In the figure above, each panel represents a different lead and its overall effect on metabolism. The circles within each panel represent metabolites that are significantly changed by the respective lead candidate. The control is the third panel in the bottom row. This data set allows one to understand which variations of an inhibitor are linked to non-targeted side effects. Such information is critical for lead prioritization and optimization. (Source: Metabolon Inc.)

Metabolic challenges

As in any emerging field, metabolite researchers face a number of challenges, ranging from biochemical issues to technological ones. No comprehensive public database of metabolites exists, although some researchers estimate there are at least 2,500 metabolites in humans. Identifying unknown metabolites is difficult, as is placing them in pathways and understanding how those pathways interact. Physiological and environmental variations unrelated to a disease will remain a challenge. Mass spectrometry and NMR instruments and analysis tools can be costly for many labs, and existing software is not yet sophisticated enough.

Getting experts to interpret the data can be difficult because it requires a multidisciplinary approach. And as the field moves forward, integrating gene expression, protein, and metabolite information at the systems biology level will be one of the biggest challenges in the next few years, says Holmes (see related story on page 47 on metabolite profiling data management).

Despite the many hurdles, Holmes and other researchers have been pushing ahead. Imperial College and University of Cambridge researchers have reported that they were able to test serum or plasma using proton NMR and pattern-recognition techniques to not only diagnose the presence of coronary heart disease in 66 patients (36 with triple vessel disease and 30 normals), but also identify the severity of the coronary arterial stenosis in 76 patients with the disease.

In addition, Holmes' lab is almost finished with an epidemiological study on hypertension that relates blood pressure to dietary factors and population differences. The lab has run 10,000 urine samples from people from China, Japan, the United States, and the United Kingdom in

multiple centers. "Until very recently we wouldn't have been able to cope with that," she says of the large study. Two things enabled her team to get to this stage. First, the automation has evolved to the point where such high-throughput screening is possible, and second, chemometric methods have been developed to help compensate for physiological variations or factors not of interest.

In addition to research on diseases, her lab is using metabonomics to study the toxicity of rescued drugs. "The big advantage of looking at these drugs is that you might be able to find out what the mechanism of toxicity is and then modulate the drug slightly to a chemical structure that isn't going to be toxic but that will keep the same pharmacological activity," she says, adding that could save a lot of research time and money.

Applying COMET

Pharmaceutical companies involved in COMET have brought their experience at Imperial to their companies

Treatment by Early Diagnosis

Predicting disease early is not just for newborns. Metabolomics company Metabolon Inc., Research

and have also set up more expansive in-house programs in metabonomics. "The expertise we're getting from COMET is feeding back into our in-house efforts," says Glenn Cantor, PhD, principal veterinary pathologist at Bristol-Myers Squibb's Pharmaceutical Research Institute, Princeton, N.J.

"The COMET database is a treasure trove of information, but the surface is just being scratched," adds Don Robertson, PhD. Robertson is director of the Metabonomics Evaluation Group in the Department of Safety Sciences, Pfizer Global Research and Development, Ann Arbor, Mich. He says one issue with metabonomics is that a lot of unique biomarkers will not be found in a data set. "But we may find patterns of two to five things that will be helpful in pigeonholing a specific toxicity to a specific mechanism or a specific pathophysiology," he says.

Typically, his team will have five chemical leads that have shown some kind of *in vivo* or *in vitro* efficacy, and so the question is which chemical lead to take forward. They will use the traditional approach to look for the chemical with the best pharmacokinetics, dynamics, and metabolism (PDM) characteristics. "And now metabonomics gives us the chance to actually add safety into that mix. We can take those five potential leads, do *in vivo* metabonomics in a mouse with a limited amount of a compound, about 50 milligrams, and then look at the spectral changes across those five compounds." Frequently, the results are not identical. Even if he doesn't know anything about what is changing, the fact that the results are not identical tells him there are some differences in at least the off-target effect, assuming they are hitting their target.

Network in place

Robertson says Pfizer has set up a metabonomics practice network involving all four company sites doing metabonomics work. The loose-knit network has upwards of 40 people; part of that network is the dedicated Metabonomics Evaluation Group of eight people in Ann Arbor that Robertson heads. His group has been researching vasculitis, an inflammation of the blood vessels, for about four years. Vasculitis is challenging in that there are no good biomarkers for it from a toxicity perspective, he says. Metabonomics has proven to be a powerful technology to assess vasculitis from urinary spectral patterns in animals. It has enabled his team to distinguish between changes in pathology and changes caused by using dexamethasone, an anti-inflammatory drug. His group conducted a study in which it suppressed inflammation using dexamethasone, and it still was able to see metabonomic effects. "This suggested the changes we were seeing were indeed due to the vessel damage and not just the inflammatory response."

Robertson adds that initially he thought metabonomics would make the most difference in the toxicity part of drug discovery and development. "But I'm not so sure anymore. The clinical applications could be as big or bigger than anything we do preclinically, because being able to do peripheral samples like urine and blood is amenable to clinical trials. It only takes one success in the clinical trial area to outbalance lots of success in the preclinical arena."

Ryals predicts that metabolomics could become a larger field than pharmacogenomics in five to 10 years. "The effect of the environment on drugs and on diseases in terms of drugs is going to be at least as big if not bigger than the genetic impact." Ryals points to the need for pharmaceutical companies to be able to develop drugs more efficiently. The Tufts Center for the Study of Drug Development estimated in 2002 that it costs \$802 million to bring one new drug to market. "But if you take a single drug and follow it through development, it's probably only about \$300 million or \$400 million. The difference is the cost of the failure rate of pharmaceuticals," says Ryals. "So there's a huge inefficiency in that process that needs to be improved dramatically." And new technologies like metabolomics will help.

Triangle Park, N.C., has been collaborating with various researchers on later-life neurodegenerative diseases such as ALS and Alzheimer's. "In neurodegenerative disease one of the key issues in treatment is to get an early-stage diagnosis," says John Ryals, PhD, the company's CEO. "If we could do an objective determination of Alzheimer's disease at the time the patient is diagnosed with mild cognitive impairment, we could probably do a lot for that disease, because we could come in 10 to 15 years earlier with therapy. Right now, by the time we start applying therapy for some of these neurodegenerative diseases, most of the neurological damage has been done."

In an ongoing collaboration with Massachusetts General Hospital, Boston, Metabolon analyzed 60 plasma samples from what the company thought were ALS patients and controls. The blinded samples were analyzed metabolically, which Ryals says produced some unexpected results. "Instead of having two groups of patients, we found we had four groups of patients." Metabolon discussed the situation with the clinicians, who then went back to the patients for more information. As it turned out, some of the patients had a related ailment called lower motor neuron disease, and others were ALS patients who were taking a drug called riluzole. The metabolomic study was picking up indicators for the effects of the drug as well as the related disease. "We now can follow the action of riluzole in these patients, and that's interesting," says Ryals.

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