Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

Metabolomics: A Global Biochemical Approach to Drug Response and Disease

Rima Kaddurah-Daouk,[1] Bruce S. Kristal,[2] and Richard M. Weinshilboum[3]

[1]Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, North Carolina 27710; email: kaddu001@mc.duke.edu

[2]Department of Neurosurgery, Brigham and Women's Hospital, Boston, Massachusetts 02115

[3]Division of Clinical Pharmacology, Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic College of Medicine, Mayo Medical School-Mayo Foundation, Rochester, Minnesota 55905

Annu. Rev. Pharmacol. Toxicol. 2008. 48:653–83

The *Annual Review of Pharmacology and Toxicology* is online at http://pharmtox.annualreviews.org

This article's doi:
10.1146/annurev.pharmtox.48.113006.094715

Copyright © 2008 by Annual Reviews.
All rights reserved

0362-1642/08/0210-0653$20.00

Key Words

pharmacometabolomics, metabolomic signatures, HPLC, mass spectroscopy, NMR, electrochemical array detection

Abstract

Metabolomics is the study of metabolism at the global level. This rapidly developing new discipline has important potential implications for pharmacologic science. The concept that metabolic state is representative of the overall physiologic status of the organism lies at the heart of metabolomics. Metabolomic studies capture global biochemical events by assaying thousands of small molecules in cells, tissues, organs, or biological fluids—followed by the application of informatic techniques to define metabolomic signatures. Metabolomic studies can lead to enhanced understanding of disease mechanisms and to new diagnostic markers as well as enhanced understanding of mechanisms for drug or xenobiotic effect and increased ability to predict individual variation in drug response phenotypes (pharmacometabolomics). This review outlines the conceptual basis for metabolomics as well as analytical and informatic techniques used to study the metabolome and to define metabolomic signatures. It also highlights potential metabolomic applications to pharmacology and clinical pharmacology.

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

INTRODUCTION

The final decades of the twentieth, and the beginning of the twenty-first, centuries have witnessed a revolution in biomedical research that has made it possible to move from the study of single genes, single mRNA transcripts, single proteins, or single metabolites to studies that encompass entire genomes, transcriptomes, proteomes, and metabolomes (**Figure 1**). Those changes have occurred in parallel with advances in molecular pharmacology that resulted in a therapeutic revolution (1), with the development of drugs that have made it possible for the first time in human history to treat or control diseases that range from childhood leukemia to hypertension, from breast cancer to depression. However, major challenges facing pharmacologic science include the integration and application of the analytical techniques and data analysis methods of the new biology. The development of powerful and effective pharmacologic agents has also highlighted the necessity for individualizing drug therapy to select those patients most likely to respond to treatment, to minimize the occurrence of adverse drug reactions, and to maximize the desired therapeutic effect. Initial efforts to individualize pharmacologic therapy have focused on genomics, i.e., pharmacogenomics, with a series of notable success stories (2). However, those efforts have also served to clarify the need to unite well-defined phenotypes with increasingly detailed genotypic data. Metabolomics promises to contribute significantly to the achievement of that goal, particularly if we succeed in combining pharmacometabolomics with pharmacogenomics.

The development of analytical techniques that make it possible to assay and quantitate components of the metabolome and to extract useful signatures from those data promises to increase our understanding of disease pathophysiology, our knowledge of mechanisms responsible for drug effect, and our ability to approach the goal of individualized drug therapy. In this review, we briefly outline the current status

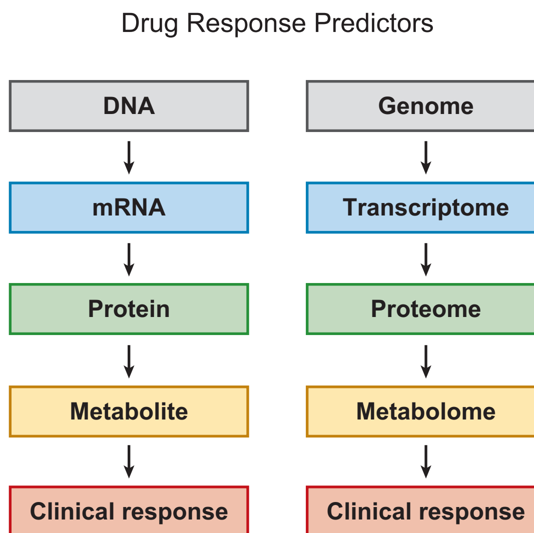


Figure 1
Drug response predictors in the pre- and post–new biology eras.

of metabolomics—its conceptual basis, the analytical techniques that are used to perform metabolomic studies, and the informatic tools that are required to analyze metabolomic data—with specific examples to illustrate each topic. Special emphasis will be placed on the way in which this new and rapidly developing discipline might contribute to pharmacology research, i.e., on pharmacometabolomics.

METABOLICS: AN OVERVIEW

Metabolomics, the study of metabolism at the global, or -omics, level, has the potential to contribute significantly to biomedical research and, ultimately, to clinical medical practice. This rapidly developing discipline involves the study of the metabolome, the total repertoire of small molecules present in cells, tissues, organs, and biological fluids (3–15). The identities, concentrations, and fluxes of these compounds result from a complex interplay among gene expression, protein expression, and the environment. In contrast to classical biochemical approaches that often focus on single metabolites, single metabolic reactions and their kinetic properties, and/or defined sets of linked reactions and cycles (i.e., precursor/product, intermediary metabolism), metabolomics involves the collection of quantitative data on a broad series of metabolites in an attempt to gain an overall understanding of metabolism and/or metabolic dynamics associated with conditions of interest, including drug exposure (16). Many names have been used to refer to this new field, including metabonomics, metabolic profiling, metabolic fingerprinting, and metanomics, among others (3, 17). However, metabolomics has been used most often, so that term is applied throughout this review.

The overall size of the metabolome remains a subject of debate and depends on the definition of exactly what components should be included and on the analytical platform used. Numbers that range from a few thousand to tens of thousands of small molecules have been proposed. As implied earlier, metabolomic information complements data obtained from other fields that comprise the new biology—genomics, transcriptomics, and proteomics—adding a final piece to a systems approach for the study of drug action, individual variation in drug response, and disease pathophysiology. Ideally, metabolomics will ultimately contribute a detailed map of the regulation of metabolic pathways, and, therefore, of the interaction of proteins encoded by the genome with environmental factors, including drug exposure. Therefore, the metabolome represents a state function for an individual at a particular point in time or after exposure to a specific environmental stimulus, e.g., a specific drug or xenobiotic.

Unlike earlier analytical methods, metabolomics utilizes instruments that can simultaneously quantitate thousands of small molecules in a biological sample. This analytical capability must then be joined to sophisticated mathematical tools that can identify a molecular signal among millions of pieces of data (18). Disease disrupts metabolism and, as a result, causes changes that are long lasting and can be captured as metabolic signatures. Initial metabolomic signatures have already been reported for several disease states, including motor neuron disease (19), depression (20), schizophrenia (21–23), Alzheimer's disease (24), cardiovascular and coronary artery

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

disease (25, 26), hypertension (27), subarachnoid hemorrhage (28), preeclampsia (29), type 2 diabetes (13, 30, 31), liver cancer (32), ovarian cancer (33), breast cancer (34), and Huntington's disease (35). These signatures are made up of tens of metabolites that are deregulated, with concentrations that are modified in the disease state or after drug exposure. As a result, analysis of these signatures and their components can potentially provide information with regard to disease pathophysiology. Metabolic signatures have also been identified for several drugs where the signatures represent changes that occur secondary to drug treatment and in which those signatures capture information from pathways that are targets for, or are affected by, drug therapy (23, 36–40). In summary, metabolomics promises to have broad implications for both basic biomedical research and medical practice because it can capture information with regard to mechanisms of disease and of drug action, making it possible to map disease risk or drug action to metabolic pathways.

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

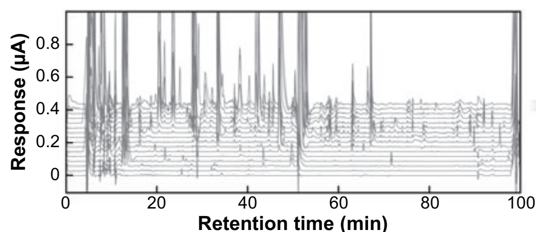
THE METABOLOMICS PROCESS

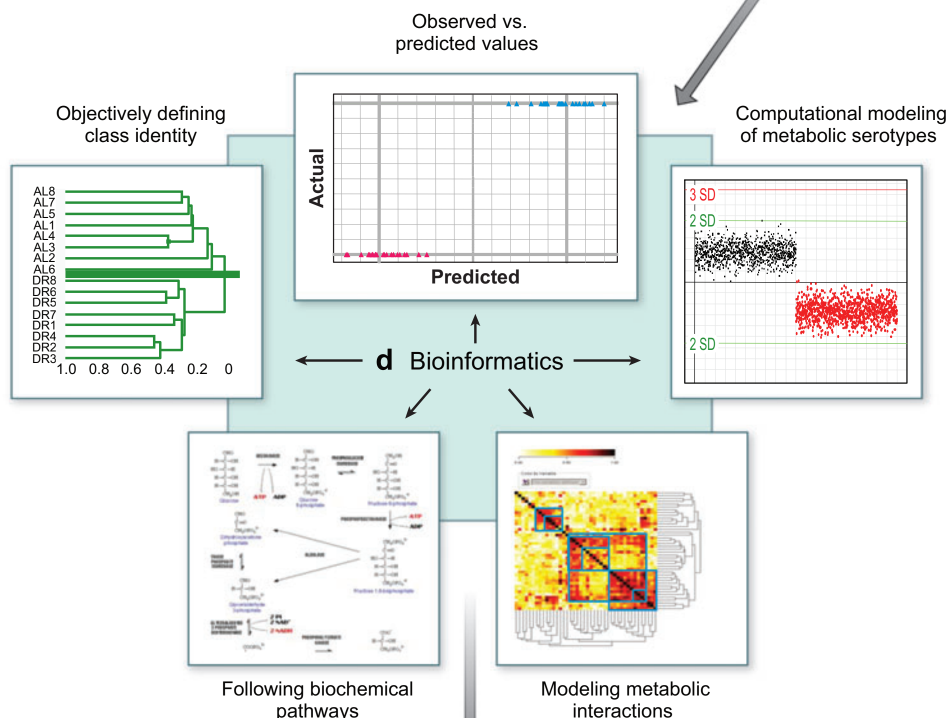
A typical metabolomics study is depicted schematically in **Figure 2** (16). Samples of interest (e.g., plasma, cerebral spinal fluid, or tissue biopsies) are collected. Small molecules are extracted from the sample and are analyzed using techniques that separate and quantitate the molecules of interest. Those analytical techniques include, among others, liquid and gas chromatography, mass and nuclear magnetic resonance (NMR) spectroscopy, and liquid chromatography with electrochemical detection (see subsequent detailed discussion). Combinations of these techniques can also be used to augment separations and/or to expand the analyte information collected. These datasets must then be collected and curated, a process that can take significant time. After curation, the data are analyzed by one or more software packages designed for use with large datasets. A database is then generated for the same patient before and after drug therapy or for diseased patients and control subjects. These databases include levels of detectable metabolites and the identity or a description of the properties of the metabolites, i.e., oxidation reduction potential, mass/charge ratio, etc. Software tools can then be used to (*a*) identify disease signatures (e.g., compounds that highlight a disease state), (*b*) predict class (e.g., pre- or postdrug exposure, disease or control), (*c*) identify unrecognized groups in the data (e.g., drug response subgroups), (*d*) identify interactions among variables, and (*e*) map variables to known biochemical pathways. A critical metabolomics concept is that a biomarker that predicts disease or

Figure 2

Typical metabolomics experiment flow diagram. (*a*) Samples are collected and (*b*) individual metabolites are quantitatively analyzed using one or more high–data density analytical instruments. (*c*) The datasets are curated and (*d*) analyzed using a series of high–data density informatics approaches. The informatics outputs shown here include class prediction (SIMCA-P, Umetrics), principal components analysis of a computationally modeled dataset (SIMCA-P, Umetrics), 2D cluster analysis (GeneLinker Platinum, Improved Outcomes Software), metabolic analysis (**http://www.biotech.icmb.utexas.edu**), and cluster analysis (from Piroutte, Infometrix). Adapted from Reference 16.

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.





→ **Mechanistic insight**
→ **Drug development**
→ **Toxicology**
→ **Classification**
→ **Prediction**
→ **Functional genomics**
→ **Sub-threshold studies**
→ **Others**

helps to monitor drug therapy is most often not a single molecule, but rather a pattern of several molecules. That concept determines the need for quantitative precision and the careful avoidance of artifacts during this type of research. Although this can be a difficult analytical task in the early stages of metabolite pattern detection, if the relevant metabolomic species can be defined and identified, appropriate techniques can then be used to develop rapid targeted assays suitable for more routine application, both in the research laboratory and/or in a clinical setting.

The choice of metabolomic analytical instrumentation and software is often goal specific because each type of instrument has, as discussed subsequently, specific strengths and limitations. For example, liquid chromatography (LC) followed by coulometric array detection is ideal for mapping neurotransmitter pathways (17, 41, 42). Gas chromatography (GC) in conjunction with mass spectrometry (MS) is often used in the analysis of lipid subsets (lipidomics) (43). Liquid chromatography together with mass spectroscopy (LC-MS) is often used to obtain the largest possible biochemical profile, and NMR has been used successfully to perform toxicology studies (44, 45). In a similar fashion, different software packages include specific tools designed to address questions distinct to each study. Because of the importance of analytical platforms for metabolomic studies, the following section briefly reviews, in turn, each of the major metabolomic analytical methods.

METABOLOMICS ANALYTICAL METHODS

Metabolomics involves the study of the repertoire of small molecules, or metabolites present in a cell, tissue, organ, or biological fluid. Small molecule in this setting refers to endogenous molecules involved in, or resulting from, primary and intermediary metabolism, as well as exogenous compounds, such as drugs and other xenobiotics. Representative endogenous small molecules include well-known and well-studied compounds, such as glucose, cholesterol, ATP, biogenic amine neurotransmitters, and lipid signaling molecules. By choosing appropriate separation and detection technologies, these molecules can be analyzed on the basis of their individual properties. A wide variety of methods have been used to separate and quantitate components of the metabolome, and no single analytical platform can capture all metabolomic information in a sample. At one level, an analytical platform may be described in the context of its instrumentation. Therefore, GC-MS, LC-MS, and NMR-based metabolomics platforms are suited for mapping global biochemical changes in nontargeted ways; and LC-electrochemistry array metabolomics platforms (LCECA) are excellent for mapping neurotransmitter pathways and pathways involved in oxidative stress (for targeted and untargeted studies). At another level, an analytical platform may be described in the context of the analytical goal. Lipidomics platforms are designed for mapping lipid biochemical pathways. Recent work has described approaches for the analysis of specific subsets of compounds, for example, thiol-containing metabolites (46), acylcarnitines, amino acids, and carbohydrates, among others. Affinity-based techniques promise to broaden this type of approach further in the future. Most or all of these analytical platforms are already familiar to investigators involved in pharmacology and toxicology research. The difference in metabolomics lies not in

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

the platforms, but rather in the specific way in which these analytical tools are applied, the samples they are used to analyze and the approaches taken to control the experiments and to analyze the data.

The logistics of metabolomic analysis require significant planning, and the scope of results obtained is generally linked to the instrument used to collect the data. Analytical aspects of metabolomics can be divided into four functions: sample acquisition, sample storage, sample extraction, and metabolome analysis. Although each is important, we focus only on the final two: sample extraction and the analytical step. In practice, these two activities are inseparably linked. Perhaps one way to illustrate the issues involved is by contrasting this field with mRNA microarray analysis. Extraction procedure(s) used to isolate RNA prior to microarray analysis are thought to be essentially universal, i.e., all RNAs are extracted more or less equally. In contrast, different metabolomic extraction procedures can reveal orthogonal or overlapping metabolomes, depending on the choice of reagents (e.g., hexane for highly lipophilic versus acidified acetonitrile for more hydrophilic species). Some sample extraction procedures have been developed that are highly specific for given subsets of compounds, whereas others are more general. Liquid-liquid and solid phase extractions have the advantage that they can be tailored, for example, to remove specific confounding species and/or to focus on subsets of compounds (see the Web sites of specific vendors for descriptions of applications using tools such as solid phase extraction). Obviously, the extraction procedure must be matched to the analytical subset of interest. Second, although many microarray platforms exist, each can measure most or all RNA species. A custom array is limited by choice, but theoretically is unlimited in scope. In contrast, as discussed subsequently, analytical platforms for metabolite analysis are more limited, and they differ in at least six operating parameters other than cost: universality, specificity, sensitivity, quantitative precision, their ability to provide structural information, and throughput capacity. There are at least four major analytical platforms with proven utility for metabolomic applications: NMR, GC-MS, LC-MS, and LCECA (14–16). Each of these platforms has specific advantages and disadvantages. In subsequent paragraphs, each of these key platforms is described. Their strengths, limitations, and examples of their application in metabolomics are also summarized briefly, followed by comments with respect to ways to obtain more detailed information with regard to these platforms.

NMR Spectroscopy

There are numerous reasons for employing NMR as a primary tool for structure-based metabolomic investigations, many of which are the same as those that have attracted structural biologists to NMR for the structural and dynamic analysis of proteins and nucleic acids. Modern NMR makes it possible to perform rigorous structural analysis of many metabolites in crude extracts, cell suspensions, intact tissues, or whole organisms. Structural determination of known metabolites using various one-dimensional (1D) and 2D NMR methods is straightforward, whereas de novo structural analysis of unanticipated or even unknown metabolites is also feasible. The latter can bypass the need for authentic standards (often a major barrier to structure

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

determination) and is unparalleled by other molecular structural techniques, such as MS or infrared spectroscopy. In addition to popular high-sample-throughput applications, NMR is particularly powerful for metabolite structural determinations, including the atomic positions of isotopic labels (e.g., ^{13}C, ^{15}N, or ^{2}H) in different isotopomers generated during stable isotope tracer studies (34, 47–49). These latter applications provide detailed maps of biochemical pathways or networks, which can also serve as crucial inputs for in silico quantitative flux analysis (50, 51). As a result, metabolic pathways can now be systematically mapped by NMR with unprecedented speed.

In summary, NMR offers essentially universal detection, excellent quantitative precision, and the potential for high throughput (>100 samples/day is attainable). Because a single compound can give multiple peaks, statistical approaches have been developed that enable the deconvolution of this type of complexity. The major disadvantage of NMR is its relatively poor sensitivity (approximately 1 nmol solute is required). Another disadvantage is high initial cost because NMR instruments can cost well over one million dollars, but FT-ICR MS instruments can be equally expensive. NMR has been particularly successful when applied to toxicology studies.

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

MS-Based Platforms

MS represents a universal, sensitive tool that can be used to characterize, identify, and quantify a large number of compounds in a biological sample where metabolite concentrations might cover a broad range (52–56). With carefully chosen upstream sample handling, MS can be used to measure low abundance signals, such as those from signaling molecules or hormones. That is particularly true for targeted analyses. Metabolomics requires proper separation of the compounds to be assayed, and chemical separation techniques such as GC and LC or capillary electrophoresis can all be joined to MS detection. In the case of molecules for which authentic biochemical standards exist, metabolites can be identified and quantified by the use of these combined separation techniques as a result of two orthogonal parameters, compound separation time and molecular mass. MS also makes it possible to monitor the presence of molecules that are detected reproducibly but are as yet unidentified. Those compounds might include unknown drug metabolites, byproducts of gut flora, or oxidative damage products. Structural identification can also be attempted, but is not always straight-forward. Structural identification is aided by a combination of three factors: high mass accuracy, ion fragmentation capability, and software designed to recognize the rules by which nature assembles compounds (57). The availability of these three factors, alone and in combination, has greatly aided efforts to assign compounds their most likely elemental composition, and, in many cases, key substructures. Subsequent paragraphs specifically highlight the relative advantages and special features of GC-MS and LC-MS platforms.

GC-MS offers structural information (excellent when the compounds are already present in existing libraries), reasonable quantitative precision, and high throughput (once again >100 samples/day is possible). Mid-level instrumentation costs fall between $100–$300,000. Sensitivity is at least 2 orders of magnitude higher than is

the case for NMR. One limitation of GC-MS is its inability to study molecules that cannot be readily volatilized. Another is the relatively low mass accuracy of these instruments (often unit resolution) unless magnetic sector machines are used, but that is done at the expense of a higher initial cost and reduced throughput. It is also worth noting that GCxGC MS provides an additional orthogonal degree of separation, and has particular utility where changes are anticipated in related metabolites as occurs in studies of a series of related compounds.

The greatest advantage of LC-MS for application to metabolomic studies in pharmacology and toxicology is its flexibility. Different combinations of mobile phase and columns make it possible to tailor separations to the compounds of interest, including chiral compounds when appropriate conditions are used. As a result, most compounds can be analyzed by LC-MS. Instruments exist that enable low, medium, or high mass accuracy, and linear ion traps can enable MS^n, providing fragmentation profiles specific for given molecules. This technique makes it possible to trade off sensitivity for throughput (with typical metabolomic throughputs ranging from 20–100 samples/day). The cost of LC-MS instruments ranges from approximately $100,000 for a basic single quadruple MS to well over one million dollars for an FT-MS. The combination of high mass accuracy, MS^n fragmentation, and appropriate software makes it possible for MS instruments to determine the exact molecular composition of many compounds of interest. One limitation of LC-MS is relative difficulty in obtaining consistent quantitative precision. In the context of pharmacometabolomics, LC-MS is well suited to broad survey studies. Defined fragmentation patterns have been shown to be useful for following drug metabolites, which can also be done with labeled drugs. LC-MS can also be used for stable isotope/flux experiments. The flexibility of LC-MS can be applied to advantage when investigators have specific subsets of metabolites in mind. Another useful feature of MS is the ability to target specific classes of compounds by examining loss of a fragment of the molecule in a collision cell. Triple-Quad mass spectrometers can be used in that way to conduct semitargeted analyses.

LCECA Platforms

LCECA detection metabolomics platforms generally contain 16 coulometric electrodes in an array (58–61), allowing differential detection and quantification of small molecules on the basis of their oxidation-reduction potentials (**Figure 3**). These compounds represent a subset of the metabolome that includes molecules amenable to detection by oxidation-reduction. For example, this platform is ideal for application to studies of the tryptophan and tyrosine pathways that lead to monoamine neurotransmitters because many metabolites within these pathways can be measured quantitatively with LCECA. The robust nature of this platform, its reproducibility and its sensitivity have been well described in a series of peer-reviewed publications (58–62). Preliminary experiments described later in this review demonstrate the power and promise of the electrochemistry-based platform for metabolomics analysis for defining signatures for central nervous system (CNS) disorders and drugs that are used to treat those diseases. Examples of studies performed with an LCECA platform

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

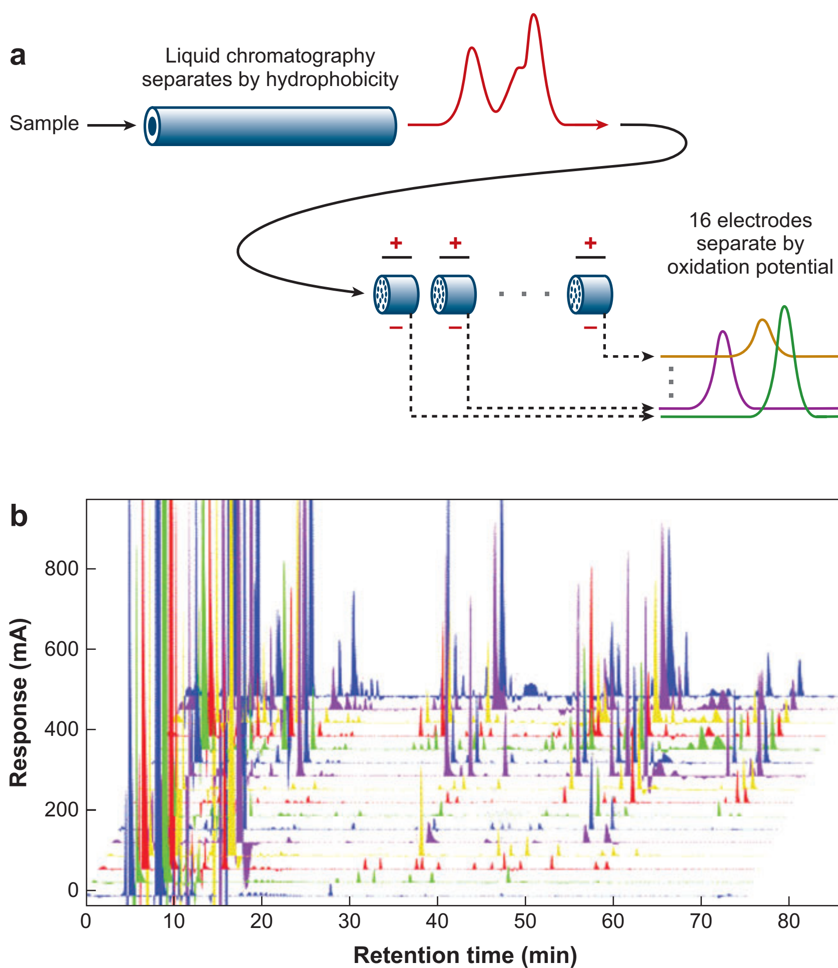


Figure 3

Metabolic profiling using high-performance liquid chromatography (HPLC) and coulometric electrochemical array detection (LCECA). (*a*) LCECA detection separates in two dimensions: hydrophobicity and oxidation potential. Samples are injected into an HPLC column where they are fractionated by hydrophobicity. The eluent from the column flows through porous electrodes representing 16 different electrical potentials—the coulometric array. These electrodes detect redox-active metabolites and measure their oxidation potentials. (*b*) Each electrode generates one chromatogram. Therefore, the output consists of 16 parallel chromatograms corresponding to 16 different oxidation potentials. The height of a peak in one of the output chromatograms indicates the concentration of a metabolite with a particular hydrophobicity and oxidation potential. As indicated schematically in the figure, this method is able to use oxidation potential to separate peaks that overlap after separation by hydrophobicity. With kind permission from Springer Science and Business Media (19).

where it was used for targeted analysis include analyses of the serotonin system in Alzheimer's disease (63), kynurenic acid (a tryptophan metabolite) in mammalian cerebral spinal fluid and brain (64), the kynurenine pathway in Huntington's disease (41), and alterations in dopamine and serotonin metabolism in Parkinson's disease (65).

In LCECA, the detector is a powdered graphite electrode that detects only electrochemically active compounds—specifically those that can react under 900 mV. Higher voltages can be used for short times, but they damage the electrodes and degrade performance. The LCECA system is extremely sensitive, perhaps 2–3 orders of magnitude better than GC-MS, and it displays strong run-to-run precision over long periods of time. The initial cost is also low, at slightly under $100,000. Disadvantages include the lack of structural information and low throughput (12 samples/day in the most commonly used metabolomic configurations). For pharmacometabolomic studies, the specific nature of the molecules that can be detected can either be a significant plus or a minus. The system can, as mentioned previously, detect molecules such as tyrosine and tryptophan metabolites, as well as antioxidants and oxidative damage products, but it is "blind" to molecules such as glucose, ketoglutarate, and most fatty acids. When the molecule of interest is one that can be detected, the specificity of this platform is an advantage because of noise reduction. The sensitivity of LCECA and its ability to target key metabolic pathways that are sensitive to changes in the environment is a great advantage, as is the ability of the system to detect changes in redox potential and low levels of oxidant damage, each of which can be a hallmark of drug effect.

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

Selecting a Platform

In an ideal setting, one platform would be able to accurately measure all compounds of interest. In another ideal setting, a researcher would be able to examine the preceding descriptions of platforms and make straight-forward, logical choices about the best platform for use in his or her studies. A simple analogy would be the investigator who sees a procedure of interest in a journal article and then follows that protocol in their own laboratory. Unfortunately, metabolomics does not lend itself well to this model. Instruments are expensive, and many of them require considerable skill to use. Even an apparently standard analysis may be difficult to adapt to a slightly different instrument, and many metabolomic analyses are currently being performed in ways that involve at least one propriety reagent, piece of equipment, or software for data reduction.

This complexity highlights at least two additional factors that must be considered: instrument and sample availability. One approach, taken by some pharmaceutical and biotechnology companies that specialize in metabolomic analysis, is the use of multiple instruments and/or multiple extraction regimens. This approach strengthens the data obtained by playing to the strengths of individual instruments. For example, one could obtain data for glucose and energy metabolism using NMR, assay lipids with a GC platform, and measure neurotransmitters with an LCECA platform. However, the majority of laboratories, especially in academic settings, lack access to

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

multi-instrument platforms. Therefore, it is often necessary to consider the practical limitations of available, adaptable, or obtainable instrumentation prior to beginning an analysis. If the available instrumentation is not appropriate, then one might need to establish collaborative interactions or explore metabolomic service facilities. A similar issue is sample availability. In many cases biological specimens are limiting—sometimes severely so—and multiple uses of these samples must also be taken into account (e.g., clinical chemistry, proteomics, microarrays, specialized hormone assays). As a result, it is often critical to also consider the logistics of analysis from the standpoint of what instrument would give the most information while requiring the least sample? What information is most critical? Is the targeted analysis of a few metabolites the most important goal? The trade-off is often between number of metabolites, sensitivity, and precision of the measurements. These decisions must be made before analyses are begun—ideally before the samples are collected.

Owing to space limitations, this review only addresses analytical platforms in a simplified fashion. Many books have been written on NMR and MS, and annual MS meetings include thousands of abstracts on improvements in technique. Therefore, readers interested in pursuing metabolomic analyses should turn to primary sources for more detailed information. The following references provide a starting point (3, 14–16). Major additional sources of information include (*a*) Pubmed and related sources; (*b*) Web sites, e.g., **http://www.metabolomicssociety.org**; (*c*) instrument manufacturers (instruments are changing rapidly, and instrument manufacturers can provide up-to-date data); (*d*) analytical meetings; and (*e*) investigators active in the field.

METABOLOMIC INFORMATICS TOOLS

The sheer size of the datasets obtained during metabolomic studies, as with any -omics field, places limits on the utility of classical statistics, in particular the univariate and other standard statistics most familiar to biologists. The art of informatics, and in metabolomics it is still largely an art as opposed to a science, rests on the ability to make the experimental design and the specific approaches taken match the critical questions addressed by the study. Broadly speaking, the questions relevant for pharmacometabolomic studies fall into one or more of the following groups: (*a*) What happens to drug X in the context of condition Y?; (*b*) How does condition Y alter the metabolism of drug X?; (*c*) Is drug X present?; (*d*) How does drug X alter the metabolism of compound Z or a family of compounds Z′?; (*e*) Can we use metabolomics to make early, accurate predictions about whether drug X does (or will do) action A?; and (*f*) Is drug X doing anything detectable?, and the closely related statement, Tell me everything it does. Questions *a*, *b*, *c*, and *d* may be approached using the analytical tools of metabolomics, but the informatic analysis essentially reduces to a problem with few variables, which are likely to be highly related. These studies are appropriately addressed with classical statistical methods such as ANOVA or t-tests with appropriate Bonferroni/false discovery rate corrections (66–68).

Question *e* covers all aspects of predictive pharmacology, including issues of efficacy, safety, pharmacogenomics/pharmacometabolomics, etc. As a general rule, the

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

informatics approaches required are conceptually well defined, and they fall exclusively, or almost exclusively, in the realm of supervised analysis. In supervised analysis, the investigator starts with one or more previously defined classes, and a series of prior examples (termed a training set) that fall into each of these classes. One then learns to recognize the training set using a series of mathematical approaches. As a result, supervised techniques uncover the features (variables) that best discriminate between those groups. Broadly speaking, supervised approaches involve tools that use projection methods to define planes of maximal separation [SIMCA (soft independent modeling of class analogy); PLS-DA (partial least squares projection to latent structures discriminant analysis); O-PLS (orthogonal partial least squares) (69–71); modified clustering/distance algorithms, e.g., kNN (k-nearest neighbor analysis); and machine learning tools, e.g., genetic algorithms, genetic programs, artificial neural networks]. Other supervised methods have also been applied to molecular fingerprinting data, including ANOVA (72), partial least squares (PLS) (73), and discriminant function analysis (DFA) (74). Each of these techniques has strengths and limitations (e.g., severe overfitting concerns) that are beyond the scope of this brief overview. However, essentially all use of supervised analysis requires eventual confirmation with a test set—a series of examples independent of those used in the analysis of the training set.

Question *f* covers situations that are orthogonal to the understood or predicted aspects of drug effect. One example might include concerns over whether a drug targeted elsewhere also alters cardiac or hepatic metabolism. Another example might include an attempt to determine which of a series of drugs has more off-target effects. Alternative examples include a search for potential subsets of patients who do and do not respond to drug therapy or a search for potential interactions. Note that the latter two examples, given sufficient background information, might also be covered under question *e*. In the case where little is known, metabolomic analysis offers the possibility of a high potential payoff.

At the data analysis level, the primary limitation to the analysis of data from human subjects lies in the sheer complexity of the data. Therefore, the tools of most interest are those that simplify the data in some way. In general, the algorithms of interest conduct what is referred to as unsupervised analysis. Unsupervised algorithms identify patterns in the data without bias and are typically driven by the largest changes (variance) in the dataset (75, 76). Examples of unsupervised methods that have been used routinely in analyzing molecular fingerprinting data are hierarchical clustering (77), principal component analysis (PCA) (77, 78), and self-organizing maps (77, 79). These methods are generally very sensitive to subtleties of experimental design (59); outliers; and the way in which data has been collected, scaled, normalized, or winsorized (a tool for reducing outlier effects) (61). These methods are also sensitive to the specifics of the informatics analysis. These sensitivities are such that, in some cases, apparently diametrically opposed results can occur. Thus, exploratory analyses are primarily used in one of three ways: (*a*) to look for very large and unexpected results that are stable across most or all conditions tested; (*b*) to generate hypotheses for testing in the course of future studies, i.e., to explore the dataset; and (*c*) to provide the best test possible for the absence

of an effect, bearing in mind the fact that absence of evidence is not evidence of absence.

Finally, the confines of this review preclude an in depth discussion of methods for the analysis of metabolomic data. Although excellent texts exist (80, 81), readers might be best served by following up this topic either with local experts in informatics (including those conducting studies outside of metabolomics, such as investigators involved in microarray analysis) and/or with the authors of relevant publications.

METABOLOMIC SIGNATURES AND DISEASE

Disease states perturb biochemical networks, resulting in new metabolomic signatures. Several early metabolomic analyses of neurologic disease focused on motor neuron disease (MND) in which the electrochemistry-based metabolomic platform (LCECA) described above made it possible to map metabolic patterns that differentiated patients from matched healthy controls. This example of the application of metabolomics to disease pathophysiology is presented because it also introduces the concept of signatures for drug response—a topic presented in greater detail below.

MNDs are a heterogeneous group of disorders that include amyotrophic lateral sclerosis (ALS), upper motor neuron (UMN) disease, and lower motor neuron (LMN) disease—all of which result in the death of motor neurons. It is unclear if these diseases are related or whether similar pathways are deregulated during the disease process. Metabolomic analysis of plasma from 30 healthy controls and of 28 patients with MND (19) using an LCECA platform resulted in the identification of 50 metabolites that were elevated in MND patients (**Figure 4**) and more than 70 that were decreased ($P < 0.05$). Included among the elevated compounds were 12 that were associated with riluzole therapy (**Figure 4**). Riluzole is a drug that is used to treat these patients that inhibits glutamate release and is an antagonist at MNDA and kainate-type glutamate receptors. This study was one of the first to define a metabolic signature for a drug that reflected its pharmacodynamics because these metabolites were not related to metabolism of the drug itself, but rather its effects on biochemical pathways (19). It was possible to separate MND patients from controls on the basis of their metabolomics signatures, as well as patients on and off drug therapy (19) (**Figure 5**). In a subsequent study of 19 subjects with MND who were not taking riluzole and 33 healthy control subjects, six compounds were found to be significantly elevated in MND, whereas the number of compounds with decreased concentrations was similar to that observed in the initial study (19). These MND data also revealed a distinctive signature of highly correlated metabolites in a set of four patients with slow disease progression, three of whom had LMN disease (**Figures 4** and **5**, indicated with an asterisk). These observations resulted in the initiation of much larger studies in patients with MND that are ongoing (a project initiated by the National ALS Association that includes Metabolon Inc., MGH Neurology, Duke Medical Center, and University of Pittsburgh), in which signatures are being defined in the plasma and CSF of these patients using GC-MS and LC-MS platforms to define the nature of the compounds that differentiate MND patients from healthy control subjects and to define subsignatures for each class. The chemical identity of these metabolites may highlight pathways

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

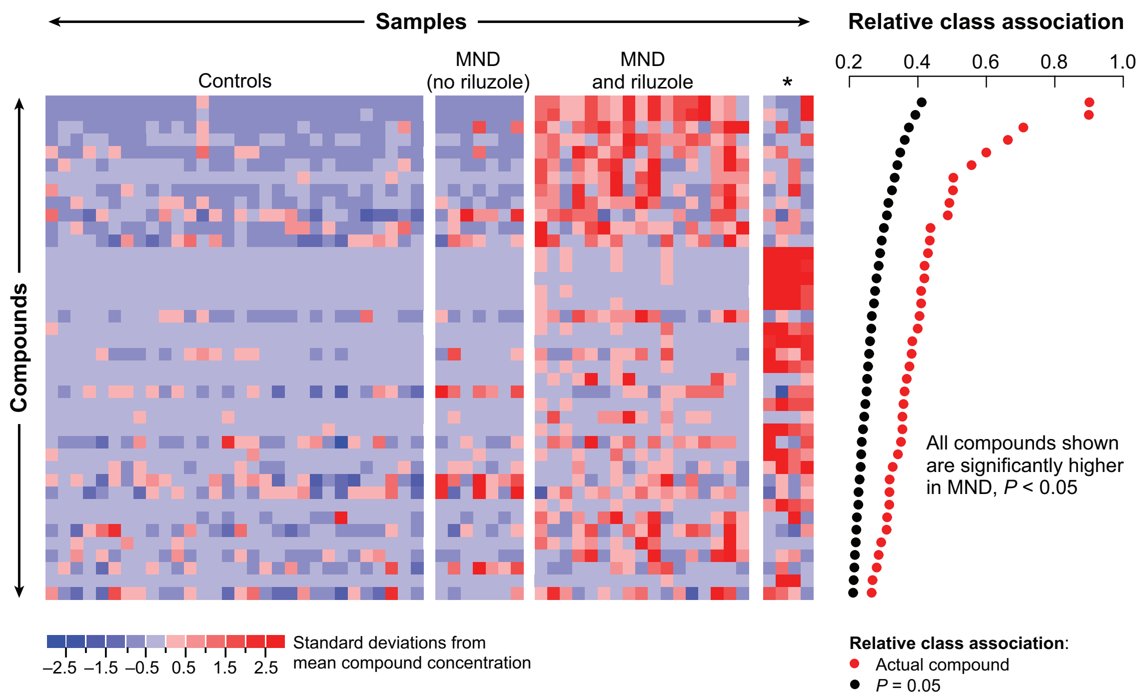


Figure 4

The heat map of metabolomic data for MND patients and controls. The figure shows metabolites with significantly higher concentrations in plasma samples from MND patients than in control subjects. Each row represents a metabolite, and each column represents a healthy control or a patient, with each colored square representing the relative concentration of a single metabolite in a single subject. Compounds are on the basis of decreasing association with MND. Significant association measures at $P = 0.05$ are indicated by black dots to the right. Association measures for actual data are indicated by red dots. The metabolites that are elevated in MND define three subgroups that consist of patients not taking riluzole, patients taking riluzole, and four patients (indicated by an *asterisk*) with a distinctive signature. Three of these distinctive patients had LMN disease. With kind permission from Springer Science and Business Media (19).

related to disease pathophysiology and/or response to drug therapy. Metabolomic signatures for patients with MND are also being compared with signatures for other CNS disorders to define the sensitivity and specificity of these signatures as potential diagnostic biomarkers. They are also being reevaluated as the disease process progresses in an attempt to define biomarkers for disease progression. Additionally, metabolic profiling for patients with ALS, UMN, and LMN might provide further insights about how closely related these motor neuron disease are and could help define common and unique pathways implicated in disease pathogenesis.

This single example illustrates the fact that it requires significant effort to define biomarkers that are predictive and disease-specific. In this case, it was important to compare central and peripheral effects to define a set of metabolites that might be

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

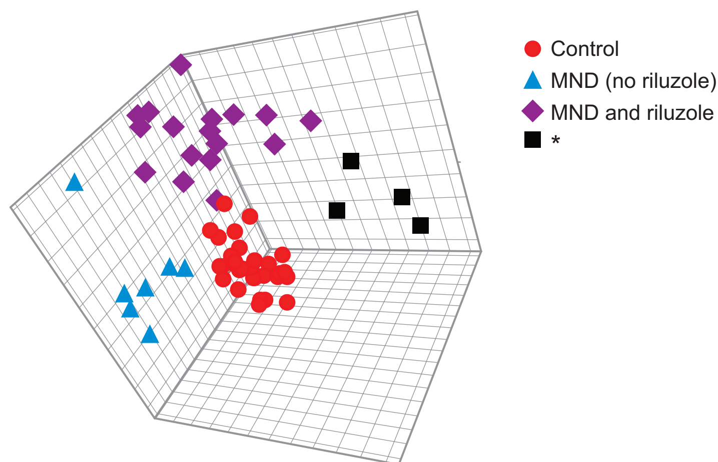


Figure 5

PLS-DA distinguished subgroups of MND patients and controls. Models using projections into three dimensions provided statistically significant separations between subgroups ($P < 0.01$ by permutation test—random assignment of samples to subgroups). Red are control subjects; purple are MND patients on riluzole; blue are MND patients not on riluzole; and black are atypical MND patients, three of whom had LMN disease (indicated by an *asterisk*). With kind permission from Springer Science and Business Media (19).

disease-specific. A great deal of work will still be required to determine the significance of these observations, to identity the structures of the molecules that underlie these signatures, and to confirm these preliminary findings in adequately powered clinical studies. The possible contributing effects of confounding factors such as life style, other disease conditions, and effects of other medications have to be dealt with and factored out of metabolic signatures. However, examples of this type support the hypotheses that a disease can result in a new biochemical state that is long lasting and can be captured as a metabolic signature. Subclasses of disease based on metabolomic signatures are also starting to emerge, and these early examples are setting the stage for studies of other neurological and neuropsychiatric disorders, such as Parkinson's, Huntington's, depression, schizophrenia, substance abuse, and dementia. The next section focuses on the application of metabolomics to characterize drug-response signatures.

METABOLOMIC SIGNATURES AND DRUG-RESPONSE PHENOTYPES

A major potential application of metabolomics involves the definition of pathways that contribute to drug response phenotypes. That type of study could provide information with regard to the pharmacokinetic and pharmacodynamic properties of a drug, as well as insight into mechanisms responsible for individual variation in drug response. The global mapping of signatures pre- and post drug treatment is already teaching us that metabolomic signatures can highlight biochemical pathways that may

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

be targets for drugs (9, 44, 45, 82–87). That information can confirm what is already known with regard to drug effect. However, we also often observe new pathways that have not previously been identified as therapeutic targets. The mapping of signatures in good and poor responders could also identify pathways of importance for variation in response to therapy. A series of recent examples are used to illustrate the potential of metabolomics to inform research related to drug response. The examples highlighted subsequently utilized different metabolomic analytical platforms and applied them to study samples from both humans and experimental animals, but, in all cases, the purpose was to take a global approach to help define drug mechanisms and/or mechanisms responsible for drug side effects. Examples described in subsequent paragraphs include metabolomic studies of atypical antipsychotic agents in patients with schizophrenia, of HMG-CoA reductase inhibitors in hyperlipidemic patients, of the antidiabetic drug rosiglitazone in both humans and mice, and of the antineoplastic agent cisplatin, as well as a series of potentially hepatotoxic compounds in rodents. As a group, these studies provide an outline of the nature and breadth of data that metabolomics could potentially provide to help inform pharmacologic research.

Schizophrenia is a debilitating psychiatric disease characterized by psychosis, negative symptoms and neurocognitive deficits (88). Theories of the pathophysiology of schizophrenia have centered on neurotransmitters and their receptors, and drugs used to treat this disease have largely targeted the dopamine, serotonin, and glutamate neurotransmitter systems (89–91). Although those drugs are effective, there are large individual variations in response to treatment and development of side effects (92–95). Phospholipids, compounds that play a critical role in the structure and function of membranes, seem to be impaired in schizophrenia (96). In addition, there has been growing concern with regard to the potential for antipsychotic drugs, especially clozapine and olanzapine, to cause adverse metabolic effects, such as weight gain, hyperglycemia, and hypertriglyceridemia (97). However, not all patients develop metabolic side effects, and mechanisms responsible for this individual variation are poorly understood. Furthermore, it is not known if these side effects are correlated with drug efficacy, and some antipsychotics, e.g., aripiprazole, have fewer of these side effects than do other drugs (98).

Metabolomics has recently been applied in an attempt to better define pathways modified by antipsychotic drugs. One study (23) used a specialized lipidomics platform that measures more than 300 polar and nonpolar lipid metabolites across 7 lipid classes to evaluate global lipid changes in schizophrenia after treatment with three commonly prescribed atypical antipsychotics, olanzapine, risperidone, and aripiprazole. Lipidomics is a branch of metabolomics that specifically focuses on a range of polar and nonpolar lipid metabolites, making a comprehensive assessment of lipid biochemistry possible (36, 99, 100). In this particular study, lipid profiles were obtained for 50 patients with schizophrenia before and after 2–3 weeks of treatment with olanzapine ($N = 20$), risperidone ($N = 14$), or aripiprazole ($N = 16$) (23). At baseline, and prior to drug treatment, major changes were noted in two phospholipid classes, phosphotidylethanolamine (PE) and phosphotidylcholine (PC) (23), suggesting that phospholipids that play a key role in proper membrane structure and function seem to be impaired in patients with schizophrenia. Detailed perturbations within

the omega 3 and omega 6 subclasses in PE and PC were also mapped as well as shifts between saturated and ploy unsaturated fatty acids (23). Effects of three antipsychotic drugs, olanzapine, risperidone, and aripiprazole, on lipid biochemical pathways were then evaluated by comparing metabolic profiles at baseline to post treatment (23; **Figures 6, 7,** and **8**).

It was of interest that each of the three drugs studied had a unique signature (23; **Figure 6**) suggesting that these drugs while they have few effects in similar they also have many effects that are unique for each. Phosphatidylethanolamine concentrations that were decreased at baseline in patients with schizophrenia were elevated after treatment with all three drugs. However, olanzapine and risperidone affected a much broader range of lipid classes than did aripiprazole, with approximately 50 lipids that were increased after exposure to these drugs, but not after aripiprazole therapy (23; **Figure 6**). On balance, aripiprazole induced minimal changes in the lipidome (**Figure 6**), consistent with its limited metabolic side effects.

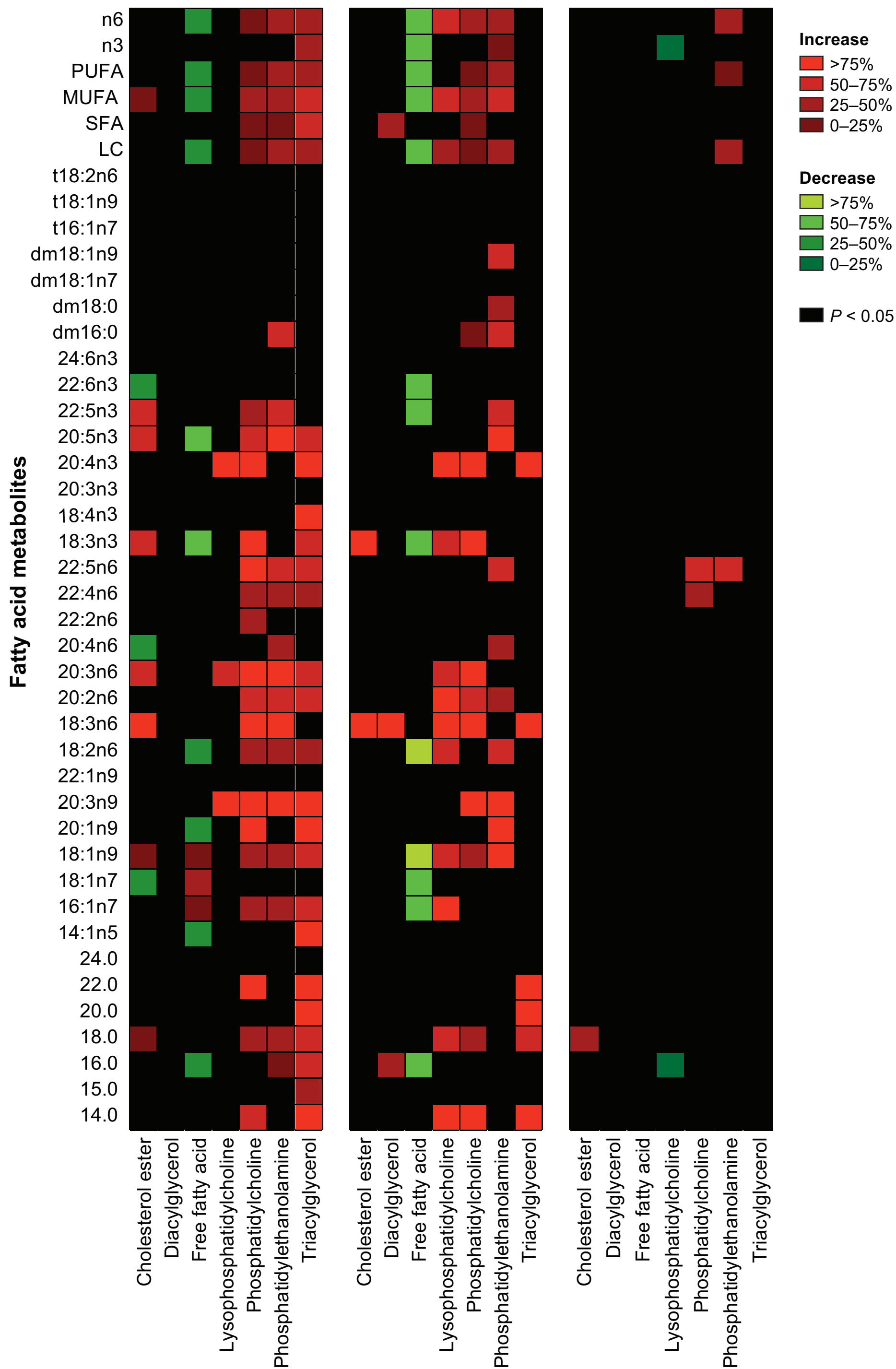
Figure 7 shows metabolites that were down regulated in patients with schizophrenia as compared to controls and the effects of the three drugs on reverting some of these baseline defects (for full analysis see 23). **Figure 8** shows key changes that were noted after treating with olanzapine (green represents down regulated and red represents upregulated) and compares which of these changes were also seen with the other drugs.

There were also increased concentrations of triacylglycerols and decreased free fatty acid concentrations after both olanzapine and risperidone, but not after aripiprazole therapy (23; **Figure 6**). All of these changes suggest peripheral effects that might be related to the metabolic side effects that have been reported for this class of drugs and highlights lipases in the liver as possibly targets for these drugs. Finally, a principal component analysis identified baseline lipid alterations that seemed to correlate with acute treatment response (**Figure 9**). These results raised the possibility that a more definitive long-term randomized study of these drugs in which global lipid changes would be correlated with clinical outcomes might yield biomarkers related to response and development of side effects. This study of atypical antipsychotic drugs illustrates the way in which metabolomics might contribute to our understanding of drug response phenotypes and how it provides tools to analyze pathways implicated in variation to response for this class of drugs. The next example involves the statins, a major class of drugs used to treat cardiovascular disease.

Figure 6

Heat map showing differences in individual lipid metabolites in the plasma of patients with schizophrenia posttreatment as compared with pretreatment with olanzapine (*top panel*), risperidone (*middle panel*), and aripiprazole (*bottom panel*). Fatty acid metabolites are shown as they appear in each distinct lipid class. The percent increase in any lipid upon treatment with drug is shown in red squares and decrease in green squares as described in Reference 23. The brightness of each color corresponds to the magnitude of the difference in quartiles. The brighter the square the larger the difference. Reprinted by permission from Macmillan Publishers Ltd, Mol. Psychiatry, Apr 17 [Epub ahead of print], copyright 2007.

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.



Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

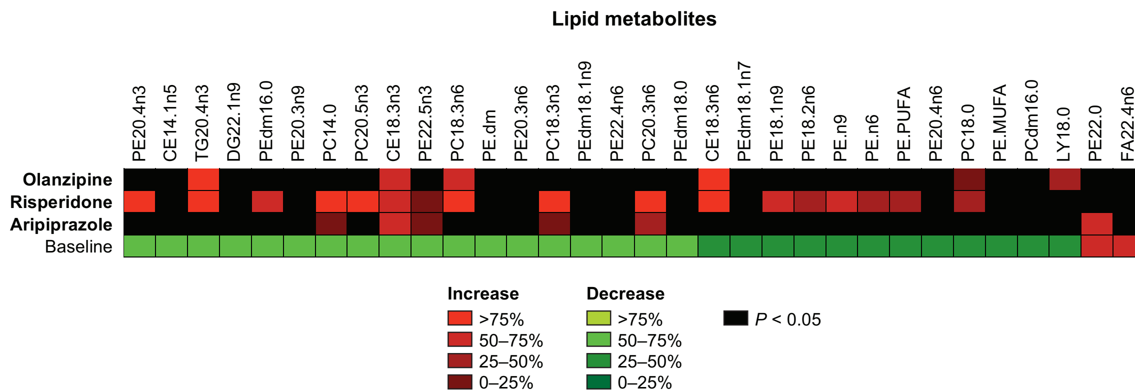


Figure 7

Lipidomic analysis of defects in patients with schizophrenia and effect of three antipsychotic drugs. The heat map shows the most significantly down regulated lipid metabolites in plasma of patients with schizophrenia as compared with controls and the effect of the three drugs in reversing some of these differences. Quantitative data (expressed in nanomole per milliliter sample) were used to calculate the percent increase (*red squares*) or decrease (*green squares*) in lipids in unmedicated patients with schizophrenia pretreatment as compared with control subjects (*baseline*). For full analysis of metabolites that are both up- and downregulated at baseline, see Reference 23. The significance of differences was analyzed by unpaired t-test. Similar quantitative data were used to calculate the percent increase (*red squares*) or decrease (*green squares*) of lipids in patients post treatment as compared with pretreatment (23). Significance of differences was analyzed by paired t-test. The four brightness levels correspond to percentage differences between the groups of 0%–25% (*darkest*), 25%–50% (*next brightest*), 50%–75% (*next brightest*), and >75% (*brightest*). Differences not meeting the $P < 0.05$ value are shown in black. Modified from Reference 23.

In the case of the statin study, a lipidomics platform and gene expression assays were used by Laaksonen et al. (101) to map the effects on muscle pathways of two HMG-CoA reductase inhibitors, atorvastatin, and simvastatin. Myopathy is a rare side effect of statins that appears especially when these drugs are used at high doses. This group of investigators observed that multiple skeletal muscle metabolic and signaling pathways, including proinflammatory pathways, seemed to be targets for high doses of simvastatin, but not atorvastatin. A parallel analysis of the effect of these drugs on blood lipid profiles was performed in an attempt to define biomarkers for statin-induced metabolic alterations in muscle that might make it possible to identify patients who should be treated with a lower dose of drug to prevent myopathy. Atorvastatin and simvastatin treatment resulted in specific plasma lipidome signatures, suggesting that lipidomic analysis might help to make it possible to select specific lipid-lowering agents for use by individual patients. Ongoing studies conducted by the NIH-Metabolomics Research Network for Drug Response Phenotype are defining global lipid changes in good and poor responders—based on changes in LDL levels—to simvastin treatment. Initial findings have demonstrated that far more lipid classes are changed in responders than in nonresponders (R. Kaddurah-Daouk, S. Watkins, M. Wiest, R. Baillie, R. Weinshilboum, and

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org by Karolinska Institute on 06/03/08. For personal use only.

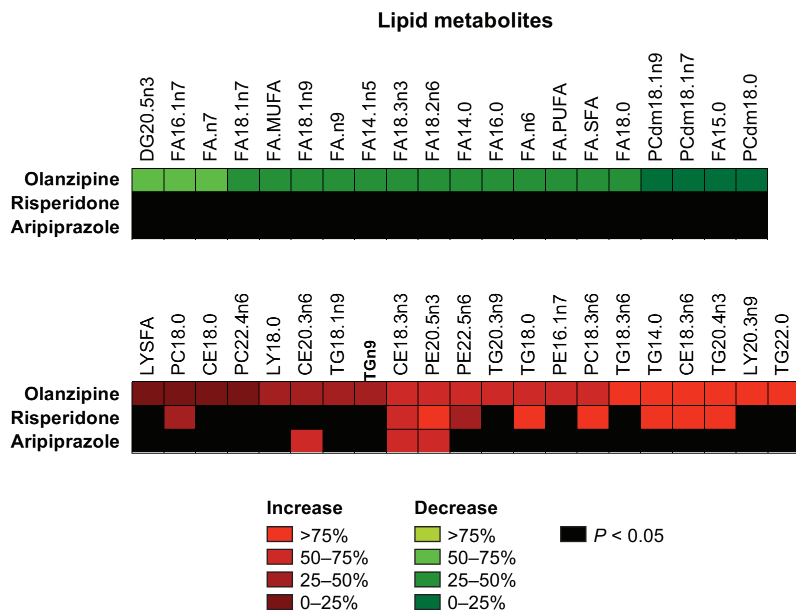


Figure 8

Lipidomic analysis of effect on olanzapine on patients with schizophrenia and a comparison to effects seen by other drugs. The heat maps show the most significantly modified lipid metabolites in plasma of patients treated with these drugs. The top panel shows metabolites down regulated by olanzapine, but not by the other two drugs. The bottom panel shows metabolites upregulated by olanzapine, but only a subset of those metabolites were also modified by the other two drugs. Quantitative data (expressed in nanomole per milliliter sample) were used to calculate the percent increase (*red squares*) or decrease (*green squares*) of metabolites in schizophrenic patients post treatment as compared with pretreatment. The significance of differences was analyzed by paired t-test. The four brightness levels correspond to percentage differences between the groups of 0%–25% (*darkest*), 25%–50% (*next brightest*), 50%–75% (*next brightest*), and >75% (*brightest*). Differences not meeting the $P < 0.05$ value are shown in black. Modified from Reference 23. Permission from Macmillan Publishers Ltd., *Mol. Psychiatry*, Apr 17 [epub ahead of print], copyright 2007.

R. Krauss, unpublished data). Metabolomics tools could define pathways implicated in statin drug response phenotypes, which includes both therapeutic benefit and side effects.

In a third example involving human subjects, van Doorn et al. (37) applied ^{1}H-NMR spectroscopy to profile blood plasma and urine samples from patients with type 2 diabetes before and after treatment with rosiglitazone, a drug that activates PPARγ nuclear receptors. Metabolic profiles were compared with those of healthy volunteers. Rosiglitazone treatment led to reductions in urinary hippurate and aromatic amino acid concentrations; increases in plasma branched-chain amino acid, alanine, glutamine, and glutamate concentrations; and significant changes in plasma lipids in diabetic patients. No drug effects were noted in the healthy control subjects. This study demonstrated, once again, the potential of metabolomics to define

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org by Karolinska Institute on 06/03/08. For personal use only.

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

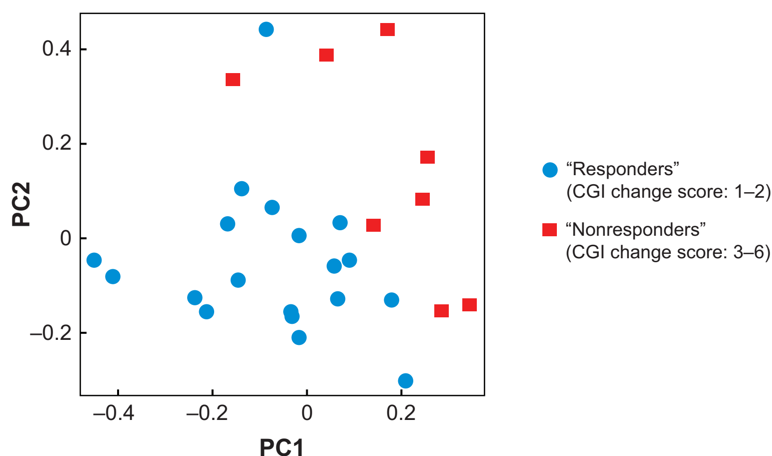


Figure 9

Lipidomic analysis of data for schizophrenic patients treated with atypical antipsychotic drugs. Principle component analysis for clinical global impressions (CGI) scale changes in schizophrenic patients after treatment. Logistic regression was used to identify pretreatment lipid metabolites that were related to response (responders, those who had a CGI change scores of 1–2; nonresponders, those who had scores of 3–6). Principle component analysis was then applied to these metabolites, and separation of the groups was visualized with a scatter plot of the first (PC1) versus the second (PC2) principal component. Red squares are subjects who responded to drug treatment with a CGI change score of 1–2. Blue circles are subjects who do not respond to drug treatment and who had a CGI change score of 3–6. Reprinted by permission from Macmillan Publishers Ltd, Mol. Psychiatry. Apr 17 [Epub ahead of print], copyright 2007.

global biochemical effects of drugs, information that—especially when wedded to genomic, transcriptomic and proteomic data—might help understand drug mechanisms. In a related study performed with mice, Watkins et al. (36) used a targeted lipidomics platform to study the effects of rosiglitazone in a genetic mouse model of diabetes in which the antidiabetic action of this drug was accompanied by excessive weight gain. They observed significant tissue-specific effects of drug treatment on lipid metabolism. A cross-species comparison in which metabolic signatures of drugs in animal models, such as this genetic mouse model, and in humans could provide information with regard to the potential relevance of specific animal models in drug discovery, as well as additional insight into the mechanism(s) of drug action.

Another example of the application of metabolomics to study drug mechanisms in experimental animals is the work of Portilla et al. (39). Those investigators used ^{1}H-NMR spectroscopy to study the response of mice to a single injection of the antineoplastic agent cisplatin. Nephrotoxicity is a side effect when cisplatin is used in the clinic. They observed marked changes in the urinary metabolic profile after drug exposure that preceded changes in common biomarkers of nephrotoxicity, such as blood urea nitrogen or serum creatinine. PCA demonstrated the presence of glucose, amino acids, and trichloroacetic acid cycle metabolites in the urine 48 h after cisplatin administration. These metabolic alterations preceded changes in serum

creatinine levels. Biochemical studies confirmed the presence of glucosuria, but also demonstrated the accumulation of nonesterified fatty acids and triglycerides in serum, urine, and kidney tissue, in spite of increased levels of plasma insulin. Most of these metabolic alterations were ameliorated by the administration of a PPARα ligand. Although it remains unclear as to which of these metabolic changes, if any, might be related to nephrotoxicity, this work may represent a step toward defining predictive biomarkers for this adverse drug reaction.

The final example involves the use of experimental animals to perform a study of drug-induced hepatotoxicity. Hepatotoxicity is a common and potentially serious adverse response to drug exposure. For example, acetaminophen (paracetamol) can cause potentially life-threatening drug-induced hepatotoxicity (102, 103). In a recent metabolomic study, male Sprague-Dawley rats were treated with three hepatotoxins, galactosamine, allyl alcohol, and acetaminophen, and both pre- and postdrug exposure urine samples were subjected to NMR analysis (38). A model was then developed that used predrug metabolomic data to predict both acetaminophen glucuronide conjugate to parent drug ratio and postacetaminophen hepatotoxicity (class 1, no or minimal hepatic necrosis, to class 3, moderate necrosis). The major predrug compounds in the urine that were associated with postacetaminophen hepatotoxicity were taurine, trimethylamine-N-oxide (TMAO), and betaine. A higher predrug urinary taurine level was associated with more class 1 than class 3 hepatic histology, whereas higher combined predrug concentrations of TMAO and betaine were associated with more class 3 than class 1 histology (38).

These examples of pharmacometabolomics, studies conducted with both humans and experimental animals, studies that used several different analytical platforms, all serve to demonstrate the potential of metabolomics to enhance our understanding of drug mechanisms or adverse drug reactions. Metabolomics also could, when united with other high-throughput, data-intense techniques (**Figure 1**), help us move toward the goal of individualized drug therapy.

METABOLOMICS AND INDIVIDUALIZED DRUG THERAPY

Metabolomics, as illustrated in the preceding paragraphs, has the potential to contribute significantly to our understanding of mechanisms of drug action. However, it also provides comprehensive and accurate biochemical phenotypes for drug response well beyond those previously available, so an additional application of metabolomics in pharmacology would involve individualized drug therapy (104–109). The range of types of data available to help us move toward individualized approaches that will make it possible to understand and predict variation in drug response is depicted schematically in **Figure 1**. That figure shows the genome at one end of the spectrum, with the metabolome at the other, followed by clinical response. Our ability to systematically query the human genome has grown exponentially—culminating in a recent series of successful genome-wide association studies in which over 500,000 single nucleotide polymorphisms (SNPs) were assayed across the genome in every DNA sample studied—using DNA from thousands of individual subjects in each study (110–112). Genomics and metabolomics can identify genes and metabolites,

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

respectively, that might lie in pathways outside of our current knowledge of drug pharmacokinetics and pharmacodynamics. The union of genome-wide genotyping techniques with metabolome-wide data that can provide sophisticated biochemical phenotypes based on the assay of thousands of small molecules opens the way for major advances in our ability to define biological mechanisms responsible for differences among patients in risk for the occurrence of variation in drug efficacy, as well as adverse drug reactions.

CONCLUSIONS

Metabolomics, the study of the complete repertoire of small molecules in cells, tissues, organs, and biological fluids, represents a major and rapidly evolving component of the new biology. The development of a series of analytical platforms, NMR, GC-MS, LC-MS, and LCECA, all capable of accurately measuring hundreds or thousands of small molecules in biological samples, promises to substantially advance our understanding of disease pathophysiology and to make it possible to discover biomarkers for disease risk. However, few areas of biomedical research stand to benefit more from the application of metabolomics than do pharmacology and toxicology. There can be little doubt that the addition of pharmacometabolomic analyses to genomic, transcriptomic and proteomic assays will greatly enhance our understanding of mechanisms of drug effect, of adverse drug reactions, and of the biology underlying individual variation in drug response phenotypes.

DISCLOSURE STATEMENT

Drs. Kaddurah-Daouk and Kristal are equity holders in Metabolon Inc., a biotechnology company in the metabolomics domain, and they also hold IP interest in this field.

ACKNOWLEDGMENTS

We thank Luanne Wussow for her assistance with the preparation of this manuscript. Supported in part by National Institutes of Health grants R24 GM078233, "The Metabolomics Research Network" (R.K.D., B.S.K., R.M.W.); SMRI (R.K.-D.), NARSAD (R.K.-D.), U01 GM61388, "The Pharmacogenetics Research Network" (R.M.W.); R01 GM28157 (R.M.W), R01 CA102536, R01 AG28996, R01 AG25872 (B.S.K); and a PhRMA Foundation "Center of Excellence in Clinical Pharmacology" Award (R.M.W.).

LITERATURE CITED

1. Weinshilboum RM. 1987. The therapeutic revolution. *Clin. Pharmacol. Ther.* 42:481–84
2. Weinshilboum RM, Wang L. 2006. Pharmacogenetics and pharmacogenomics: development, science, and translation. *Annu. Rev. Genomics Hum. Genet.* 7:223–45

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org by Karolinska Institute on 06/03/08. For personal use only.

3. Harrigan G, Goodacre R. 2003. *Metabolic Profiling: Its Role in Biomarker Discovery and Gene Function Analysis*. Boston: Kluwer Acad. Publ.
4. Van Der Greef J, Verheij ER, Vogels J, Van Der Heijden R, Davidov E, Naylor S. 2003. The role of metabolomics in systems biology, a new vision for drug discovery and development. See Ref. 3, pp. 170–98
5. Lindon JC, Nicholson JK, Holmes E, Everett JR. 2000. Metabonomics: metabolic processes studied by NMR spectroscopy of biofluids. *Concepts Magn. Reson.* 12:289–320
6. Dettmer K, Hammock BD. 2004. Metabolomics—a new exciting field within the "omics" sciences. *Environ. Health Perspect.* 112:A396–97
7. Schmidt CW. 2004. Metabolomics: what's happening downstream of DNA. *Environ. Health Perspect.* 112:A410–15
8. Schmidt C. 2004. Metabolomics takes its place as latest up-and-coming "omic" science. *J. Natl. Cancer. Inst.* 96:732–34
9. Lindon J, Holmes E, Bollard M, Stanley E, Nicholson J. 2004. Metabonomics technologies and their applications in physiological monitoring, drug safety assessment and disease diagnosis. *Biomarkers* 9:1–31
10. Vaidyanathan S, Harrigan GG, Goodacre R. 2005. *Metabolome Analysis, Strategies for Systems Biology*. New York: Springer
11. Mendes P. 2006. Metabolomics and the challenges ahead. *Brief. Bioinform.* 7:127
12. Maddox JF, Luyendyk JP, Cosma GN, Breau AP, Bible RH, et al. 2006. Metabonomic evaluation of idiosyncrasy-like liver injury in rats cotreated with ranitidine and lipopolysaccharide. *Toxicol. Appl. Pharmacol.* 212:35–44
13. Van Der Greef J, Martin S, Juhasz P, Adourian A, Plasterer T, et al. 2007. The art and practice of systems biology in medicine: mapping patterns of relationships. *J. Proteome Res.* 6:1540–59
14. Villas-Boas SG, Roessner U, Hansen MAE, Smedsgaard J, Nielsen J. 2007. *Metabolome Analysis, An Introduction*. Hoboken, NJ: Wiley
15. Weckwerth W. 2007. *Metabolomics: Methods and Procedures*. Totowa, NJ: Humana Press
16. Kristal BS, Kaddurah-Daouk R, Beal MF, Matson WR. 2007. Metabolomics: concept and potential neuroscience application. In *Handbook of Neurochemistry and Molecular Neurobiology: Brain Energetics. Integration of Molecular and Cellular Processes*, pp. 889–912. New York: Springer
17. Kristal BS, Shurubor YI, Kaddurah-Daouk R, Matson WR. 2007. High-performance liquid chromatography separations coupled with coulometric electrode array detectors: a unique approach to metabolomics. *Methods Mol. Biol.* 358:159–74
18. Kell DB. 2004. Metabolomics and systems biology: making sense of the soup. *Curr. Opin. Micro.* 7:296–307
19. Rozen S, Cudkowicz ME, Bogdanov M, Matson WR, Kristal BS, et al. 2005. Metabolomic analysis and signatures in motor neuron disease. *Metabolomics* 1:101–8
20. Paige LA, Mitchell MW, Krishnan KR, Kaddurah-Daouk R, Steffens DC. 2006. A preliminary metabolomic analysis of older adults with and without depression. *Int. J. Geriatr. Psychiatry* 22:418–23

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

21. Holmes E, Tsang TM, Huang JT, Leweke FM, Koethe D, et al. 2006. Metabolic profiling of CSF: evidence that early intervention may impact on disease progression and outcome in schizophrenia. *PLoS Med.* 3:e327
22. Kaddurah-Daouk R. 2006. Metabolic profiling of patients with schizophrenia. *PLoS Med.* 3:e363
23. Kaddurah-Daouk R, McEvoy J, Baillie RA, Lee D, Yao JK, et al. 2007. Metabolomic mapping of atypical antipsychotic effects in schizophrenia. *Mol. Psychiatry.* Apr 17 [EPub ahead of print]
24. Han XM, Holtzman D, McKeel DW, Kelley J, Morris JC. 2002. Substantial sulfatide deficiency and ceramide elevation in very early Alzheimer's disease: potential role in disease pathogenesis. *J. Neurochem.* 82:809–18
25. Sabatine MS, Liu E, Morrow DA, Heller E, McCarroll R, et al. 2005. Metabolomic identification of novel biomarkers of myocardial ischemia. *Circulation* 112(25):3868–75
26. Brindle JT, Antti H, Holmes E, Tranter G, Nicholson JK, et al. 2002. Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using 1H-NMR-based metabonomics. *Nat. Med.* 12:1439–44
27. Brindle J, Nicholson J, Schofield P, Grainger D, Holmes E. 2003. Application of chemometrics to 1H NMR spectroscopic data to investigate a relationship between human serum metabolic profiles and hypertension. *Analyst* 128:32–36
28. Dunne VG, Bhattachayya S, Besser M, Rae C, Griffin JL. 2005. Metabolites from cerebrospinal fluid in aneurysmal subarachnoid haemorrhage correlate with vasospasm and clinical outcome: a pattern-recognition 1H NMR study. *NMR Biomed.* 18:24–33
29. Kenny LC, Dunn WB, Ellis DI, Myers J, Baker PN, et al. 2005. Novel biomarkers for pre-eclampsia detected using metabolomics and machine learning. *Metabolomics* 1:277–34
30. Wang C, Kong H, Guan Y, Yang J, Gu J, et al. 2005. Plasma phospholipid metabolic profiling and biomarkers of type 2 diabetes mellitus based on high-performance liquid chromatography/electrospray mass spectrometry and multivariate statistical analysis. *Anal. Chem.* 77:4108–16
31. Yuan K, Kong H, Guan Y, Yang J, Xu G. 2007. A GC-based metabonomics investigation of type 2 diabetes by organic acids metabolic profile. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* 850:236–40
32. Yang J, Xu G, Zheng Y, Kong H, Pang T, et al. 2004. Diagnosis of liver cancer using HPLC-based metabonomics avoiding false-positive result from hepatitis and hepatocirrhosis diseases. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* 813:59–65
33. Odunsi K, Wollman RM, Ambrosone CB, Hutson A, McCann SE, et al. 2005. Detection of epithelial ovarian cancer using 1H-NMR-based metabonomics. *Int. J. Cancer.* 113:782–88
34. Fan X, Bai J, Shen P. 2005. Diagnosis of breast cancer using HPLC metabonomics fingerprints coupled with computational methods. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 6:6081–84

35. Underwood B, Broadhurst D, Dunn WB, Ellis DI, Michell AW, et al. 2006. Huntington disease patients and transgenic mice have similar procatabolic serum metabolite profiles. *Brain* 129:877–86
36. Watkins SM, Reifsnyder PR, Pan HJ, German JB, Leiter EH. 2002. Lipid metabolome-wide effects of the PPARgamma agonist rosiglitazone. *J. Lipid. Res.* 43:1809–11
37. van Doorn M, Vogels J, Tas A, van Hoogdalem EJ, Burggraaf J, et al. 2007. Evaluation of metabolite profiles as biomarkers for the pharmacological effects of thiazolidinediones in Type 2 diabetes mellitus patients and healthy volunteers. *Br. J. Clin. Pharmacol.* 63(5):562–74
38. Clayton TA, Lindon JC, Cloarec O, Antti H, Charuel C, et al. 2006. Pharmaco-metabonomic phenotyping and personalized drug treatment. *Nature* 440:1073–77
39. Portilla D, Li S, Nagothu KK, Megyesi J, Kaissling B, et al. 2006. Metabolomic study of cisplatin-induced nephrotoxicity. *Kidney Int.* 69:2194–204
40. Morvan D, Demidem A. 2007. Metabolomics by proton nuclear magnetic resonance spectroscopy of the response to chloroethylnitrosourea reveals drug efficacy and tumor adaptive metabolic pathways. *Cancer. Res.* 67:2150–59
41. Beal MF, Matson WR, Swartz KJ, Gamache PH, Bird ED. 1990. Kynurenine pathway measurements in Huntington's disease striatum: evidence for reduced formation of kynurenic acid. *J. Neurochem.* 55:1327–229
42. Ogawa T, Matson WR, Beal MF, Myers RH, Bird ED, et al. 1992. Kynurenine pathway abnormalities in Parkinson's disease. *Neurology.* 42:1702–6
43. German JB, Gillies LA, Smilowitz JT, Zivkovic AM, Watkins SM. 2007. Lipidomics and lipid profiling in metabolomics. *Curr. Opin. Lipidol.* 18:66–71
44. Lindon JC, Nicholson JK, Holmes E, Antti H, Bollard ME, et al. 2003. Contemporary issues in toxicology—the role of metabonomics in toxicology and its evaluation by the COMET project. *Toxicol. Appl. Pharmacol.* 187:137–46
45. Lindon JC, Keun HC, Ebbels TM, Pearce JM, Holmes E, Nicholson JK. 2005. The Consortium for Metabonomic Toxicology (COMET): aims, activities and achievements. *Pharmacogenomics* 6:691–99
46. Carlson EE, Cravatt BF. 2007. Chemoselective probes for metabolite enrichment and profiling. *Nat. Methods* 4:429–35
47. de Graaf AA, Mahle M, Möllney M, Wiechert W, Stahmann PHS. 2000. Determination of full 13C isotopomer distributions for metabolic flux analysis using heteronuclear spin echo difference NMR spectroscopy. *J. Biotechnol.* 77:25035
48. Lu D, Mulder H, Zhao P, Burgess SC, Jensen MV, et al. 2002. 13C NMR isotopomer analysis reveals a connection between pyruvate cycling and glucose-stimulated insulin secretion (GSIS). *Proc. Natl. Acad. Sci. USA* 99:2708–13
49. Fan TW-M, Lane AN. 2007. Structure-based profiling of metabolites and isotopomers by NMR. *Prog. NMR Spectrosc.* In press
50. Dauner M, Bailey JE, Sauer U. 2001. Metabolic flux analysis with a comprehensive isotopomer model in *Bacillus subtilis*. *Biotechnol. Bioeng.* 76:1440156
51. Forbes NS, Meadows AL, Clark DS, Blanch HW. 2006. Estradiol stimulates the biosynthetic pathways of breast cancer cells: detection by metabolic flux analysis. *Metab. Eng.* 8:639–52

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

52. Fiehn O. 2002. Metabolomics—the link between genotypes and phenotypes. *Plant. Mol. Biol.* 48:155–71
53. Taylor J, King RD, Altmann T, Fiehn O. 2002. Application of metabolomics to plant genotype discrimination using statistics and machine learning. *Bioinformatics.* 18(Suppl. 2):S241–48
54. Fiehn O. 2003. Metabolic networks of *Cucurbita maxima* phloem. *Phytochemistry* 62:875–96
55. Tolstikov VV, Lommen A, Nakanishi K, Tanaka N, Fiehn O. 2003. Monolithic silica-based capillary reversed-phase liquid chromatography/electrospray mass spectrometry for plant metabolomics. *Anal. Chem.* 75:6737–40
56. Shellie RA, Welthagen W, Zrostlikova J, Spranger J, Ristow M, et al. 2005. Statistical methods for comparing comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry results: metabolomic analysis of mouse tissue extracts. *J. Chromatogr. A* 1086:83–90
57. Kind T, Fiehn O. 2007. Seven golden rules for heuristic filtering of molecular formulas obtained by accurate mass spectrometry. *BMC Bioinformatics* 278:105
58. Shi H, Vigneau-Callahan KE, Matson WR, Kristal BS. 2002. Attention to relative response across sequential electrodes improves quantitation of coulometric array. *Anal. Biochem.* 302:239–45
59. Shi H, Paolucci U, Vigneau-Callahan KE, Milbury PE, Matson WR, Kristal BS. 2004. Development of biomarkers based on diet-dependent metabolic serotypes: practical issues in development of expert system-based classification models in metabolomic studies. *OMICS* 8:197–208
60. Paolucci U, Vigneau-Callahan KE, Shi H, Matson WR, Kristal BS. 2004. Development of biomarkers based on diet-dependent metabolic serotypes: concerns and approaches for cohort and gender issues in serum metabolome studies. *OMICS* 8:209–20
61. Paolucci U, Vigneau-Callahan KE, Shi H, Matson WR, Kristal BS. 2004. Development of biomarkers based on diet-dependent metabolic serotypes: characteristics of component-based models of metabolic serotypes. *OMICS* 8:221–38
62. Beal MF, Matson WR, Storey E, Milbury P, Ryan EA, et al. 1992. Kynurenic acid concentrations are reduced in Huntington's disease cerebral cortex. *J. Neurol. Sci.* 108:80–87
63. Volicer L, Langlais PJ, Matson WR, Mark KA, Gamache PH. 1985. Serotoninergic system in dementia of the Alzheimer type. Abnormal forms of 5-hydroxytryptophan and serotonin in cerebrospinal fluid. *Arch. Neurol.* 42:1158–61
64. Godefroy F, Matson WR, Gamache PH, Weil-Fugazza J. 1990. Simultaneous measurements of tryptophan and its metabolites, kynurenine and serotonin, in the superficial layers of the spinal dorsal horn. A study in normal and arthritic rats. *Brain. Res.* 526:169–72
65. Loeffler DA, LeWitt PA, Juneau PL, Camp DM, DeMaggio AJ, et al. 1998. Influence of repeated levodopa administration on rabbit striatal serotonin metabolism, and comparison between striatal and CSF alterations. *Neurochem. Res.* 23:1521–25

66. Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B* 57:289–300
67. Benjamini Y, Hochberg Y. 2000. The adaptive control of the false discovery rate in multiple comparison problems. *J. Educ. Behav. Stat.* 25:60–83
68. Benjamini Y, Yekutielim D. 2001. The control of the false discovery rate in multiple testing under dependency. *Ann. Stat.* 29:1165–88
69. Cloarec O, Dumas ME, Trygg J, Craig A, Barton RH, et al. 2005. Evaluation of the orthogonal projection on latent structure model limitations caused by chemical shift variability and improved visualization of biomarker changes in 1H NMR spectroscopic metabonomic studies. *Anal. Chem.* 77:517–26
70. Eriksson L, Johansson E, Kettaneh-Wold N, Trygg J, Wikström C, Wold S. 2006. *Multi- and Megavariate Data Analysis Part I: Basic Principles and Applications*. Umea, Sweden: Umetrics. 307 pp.
71. Eriksson L, Johansson E, Kettaneh-Wold N, Trygg J, Wikström C, Wold S. 2006. *Multi- and Megavariate Data Analysis Part II: Advanced Applications and Method Extensions.* Umea, Sweden: Umetrics. 425 pp.
72. Churchill GA. 2004. Using ANOVA to analyze microarray data. *Biotechniques* 37:173–75
73. Musumarra G, Barresi V, Condorelli DF, Scire S. 2003. A bioinformatic approach to the identification of candidate genes for the development of new cancer diagnostics. *Biol. Chem.* 384:321–27
74. Raamsdonk LM, Teusink B, Broadhurst D, Zhang N, Hayes A, et al. 2001. A functional genomics strategy that uses metabolome data to reveal the phenotype of silent mutations. *Nat. Biotechnol.* 19:45–50
75. Mendes P. 2002. Emerging bioinformatics for the metabolome. *Brief Bioinform.* 3:134–45
76. Sumner LW, Mendes P, Dixon RA. 2003. Plant metabolomics: large-scale phytochemistry in the functional genomics era. *Phytochemistry* 62:817–36
77. Eisen MB, Spellman PT, Brown PO, Botstein D. 1998. Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci. USA* 95:14863–68
78. Alter O, Brown PO, Botstein D. 2000. Singular value decomposition for genome-wide expression data processing and modeling. *Proc. Natl. Acad. Sci. USA* 97:10101–6
79. Tamayo P, Slonim D, Mesirov J, Zhu Q, Kitareewan S, et al. 1999. Interpreting patterns of gene expression with self-organizing maps: methods and application to hematopoietic differentiation. *Proc. Natl. Acad. Sci. USA* 96:2907–12
80. Manly BFJ. 1994. *Multivariate Statistical Methods. A Primer.* Boca Raton, FL: Chapman & Hall/CRC
81. Beebe KR, Pell RJ, Seasholtz MB. 1998. *Chemometrics: A Practical Guide*. New York: Wiley Intersci.
82. Coen M, Ruepp SU, Lindon JC, Nicholson JK, Pognan F, et al. 2004. Integrated application of transcriptomics and metabonomics yields new insight into the toxicity due to paracetamol in the mouse. *J. Pharm. Biomed. Anal.* 35:93–105
83. Griffin JL, Bollard ME. 2004. Metabonomics: its potential as a tool in toxicology for safety assessment and data integration. *Curr. Drug. Metab.* 5:389–98

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

84. Lindon JC, Holmes E, Nicholson JK. 2004. Metabonomics and its role in drug development and disease diagnosis. *Expert. Rev. Mol. Diagn.* 4:189–99
85. Robertson DG. 2005. Metabonomics in toxicology: a review. *Toxicol. Sci.* 85:809–22
86. Kell DB. 2006. Systems biology, metabolic modelling and metabolomics in drug discovery and development. *Drug. Discov. Today* 11:1085–92
87. Serkova N, Boros LG. 2005. Detection of resistance to imatinib by metabolic profiling: clinical and drug development implications. *Am. J. Pharmacogenomics* 5(5):293–302
88. Jablensky A, Sartorius N, Ernberg G, Anker M, Korten A, et al. 1992. Schizophrenia: manifestations, incidence and course in different cultures. A World Health Organization ten-country study. *Psychol. Med. Monogr. Suppl.* 20:1097
89. Meltzer HY. 1987. Biological studies in schizophrenia. *Schizophr. Bull.* 13:77–111
90. Javitt DC, Laruelle M. 2006. Neurochemical theories. In *Textbook of Schizophrenia*, ed. JA Lieberman, TS Stroup, DO Perkins, pp. 85–116. Washington, DC: Am. Psychiatr. Publ.
91. Scolnick EM. 2006. Mechanisms of action of medicines for schizophrenia and bipolar illness: status and limitations. *Biol. Psychiatry* 59:1039–45
92. Strauss JS, Carpenter WT. 1977. Prediction of outcome in schizophrenia. III. Five-year outcome and its predictors. *Arch. Gen. Psychiatry* 34:159–63
93. Kane JM, Marder SR. 1993. Psychopharmacologic treatment of schizophrenia. *Schizophr. Bull.* 18:287–302
94. Carpenter WT, Buchanan RW. 1994. Schizophrenia. *N. Engl. J. Med.* 330:681–90
95. Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, et al. 2005. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. *N. Engl. J. Med.* 353:1209–23
96. Horrobin DF. 1998. The membrane phospholipid hypothesis as a biochemical basis for the neurodevelopmental concept of schizophrenia. *Schizophr. Res.* 30:193–208
97. Am. Diabetes Assoc., Am. Psychiatr. Assoc., Am. Assoc. Clin. Endocrinol., N. Am. Assoc. Study Obes. 2004. Consensus Development Conference on Antipsychotic Drugs and Obesity and Diabetes. *J. Clin. Psychiatr.* 65:267–72
98. McQuade RD, Stock E, Marcus R, Jody D, Gharbia NA, et al. 2004. A comparison of weight change during treatment with olanzapine or aripiprazole: results from a randomized, double-blind study. *J. Clin. Psychiatr.* 65(Suppl. 18):47–56
99. Watkins SM. 2004. Lipomic profiling in drug discovery, development and clinical trial evaluation. *Curr. Opin. Drug. Discov. Devel.* 7:112–17
100. Watson AD. 2006. Thematic review series: systems biology approaches to metabolic and cardiovascular disorders. Lipidomics: a global approach to lipid analysis in biological systems. *J. Lipid. Res.* 47:2101–11
101. Laaksonen R, Katajamaa M, Päivä H, Sysi-Aho M, Saarinen L, et al. 2006. A systems biology strategy reveals biological pathways and plasma biomarker

Annu. Rev. Pharmacol. Toxicol. 2008.48:653-683. Downloaded from arjournals.annualreviews.org
by Karolinska Institute on 06/03/08. For personal use only.

candidates for potentially toxic statin-induced changes in muscle. *PLoS ONE* 1:e97

102. Fontana RJ, Quallich LG. 2001. Acute liver failure. *Curr. Opin. Gastroenterol* 17:291–98
103. Watkins PB, Kaplowitz N, Slattery JT, Colonese CR, Colucci SV, et al. 2006. Aminotransferase elevations in healthy adults receiving 4 grams of acetaminophen daily: a randomized controlled trial. *JAMA* 296:87–93
104. German JB, Bauman DE, Burrin DG, Failla ML, Freake HC, et al. 2004. Metabolomics in the opening decade of the 21st century: building the roads to individualized health. *J. Nutr.* 134:2729–32
105. Bren L. 2005. Metabolomics: working toward personalized medicine. *FDA Consum.* 39:28–33
106. Griffin JL, Nicholls AW. 2006. Metabolomics as a functional genomic tool for understanding lipid dysfunction in diabetes, obesity and related disorders. *Pharmacogenomics* 7:1095–107
107. Van Der Greef J, Hankemeier T, McBurney RN. 2006. Metabolomics-based systems biology and personalized medicine: moving towards n = 1 clinical trials? *Pharmacogenomics* 7:1087–94
108. Schnackenberg LK. 2006. Metabolomics special focus: an introduction. *Pharmacogenomics* 7:1053–54
109. Nebert DW, Vesell ES. 2006. Can personalized drug therapy be achieved? A closer look at pharmaco-metabonomics. *Trends Pharmacol. Sci.* 27:580–86
110. Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, Novartis Institutes of BioMedical Research, Saxena R, Voight BF, et al. 2007. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 316:1331–36
111. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, et al. 2007. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science* 316:1336–41
112. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, et al. 2007. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 316:1341–45