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Review

Metabolomics tools for identifying biomarkers for neuropsychiatric diseases

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ABSTRACT

The repertoire of biochemicals (or small molecules) present in cells, tissue, and body fluids is known as the metabolome. Today, clinicians utilize only a very small part of the information contained in the metabolome, as revealed by the quantification of a limited set of analytes to gain information on human health. Examples include measuring glucose or cholesterol to monitor diabetes and cardiovascular health, respectively. With a focus on comprehensively studying the metabolome, the rapidly growing field of metabolomics captures the metabolic state of organisms at the global or "-omics" level. Given that the overall health status of an individual is captured by his or her metabolic state, which is a reflection of what has been encoded by the genome and modified by environmental factors, metabolomics has the potential to have a great impact upon medical practice by providing a wealth of relevant biochemical data. Metabolomics promises to improve current, single metabolites-based clinical assessments by identifying metabolic signatures (biomarkers) that embody global biochemical changes in disease, predict responses to treatment or medication side effects (pharmachometabolomics). State of the art metabolomic analytical platforms and informatics tools are being used to map potential biomarkers for a multitude of disorders including those of the central nervous system (CNS). Indeed, CNS disorders are linked to disturbances in metabolic pathways related to neurotransmitter systems (dopamine, serotonin, GABA and glutamate); fatty acids such as arachidonic acid-cascade; oxidative stress and mitochondrial function. Metabolomics tools are enabling us to map in greater detail perturbations in many biochemical pathways and links among these pathways this information is key for development of biomarkers that are disease-specific. In this review, we elaborate on some of the concepts and technologies used in metabolomics and its promise for biomarker discovery. We also highlight early findings from metabolomic studies in CNS disorders such as schizophrenia, Major Depressive Disorder (MDD), Bipolar Disorder (BD), Amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD).

Contents

Abbreviations: MDD, Major Depressive Disorder; CSF, Cerebrospinal fluid; PCA, Principal components analysis; NMR, 1H Nuclear magnetic resonance spectroscopy; LC, Liquid chromatography; GC, Gas chromatography; MS, Mass spectrometry; LC–MS, Liquid chromatography together with mass spectroscopy; LC-CA, LC-electrochemistry array metabolomics platforms; NT, neurotransmitter pathways; PLS, Partial least squares; DFA, Discriminant function analysis; PE, Phosphotidylethanolamine; PC, Phosphotidylcholine; ALS, Amyotrophic lateral sclerosis; HD, Huntington's disease; PD, Parkinson's disease.

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Introduction

Currently the diagnostic and follow up of most psychiatric, neurological and neuropsychiatric disorders, collectively central nervous system (CNS) disorders, is based on the identification of cluster of symptoms and scales. For many of these conditions, it remains impossible to identify individuals at risk or easily make an accurate diagnosis. This is in part due to the fact that the etiopathogenesis of many illnesses affecting the CNS remains unclear. CNS disorders are likely to arise from the dynamic dysregulation of several gene regulatory networks, proteins, and metabolic alterations, reflecting complex perturbations (genetic and environmental) of the "system". Thus, there is growing need to scale up knowledge in the study of CNS diseases in an attempt to understand at a system level the totality of changes that can contribute to the pathogenesis of these disorders. Disease specific molecular fingerprint can be defined by integrating the use of high-throughput methods at the core of genomics (aiming to measure gene expression), proteomics (assaying proteins), metabolomics (focused on the quantification of small molecules) and other "omics" data. This knowledge could help to map dysregulated networks implicated in disease pathogenesis. Furthermore, global mapping of abnormal pathways in CNS disorders, can lead to the identification of disease biomarkers and biomarkers of response.

A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal or pathogenic processes as well as responses to therapeutic interventions (Biomarkers Definitions Working Group, 2001; Vasan, 2006). The discovery of biomarkers for psychiatric, neurological and neuropsychiatric disorders and their incorporation into clinical decision-making could dramatically change the future delivery of health care. These biomarkers should be more reliable, have more precise predictive ability than current methods, and/or could be more informative about disease pathogenesis (Biomarkers Definitions Working Group, 2001; Vasan, 2006). The path for the discovery of biomarkers is now characterized by our ability to move from the study of single genes, mRNA transcripts, proteins, or metabolites, to a more global approach. Indeed, there has been a gradual transition from research solely based on a reductionistic, hypothesis-driven approach, to a more holistic (systems view), discovery-driven approach research (Ahn et al., 2006a,b; Kell, 2006; Nicholson, 2006; van der Greef et al., 2004, 2007; Kaddurah-Daouk et al., 2008; Kaddurah-Daouk et al., 2009). This systems approach may allow the discovery of panels of biomarkers that capture more accurately a disease state and provide information that is valuable for a more individualized clinical care (Nicholson, 2006; van der Greef et al., 2006, Kaddurah-Daouk et al., 2008).

The study of metabolism at the global or "-omics" level is a new but rapidly growing field that has the potential to impact medical practice (Schmidt, 2004; Harrigan, 2002; van der Greef et al., 2003, 2004, 2007; Kaddurah-Daouk et al., 2008, 2009; Kristal et al., 2007a,b; Lindon et al., 2004; Holmes et al., 2008a). Metabolomics (also referred to as metabonomics, metabolic profiling, metabolic fingerprinting, among other terms) focuses on the identification and quantification of small molecules, or metabolites in cells, tissues, and body fluids (Kaddurah-Daouk et al., 2008; Kristal et al., 2007a,b; Hollywood et al., 2006; Oldiges et al., 2007; Shulaev, 2006; Weckwerth and Morgenthal, 2005; Holmes et al., 2008a). The overall sum of these metabolites is known as the metabolome. Metabolomics captures the status of diverse biochemical pathways at a particular moment and defines a metabolic state such as health or disease state. The comprehensive study of the metabolome could lead to the identification of new disease-specific signature(s) as possible biomarkers. As we describe in detail below, this notion is highlighted by the initial identification of biochemical signatures for CNS disorders such as schizophrenia, Major Depressive Disorder (MDD), Bipolar Disorder (BD), Amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD). Metabolic signatures are expected to replace the use of one molecule as a biomarker for disease as it will capture more comprehensive information about disease pathogenesis.

This review outlines general aspects of biomarker research and how metabolic abnormalities in CNS disorders can contribute to the identification of unique biomarkers. It includes an introduction to 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. The review also underscores how evidence documenting the existence of large number of biochemical abnormalities in CNS disorders provides a rational for the application of global approaches such as metabolomics to biomarker discovery. Examples illustrating how metabolomics was used to define initial signatures for particular CNS disorders are provided. The review concludes by discussing current challenges and future promise of this technology in the process of biomarker discovery. We also

suggest some references for those interested on a more in depth examination of any of the topics highlighted in this review.

Biomarker discovery research

A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal or pathogenic processes, as well as responses to therapeutic interventions (Biomarkers Definitions Working Group, 2001; Vasan, 2006). Biomarkers, particularly as they relate to metabolomics, can be measured in any biological sample, e.g., blood, urine, or saliva (Harrigan, 2002, Kaddurah-Daouk et al., 2008, 2009; Lindon et al., 2004; van der Greef et al., 2003; Kristal and Shurubor, 2005; Bogdanov et al., 2008; Holmes et al., 2008a). They can be indicators of disease traits (or risk markers), disease states, or disease rates (progression). Accordingly, biomarkers have been classified as antecedent biomarkers (identifying the risk of developing an illness), screening biomarkers (screening for subclinical disease), diagnostic biomarkers (recognizing overt disease), staging biomarkers (categorizing disease severity), or prognostic biomarkers (predicting future disease course, including recurrence and response to therapy, and monitoring efficacy of therapy) (Biomarkers Definitions Working Group, 2001; Vasan, 2006).

A naïve expectation is that single biomarkers can capture the complex process underlying an illness. Rather, by looking as perturbations of biochemical networks (systems view), it becomes clear that a multiparameter analysis (panel of markers or multiple metabolites) may provide better insight into disease diagnosis, prognosis, and treatment (Hood et al., 2004). By surveying for global changes in metabolic pathways, metabolomics-based approaches are more likely to provide a wealth of information that may be difficult to capture by looking at only one pathway or one biomarker. An example of the usefulness of this multivariate approach, in 2007 the U.S. Food and Drug Administration (FDA) cleared for marketing a multivariate index assay that determines the likelihood of breast cancer returning within five to 10 years after a woman's initial cancer (http://www.fda.gov/bbs/topics/NEWS/2007/NEW01555.html). This assay looks at 70 genes in a sample of a woman's surgically removed breast cancer tumor, and then applying an algorithm, produces a score that determines whether the patient is deemed low risk or high risk for spread of the cancer to another site (Fan et al., 2006).

Biomarkers in CNS disorders

According to the type of information that they provide, biomarkers for CNS disorders can also be classified as genetic, neuroimaging, clinical, or biochemical markers (DeKosky and Marek, 2003). Next, we describe the information provided by these different types of biomarkers.

Genetic biomarkers capitalize on molecular genetics to identify gene variations associated with disease. Although the presence or absence of specific alleles can at times identify individuals who are at risk of developing a given disease, they generally do not predict age of disease onset accurately.

Clinical biomarkers link processes such as the loss of a certain function (e.g., episodic memory) to survival endpoints, often relying on clinicians' assessment. Examples of these markers include memory assessment in AD (DeKosky, 2008) and loss of olfaction in PD (Herting et al., 2008; Kranick and Duda, 2008; Wolters, 2008). Unfortunately, the identification of clinical biomarkers for CNS disorders has been fraught with difficulties due to the known variability in the expression of signs and symptoms (Haehner et al., 2007). This variability maybe a consequence of individual-specific topographical sequences of pathology [i.e., extent and progression of the degenerative process at defined sites) (Wolters, 2008)].

Neuroimaging biomarkers originate on the use of imaging technologies such as single photon emission computerized tomography (SPECT) and positron emission tomography (PET) for the detection of mostly biochemical changes using isotope-labeled tracers probes; Magnetic Resonance Imaging (MRI) for higher resolution structural analysis; and functional MRI (fMRI) which by measuring changes in the blood oxygenation levels in microcirculation provides indirect measures of regional changes in cerebral blood flow and neural activity. Neuroimaging biomarkers are emerging as potential supplements to clinical data in the assessment CNS disorders including PD (Berg, 2008; Brooks, 2004), Alzheimer's disease AD (Jagust, 2004), BD (Phillips and Vieta, 2007) and HD (Rosas et al., 2004); as well as potential means to assess new medication affecting the brain (Borsook et al., 2006). Indeed, these markers could provide diagnostic and prognostic information; can be performed repeatedly from an early stage of the disease and throughout progression of the disease. Some limitations for the large-scale use of neuroimaging biomarkers are the need for highly specialized equipment and personnel, imaging time and cost, as well as subjects' exposure to radioactive probes.

Finally, classical research (pre-metabolomics) on the identification of biochemical biomarkers in blood and CSF for CNS disorders has been aimed at assaying single metabolites. Often this search has been based on research hypotheses. Unfortunately, none of the single biomarkers identified to date have the desired sensitivity and specificity for diagnosis; have sufficient power to identify disorders at an early stage; or serve of CNS disorders as surrogate endpoints in clinical trials.

Evidence for metabolic changes in neuropsychiatric disorders: rationale for a metabolomics approach for biomarker discovery

A comprehensive review of the current knowledge on biochemical abnormalities in CNS disorders is outside the scope of this review. We provide an overview of biochemical abnormalities described in common CNS disorders here (Kaddurah-Daouk et al., 2009). Findings are derived from studies often focusing on quantifying single or few metabolites. This data provides a rationale for the use metabolomic approaches in the study of CNS disorders aiming to capture more global biochemical disturbances and using this information for the identification of biomarkers that reflect more accurately the disease state, disease progression or response to therapy.

Studies have used cerebrospinal fluid (CSF) or blood (plasma or serum) to conduct biochemical analysis. Despite CSF is an obvious sample to use when searching for biomarkers in conditions affecting the CNS, due to reasons that we discuss later in detail; the use of blood has several practical advantages and most of the studies used blood as a source of sample.

In CNS disorders several metabolic changes are noted. For example impairments in neuronal survival, neurotransmitters; antioxidant system and free radicals ratios, membrane composition, mitochondrial function and immune response (e.g., arachidonic acid metabolism) have been noted in many CNS disorders (Kaddurah-Daouk et al., 2009). Some of these abnormalities have been captured by classical biochemical approaches where single metabolites are measured.

Given that in each CNS disorder there are disturbances in several pathways, research based on global approaches such metabolomics would derive a more comprehensive picture of the pathways involved and their links. Furthermore, insofar as common disturbances maybe present across disorders, there might be a need to conduct studies including more that one CNS condition to clarify the specificity of findings to any given disease.

Metabolomics: a global biochemical approach for biomarker discovery

This section provides a rational for the use of metabolomics for biomarker discovery and reviews briefly the conceptual framework as well as practical aspects of the technologies applied in the field of metabolomics.

Metabolomics overview

The metabolome is the collection of small molecules that are found within a system which basically covers a broad rage of small molecules such as glucose, cholesterol, ATP, biogenic amine neurotransmitters, lipid signaling molecules among many other classes of compounds (Kristal, 2005). The identities, concentrations, and fluxes of metabolites are the final product of interactions between gene expression, protein expression, and the cellular environment (Fig. 1). Thus, metabolomic information complements data obtained from other fields such as—genomics, transcriptomics, and proteomics—adding a final piece to a systems approach for the study of disease pathophysiology, biomarkers identification and drug action (Kaddurah-Daouk et al., 2008; Kristal et al., 2007a; Lindon et al., 2004; Holmes et al., 2008a; Wishart 2008). In contrast to classical biochemical approaches that often focus on single metabolites, 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 disease and drug exposure (Kaddurah-Daouk et al., 2008, 2009; Kristal et al., 2007a; Lindon et al., 2004; Wishart 2008).

Several disease states induce long lasting changes in the metabolome and such changes can be captured using a variety of metabolomics platforms. Initial metabolomic signatures have already been reported for several disease states, cardiovascular and coronary artery disease (Sabatine et al., 2005), hypertension (Brindle et al., 2003), subarachnoid hemorrhage (Dunne et al., 2005), preeclampsia (Kenny et al., 2005), type 2 diabetes (van Doorn et al., 2007; Yi, 2006), liver cancer (Yang et al., 2004), ovarian cancer (Odunsi et al., 2005) and breast cancer (Fan et al., 2005; Holmes et al., 2008b; Beckonert, 2003). 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 concerning disease pathophysiology.

The application of metabolomics to the study of the effects of drugs captures signatures representing 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 (Kaddurah-Daouk et al., 2008; Kell, 2006; Morvan and Demidem, 2007; van der Greef et al., 2003, 2006; van Doorn et al., 2007; Wishart, 2008; Wishart et al., 2008).

Metabolomics approaches and platforms

An overview of a "typical" metabolomics study was described in (Kaddurah-Daouk et al., 2008); detail experimental protocols are provided in (Carlson and Cravatt, 2007) and (Weckwerth, 2007). Hundreds to thousands of metabolites can be separated and quantified in samples of interest such as plasma, CSF, urine or cell extracts using a variety of commonly use metabolomics platforms such as Nuclear magnetic resonance spectroscopy (NMR), Gas chromatography with mass spectroscopy (GC–MS), Liquid chromatography with mass spectroscopy LC–MS, and liquid chromatography electrochemical array detection (LCECA) (Dettmer et al., 2007; Dunn, 2008; Weckwerth, 2007). Clearly, none of these platforms alone can capture the total complexity of the metabolome. However, some of these platforms are more suited for the study of specific pathways and can be used to test hypothesis about disease mechanisms, while others are more suited for looking more globally at metabolism in a non targeted way to generate new hypothesis.

Combinations of techniques can be used to augment separations and/or expand the analyte information acquired (Williams et al., 2006). These datasets must be then collected and curated, a process that can take significant time for the overall experiment. After curation, the data are analyzed by one or more software packages designed for studies of datasets that are far too large for human evaluation. A database is then generated for the diseased patients and another for the controls or for the same patients before and after drug therapy. These databases include levels of detectable metabolites and identity (or a description of the properties) of the metabolites, i.e., oxidation–reduction potential, mass/charge ratio etc. whenever known. Next, we briefly highlight some general features of the metabolomics platforms that are currently used.

NMR spectroscopy-based metabolomics

For a comprehensive review of this platform the reader is referred to recent in depth review (Coen et al., 2008; Beckonert et al., 2007). NMR is an analytical platform that allows the reliable detection and quantification of wide range of metabolites (high universality, i.e., will detect any hydrogen containing metabolite) present in complex biological fluids at micromolar concentrations. "Whole" samples can be analyzed, thus NMR is considered to be non-destructive technique with low handling and preprocessing times. There are multiple examples in the literature of the application of NMR metabolomics in

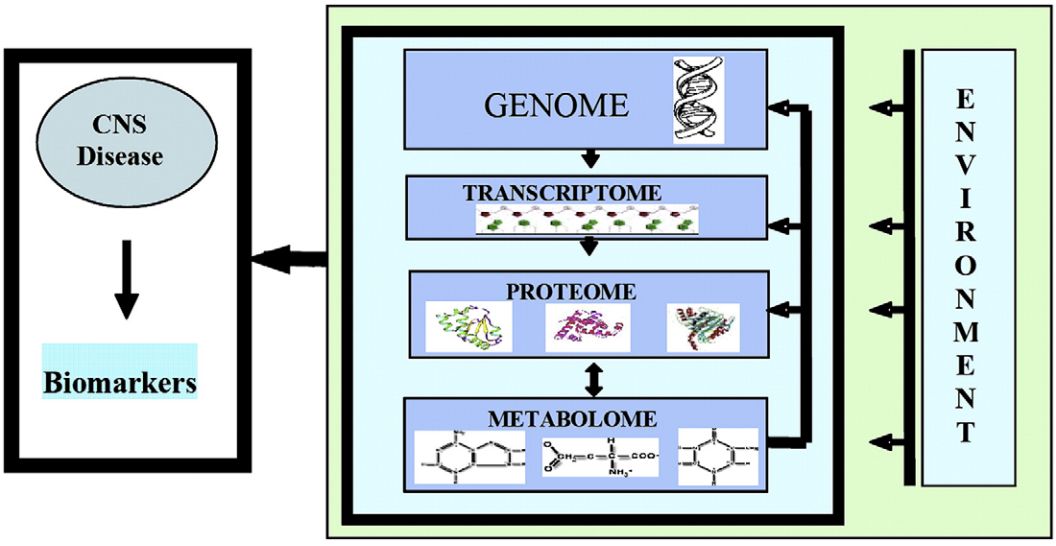

Fig. 1. Flow of information from DNA, to proteins to finally metabolites. CNS disorders are the result of the interaction between environmental factors and the genome. These interactions are manifested at the level of the transcriptome, the proteome and the metabolome. Biomarkers can be identified by focusing on any of these levels and using omics-based approaches.

CNS disorders including, Huntington's [results in a murine model (Tsang et al., 2006b)]; studies on tissue and plasma in a rodent model of traumatic brain injury (Viant et al., 2005); and studies in patients with schizophrenia (Holmes et al., 2006).

MS-based methods for metabolomics

For a comprehensive review on MS applications in metabolomics research the reader is referred to recent reviews (Dettmer et al., 2007; Dunn, 2008; Fiehn, 2008). MS-based approaches represent universal, yet sensitive tools to characterize, identify, and quantify the plethora of compounds present in a biological tissue or body fluid (Dettmer et al., 2007). Prior to analysis, biological samples need to be separated using chromatography, commonly either gas or liquid chromatography (GS and LC); and subsequently, metabolites are identified using a mass spectrometer. The use of MS for metabolomics research in CNS disorders provides a number of advantages, allowing the detection of many metabolite classes at physiological concentrations including amino and organic acids, fatty acids, sugars, sugar phosphates, bile acids, lipids and nucleotide bases. MS has the ability to identify known as well as new metabolites (not currently described in databases) in a relatively straightforward fashion. This is mainly through the measurement of molecular mass (thereby molecular formula) or by allowing the inference of molecular structure using fragmentation mass spectra.

GC–MS

The combination of Gas chromatography (GC) with MS is a well-developed and robust tool that has been applied for many years. Thus, GC–MS has been one of the major analytical drivers in the early developments of metabolomics. GC–MS allows the detection and quantification of many metabolite classes including amino and organic acids, fatty acids and some lipids, sugars, sugar alcohols and phosphates, amines, amides and thiol containing metabolites. This technique has become highly developed because of high sensitivity, high chromatographic resolution, wide range of detectable metabolite classes and the ability to identify metabolites through the production of mass spectral/retention index libraries or by comparison to commercially available libraries. GC–MS offers structural information, reasonable quantitative precision and high throughput. However, there are some disadvantages to using GC–MS for metabolite profiling and the identification of biomarkers for CNS disorders, including involved sample preparation that requires extraction as well as derivatization to improve volatility; and the limits on the size and type of molecule that can be analyzed (e.g., nonvolatile, polar macromolecules are unsuitable). In spite of these limitations, there are several successful examples of the application of GC–MS for metabolomics research in CNS disorders (Underwood et al., 2006; Paige et al. 2007).

LC–MS

This flexible and sensitive analytical platform is used to characterize, identify, and quantify a large number of compounds in a biological sample where metabolites are present at very different concentrations (Dunn, 2008). LC encompasses a range of systems including high performance liquid chromatography (HPLC) and capillary liquid chromatography, among others. Metabolic profiling using LC–MS can be hindered by issues related to the chromatographic resolution, effect of matrix effects (ionization suppression) on co-eluting metabolites and influence of column chemistries employed. Nonetheless, LC–MS has been used successfully for metabolomic studies in CNS disorders [e.g., virus infection induced neurodegeneration (Wikoff et al., 2008)]. Importantly, to handle and process LC–MS data, processing pipelines, repositories and databases have been developed (e.g., METLIN; http://metlin.scripps.edu/). Incorporation of collections of spectroscopic and chemical data aids in metabolite identification through accurate mass measurement and isotopic-pattern evaluation.

Electrochemistry based metabolomics platform—LCECA

This metabolomics platform allows differential detection and quantification of small molecules on the basis of their oxidation–reduction potentials, representing a subset of the metabolome that includes molecules amenable to oxidation–reduction, such as neurotransmitters and related pathways. This particular platform is ideal for application to the study of the tryptophan and tyrosine pathways that lead to monoamine neurotransmitters since most of the metabolites within these pathways can be measured quantitatively in this fashion. LCECA has been used to define signatures in motor neuron disease (Rozen et al., 2005) and recently in PD (Bogdanov et al., 2008).

From the above-mentioned overview, it should be apparent that none of the platforms could provide a complete characterization of the full universe of metabolites present in biological fluids. Hence, a valuable approach might be the combination of the platforms to derive comprehensive information from the same set of samples.

Extracting information from metabolomics datasets

For a comprehensive review of data analysis approaches the reader is referred to (Marie Brown et. al., 2005). The application of software tools for the analysis of the metabolomic data sets contained in a database is required for the identification of disease signatures, classification of groups of interest (e.g., disease or control, pre- or post-drug exposure) and for the identification of unrecognized groups in the data (e.g., drug response subgroups; Altmaier, 2008). Metabolomics data sets can be analyzed with a range of statistical and machine-learning algorithms (Marie Brown et al., 2005; Sajda, 2006; Shin and Markey, 2006; Lindon and Nicholson, 2008). Methods can be classified within two major classes: unsupervised and supervised. Unsupervised algorithms find patterns in the data without any biases. Examples of unsupervised methods that have been routinely used in analyzing molecular fingerprinting data include principal component analysis (PCA) and self-organizing maps (unsupervised competitive-learning network algorithms which form a nonlinear projection of a high-dimensional data manifold on a regular, low-dimensional grid) (Meinicke et al., 2008; Zou and Tolstikov, 2008). These methods are usually guided by the largest average differences between the groups, and are thus very sensitive to outliers. Given that the groupings originate from the data itself, rather than from the analyst, the methods are also very sensitive to how the experiments were carried out. These methods are best used to reveal unknown patterns in the data, but their interpretation needs to be highly connected to the experimental details. Their application is important in the sense that they provide a kind of quality control, by which we will verify which are the most salient features of the data. An example of the application of artificial neural networks in CNS disorders, includes the use of self-organizing maps to identify differences in the levels of platelets fatty acids between healthy and depressed individuals (Cocchi et al., 2008).

Supervised algorithms require that samples be labeled in groups a priori, and they uncover the features (variables) that best discriminate between those groups. Supervised methods have been applied to molecular fingerprinting data, most often ANOVA, partial least squares (PLS), and discriminant function analysis (DFA) (Mendes, 2002; Shulaev, 2006).

Metabolomics for identification of signatures and biomarkers

Metabolomics has the potential to map early biochemical changes in disease and hence provides an opportunity to develop predictive biomarkers that can trigger earlier interventions (Kaddurah-Daouk et al., 2007, 2008; Lindon et al., 2004; van der Greef et al., 2003). If that turns out to be the case then, this field fits the Biomarker Consortium's criteria defining high-impact biomarker research (http://www.fnih.org/index.php?option=com_content&task=view&id=595&Itemid=43).

The consortium is a public–private research partnership of the Foundation for NIH that has developed a number of key criteria that should be taken into account when considering any type of research aimed to the identification of biomarkers.

Although metabolomics is not explicitly described, adapting their criteria to field of metabolomics, it is possible to say that metabolomics-driven biomarker research is important by focusing on knowledge gaps, namely identifying metabolic signatures of disorders and treatment responses via the refine dissection of perturbations in biological pathways and networks; translational given that metabolomics has as a goal to improve patient care; transformational by improving the process of biomarkers discovery via the characterization of the metabolome, a sum of endogenous products which capture the dynamic interactions between the genome and the environment in health and disease; feasible by having a realistic timeframes given that many of the platforms for metabolomic research have already been developed and continue to be refined; practical by building on preexisting resources that include for instance the growing number of metabolites databases (e.g., pathway databases and viewers such as KEGG) and collaborative research networks (e.g., The Metabolomics Network for Drug Response Phenotype).

Deriving signatures and biomarkers from a highly dynamic metabolome

Metabolic signatures can be obtained using some of the platforms described above. For CNS disorders quantitative or qualitative variation in metabolites could give rise to discovery of disease biomarkers. Two implicit assumptions in this logic are that 1) under normal conditions, there are consistent patterns in the metabolome that are disturbed in the context of disease states; 2) differences between the normal and the disease state can be identified reliably, over and above, the strong day-to-day variability in the highly dynamic- and environmentally influenced-, human metabolome. Notably, a growing amount of evidence supports the notion that metabolomics could lead to the identification of biomarkers by revealing stable changes in the metabolome traceable using specific analytical platforms. However, there are pitfalls and confounding factors that need to be dealt with before a reliable biomarker that is disease-specific is identified. Next we briefly describe work demonstrating the existence of relatively stable metabolomic signatures in healthy individuals. In the next section, we review work substantiating the presence of disease-specific changes in the metabolome.

Despite that individuals' metabolic profile is partially influenced by multiple factors (including genotype, age, lifestyle, environmental factors, nutritional status, assumption of drugs, etc), the existence of an invariant part of the individual metabolome in "healthy" subjects should allow the identification of disease-associated variants that might serve as biomarkers. In other words, there are portions of the metabolome shared at the population level; as well as, individual-specific components. Indeed, biochemical analysis of human plasma samples using HPLC separations coupled with coulometric electrode array detection, allowed the identification of markers/metabolites in human plasma. It was concluded that the use of metabolomics markers in human clinical trials and epidemiological studies was warranted (Shurubor et al., 2007).

That the metabolome has an invariant part has also been documented in a recent study (Assfalg et al., 2008). In this work, metabolic fingerprints were generated by one-dimensional NMR spectroscopy from multiple urine samples collected from a group of healthy subjects. Using multivariate models that reduced inter-subject differences and minimized intra-subject variability the authors demonstrated the existence of an "individual metabolic phenotype", that was shown to constitute a strong characteristic of each donor as to allow its identification with 100% probability. Applying a projection/back-projection approach, the authors were able to obtain this "core" profile free from random daily noise factors. Many studies conducted by Drs, Matson and Kaddurah-Daouk confirm that indeed there is a metabotype that is unique for each individual (personal communication). In sum, despite being highly dynamic, the metabolome in healthy subjects might have core properties that are stable over time. This self-similarity of each of us is disrupted when a disease occurs resulting in a disease signature that is measurable above all variations (Assfalg et al., 2008; Shurubor et al., 2007, Matson et al., unpublished results).

Metabolomics–genetics interface

Importantly, there is mounting evidence suggesting that genetic variability in individuals belonging to different populations, and hence having diverse genetic make-up, can be captured by current methods used to characterize the metabolome (Dumas et al., 2007). In other words, metabolomics also has the potential to aid in the characterization of the relationships between genomic and phenotypic variation.

Metabolomics, effects of environmental factors and biomarker discovery

One of the aspects of metabolomics as a tool for discovery of biomarkers is its ability to capture characteristics that emerge from the interaction between individual and environmental factors. As demonstrated by the results of a recent landmark study (Holmes et al., 2008a,b), considering this interaction might be critical in the identification of disease biomarkers and understanding of disease pathogenesis. In their work, the researchers derived NMR spectra from two 24-hour urine specimens for 4630 subjects belonging to four human populations with contrasting geographical location, diets, diet-related major risk factors, and coronary heart disease/stroke rates. They found significant differences between populations in their NMR spectra and that; among discriminatory metabolites there were associations between specific metabolites and blood pressure (multiple regression analyses for individuals). The authors concluded that their work provide the basis for a new 'metabolome-wide association' approach in molecular epidemiology to help understand the complex interactions of lifestyles, environment and genes that determine major diseases. Remarkable, this approach provides unique opportunities for biomarker discovery.

Metabolomic applications in the study of CNS disorders

The examples provided below illustrate how the use of advanced metabolomic platforms permits a global and integrated analysis of biochemical pathways and metabolic changes occurring in a disorder. Ideally, this global mapping of biochemical abnormalities would facilitate the understating disease pathogenesis and the identification of clinically relevant biomarkers.

Metabolomic signatures in motor neuron diseases (MND)

Using high performance liquid chromatography followed by electrochemical detection (LCECA), our group profiled blood plasma from 28 patients with MND and 30 healthy controls (Rozen et al., 2005). Of 317 metabolites, 50 were elevated in MND patients and more than 70 were decreased ($p<0.05$). Among the compounds elevated, 12 were associated with the drug Riluzole, an inhibitor of glutamate release effective that has some beneficial effect on the illness (Rozen et al., 2005). Using multivariate regression techniques (Rozen et al., 2005), we were able to identify a distinctive signature of highly correlated metabolites in a set of four patients, three of whom had lower motor neuron (LMN) disease (Fig. 2). Furthermore, we defined a metabolic signature that was independent of the drug Riluzole (illness-related) by profiling patients who were off medication (Rozen et al., 2005). Collectively, these results suggest that metabolomic studies can be used to ascertain metabolic signatures of disease using easily accessible samples like plasma.

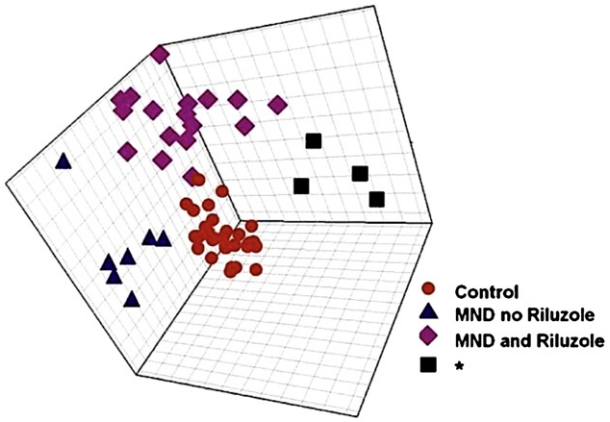

Fig. 2. Plasma Metabolomic signature of MND. Model using projections into three dimensions provided statistically significant separations between subgroups ($p<0.01$ by permutation test—random assignment of samples to subgroups). Model includes the four patients with a distinctive signature, three of whom had LMN disease, indicated by an asterisk.

Metabolomics studies in Parkinson's disease (PD) and Huntington's disease (HD)

A recent study by Bogdanov and colleges conducted interesting work defining metabolomic signatures in PD. They included 25 controls and 66 PD patients and used LCECA to create a database representing 2000 signals from plasma samples. Multivariate data analysis revealed separation of the metabolomic profile of medicated and unmedicated patients and controls. Markers of oxidative damage and the antioxidant glutathione were significantly increased in PD patients. Uric acid levels were significantly decreased. Interestingly, alike glutathione, uric acid is also an antioxidant and higher uric acid levels lower risk for PD and slow the progression of the illness. Together, these findings show that metabolomic profiling with LCECA coulometric array has great promise in the identification of biomarkers for both the diagnosis, as well as monitoring disease progression in PD.

Another neuropsychiatric disorder in which metabolomic studies have been performed is HD. In an interesting cross-species study, serum samples from a transgenic mouse model of HD and patients with HD were studied using gas chromatography-time-of-flight-mass spectrometry (Underwood et al., 2006). The investigators observed clear differences in metabolic profiles between transgenic mice and wild-type littermates (healthy mice), with a trend for similar differences in human patients and control (Underwood et al., 2006). Potential markers were related to fatty acid breakdown (including glycerol and malonate) and also to certain aliphatic amino acids. Taken together, the findings of this study suggest the interesting possibility that the metabolites responsible for distinguishing transgenic mice also comprised a metabolic signature tentatively associated with the human disease. Results from another murine model of HD have also highlighted the usefulness of metabolomics to study disease pathogenesis and identify potential biomarkers (Tsang et al., 2006b).

Metabolomics in psychiatric disorders

For more comprehensive review of this subject the reader is referred to Kaddurah-Daouk et al. (2009). Using plasma samples and sophisticated metabolomics analytical platforms and informatics tools we have begun to define biochemical pathways implicated in the pathogenesis and treatment response in schizophrenia and Major Depressive Disorder (MDD).

Findings from metabolomics studies in depression

Metabolomic analysis of blood plasma was performed on nine depressed, 11 remitted, and ten never-depressed older adults (Paige et al., 2007). Hundreds of metabolites were measured using GC–MS and a library of 800 commercially available human metabolite standards helped in compound identification. Metabolites that were altered in currently depressed patients when compared with controls included several fatty acids, glycerol and gamma-aminobutyric acid (GABA). Analyses comparing concentrations in remitted and currently depressed patients revealed a pattern of metabolite alterations similar to the control vs. currently depressed analyses (Fig. 3). One difference observed in the remitted patients relative to the depressed patients was elevation of the concentration of the ketone 3hydroxybutanoic acid (Fig. 3).

These results will need to be examined and validated in larger longitudinal cohorts. However, these findings suggest that the depressed state may be associated with alterations in the metabolism of lipids and neurotransmitters, and that treatment with antidepressants adjusts some of the aberrant pathways in disease so that the patients in remission have a metabolic profile more similar to controls than to the depressed population. An evaluation of such changes in CSF samples is needed to establish how closely these findings are to central changes.

Signatures in schizophrenia and its treatment

Several metabolomics studies have recently been conducted in an attempt to better define pathways modified in schizophrenia and its treatment (Holmes et al., 2006; Huang et al., 2007; Kaddurah-Daouk et al., 2007; Khaitovich et al., 2008; Tsang et al., 2006a). In one study (Kaddurah-Daouk et al., 2007) we used a specialized lipidomics platform and measured more than 300 polar and nonpolar lipid metabolites (structural and energetic lipids) across 7 lipid classes to evaluate global lipid changes in schizophrenia before and after treatment with three commonly prescribed atypical antipsychotics, olanzapine, risperidone, and aripiprazole. Lipidomics is a branch of metabolomics that specifically focuses on comprehensive assessment of lipid biochemistry (German et al., 2007; Wenk, 2005; Wolf and Quinn, 2008). In this particular study, lipid profiles were obtained for 50 patients with schizophrenia before and after 2–3 weeks of treatment with olanzapine, risperidone, or aripiprazole. At baseline, and prior to drug treatment, major changes were noted in two phospholipids classes, phosphotidylethanolamine (PE) and phosphotidylcholine (PC), suggesting that phospholipids that play a key role in proper membrane structure and function seem to be impaired in patients with schizophrenia (Kaddurah-Daouk et al., 2007, Fig. 4). This confirmed previous observations but establishes a far more detailed biochemical map for sites of perturbations.

The 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 (Kaddurah-Daouk et al., 2007). It was of interest that each of the three drugs studied had a unique signature suggesting that while these drugs share some effects, they also have many effects that are unique for each. PE concentrations that were suppressed 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 (Kaddurah-Daouk et al., 2007). On balance, aripiprazole induced minimal changes in the lipidome, consistent with its limited metabolic side effects. There were also increased concentrations of triacylglycerols and decreased free fatty acid concentrations after both olanzapine and risperidone, but not after aripiprazole therapy (Kaddurah-Daouk et al., 2007).

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. Collectively, 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.

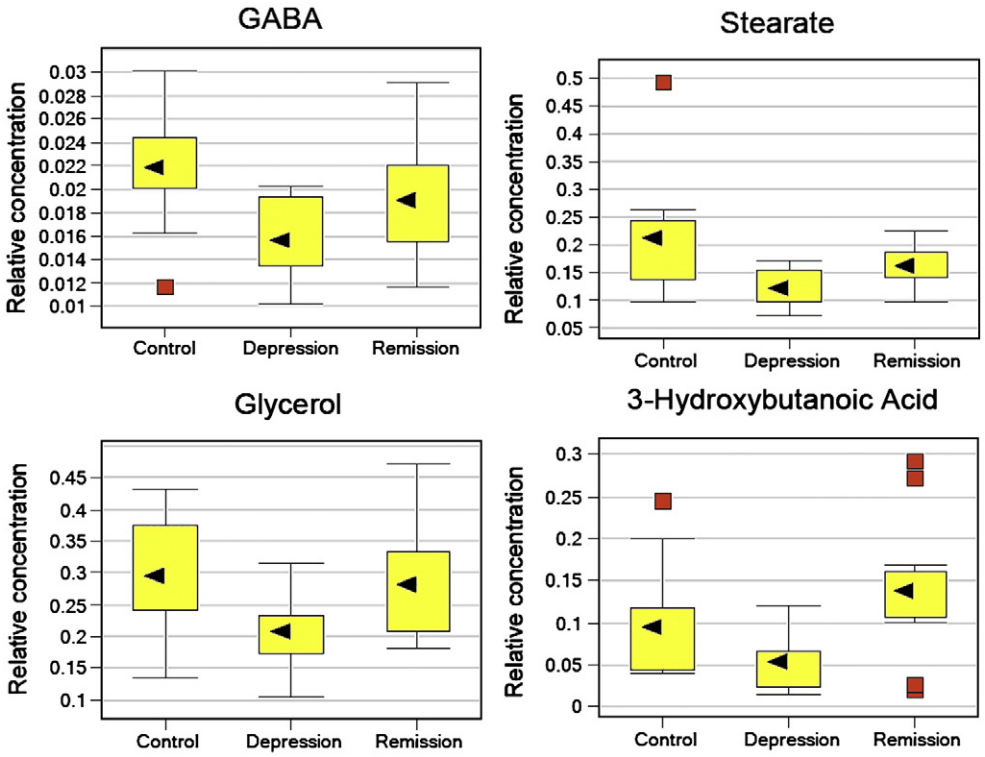

Fig. 3. Plasma metabolic signatures in active and remitted depression. Metabolomic analysis of blood plasma was performed using Gas chromatography–mass spectrometry (GC–MS) on samples from nine depressed, eleven remitted, and ten never-depressed older adults. Metabolite identification was based on a combination of chromatographic properties and mass spectra. A library of 800 commercially available human metabolite standards analyzed on a GC–MS platform helped in compound identification. Metabolites that were altered in currently depressed patients when 23 compared with controls included GABA glycerol (top and bottom left panels, respectively) and several fatty acids (e.g. stearate, top right panel). Analyses comparing concentrations in remitted and currently depressed patients revealed a pattern of metabolite alterations similar to the control vs. currently depressed analyses. One difference observed in the remitted patients relative to the depressed patients was elevation of the concentration of the ketone 3-hydroxybutanoic acid (bottom right panel). Figure from Paige et al. (2007).

In additional metabolomics studies in schizophrenia using NMR spectroscopy-based metabonomic analysis plasma samples from 21 pairs of monozygotic twins discordant for schizophrenia and 8 pairs of age-matched healthy twins demonstrated alterations in lipid profiles of both affected and unaffected schizophrenia twins (Tsang et al., 2006a). In another study of CSF samples from drug-naïve patients with first-onset schizophrenia, suggested alterations in glucose regulation an abnormality that seems to get corrected by early treatment with antipsychotics (Holmes et al., 2006). Finally, an interesting metabolomic study on postmortem tissue provides support to the notion that abnormalities at the level of glutamatergic neurotransmission and myelin synthesis play an important role in schizophrenia (Tkachev et al., 2007).

The future of metabolomics as a tool for biomarker discovery and knowledge integration

This section describes where the field of metabolomics might be in the next years and strategies to aid in the construction of a biochemical roadmap for the study of CNS disorders. The suggested roadmap could generate a wealth of information to guide the identification of biomarkers.

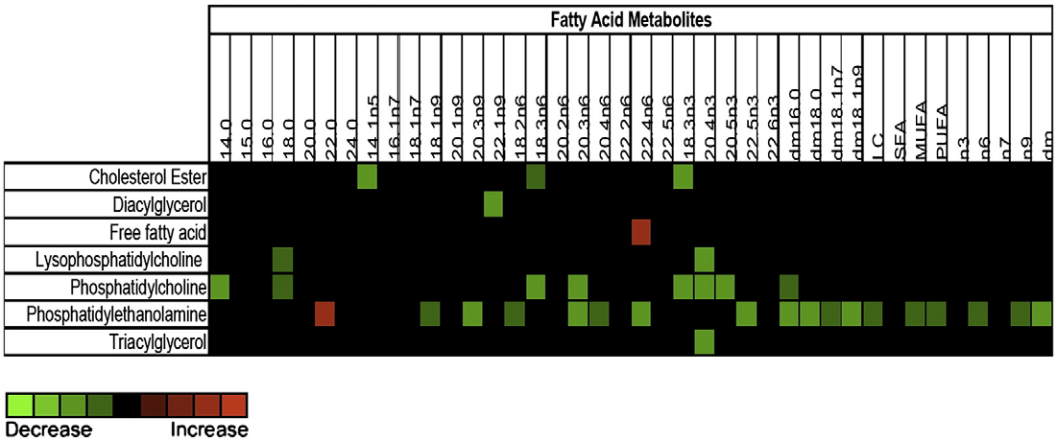

Fig. 4. Heat map showing differences in individual lipid metabolites in the plasma of patients with schizophrenia as compared to controls. The column headers indicate fatty acid metabolites as they appear in each distinct lipid class (rows). Lipids whose percent levels were higher in patients vs. controls are shown in red while those with decreased level are shown in green (Kaddurah-Daouk et al., 2007).

Metabolomics and biomarker discovery in the context of system biology, translational research and personalized medicine

The final decades of the twentieth, and beginning of the twenty-first, centuries have witnessed a revolution in biomedical research. It is now clear that understanding disease can be defined as the ability to classify, a pathological state using phenotypic observations and molecular diagnostics; explain how perturbed molecular processes cause the disease state; and define the mechanisms underlying these perturbations (e.g., genetic polymorphisms, pathogens, environmental factors, etc) (Hood et al., 2004; Kell, 2006; van der Greef et al., 2007). Reaching this high-degree of sophistication in our understanding of disease, calls for the combined use of high-throughput readouts to profile many genes, proteins and metabolites (Hood et al., 2004; Kell, 2006; van der Greef et al., 2007). This vision is at the core of systems biology, a science that combines multiple biological data streams (from gene and metabolites to organism) to enable a profound understanding of interdependent biological processes (Hood et al., 2004; Kell, 2006; van der Greef et al., 2007). This enormous network of knowledge will be critical both for understanding biological systems and for accurately preventing, diagnosing, monitoring and treating disease (Ahn et al., 2006a,b; van der Greef et al., 2007).

Metabolomics will continue to be a centerpiece in systems biology (Kell, 2004; Nicholson, 2006; van der Greef et al., 2003, 2006). An individual's metabolic state is a close representation of the individual's overall health status. This metabolic state reflects what has been encoded by the genome; modified by environmental factors; and in case of abnormal states, what is disrupted during pathological states. As described in the next section, given that complex disease states like schizophrenia or PD are likely to be underlie by disruptions at the system level, the use complimentary omic platforms may provide a more comprehensive picture suitable for the identification of biomarkers.

A powerful approach for the characterization of disturbances in biological networks that will continue to gain momentum is the concurrent use of array technologies that measure global changes at the genomic, proteomic and metabolomic level [e.g., (Pir et al., 2006; Trauger et al., 2008; Coen, 2004; Lindon and Nicholson, 2008)]. In this so called 'top-down' systems biology, the main objective is to discover new molecular mechanisms using an iterative cycle that starts with experimental data generated from array technologies, followed by data analysis and integration to capture a fuller picture of gene–protein–metabolite dynamics in a system. Thus, successful discovery of biomarkers is likely to benefit from the use of complementary approaches. For instance, functional genomics comparing gene expression profiles between normal and diseased can be hindered by gene expression not necessarily translating into changes in proteins or change of cellular processes. Thus, simultaneously capturing genomic, proteomic and metabolomic data would be helpful in determining how gene expression patterns result in specific protein expression, pathway activation and metabolomic changes. Two examples of research involving a combined genomics (gene expression) and metabolomic analysis used the brain tissue from schizophrenics, bipolar patients and control tissue and found that these platforms pointed to compromised brain metabolism and oxidative stress in BD (Prabakaran et al., 2004) and schizophrenia (Khaitovich et al., 2008).

Clinical implications of metabolomic research and identification of biomarkers

Today, clinicians capture only a very small part of the information contained in the metabolome, as revealed by a defined set of blood chemistry analyses to define health and disease states. Examples include measuring glucose to monitor diabetes and measuring cholesterol for cardiovascular health. Such a narrow chemical analysis could potentially be replaced in the near future (5–10 years from now) with a metabolic signature that captures global biochemical changes in disease and upon treatment. Replacing single-molecule biomarker analysis with metabolomics-based multiparameter diagnostics may represent an extremely promising advance toward early detection of diseases such as PD. However, sensitivity (the ability of diagnostic test to identify all patients with the illness) and specificity (the ability of a test to identify all patients without an illness) is an issue that remains to be addressed in metabolomics studies. For a biomarker to be useful in the diagnosis of any condition, it should have a sensitivity and specificity of >85%.

So in the years to come, metabolomics will continue adding significantly to our understanding of individual effects of environmental challenges (including responses to medications) as well as phenotypic correlates of genetic variation. This information, supported by the existence of an "individual metabolic phenotype" as described earlier in this review, would be helpful in the studies aimed to identify biomarkers for CNS disorders or prediction of treatment response (as well as liability to side effects).

Role of pharmacometabolomics in personalized medicine

Metabolomics is expected to play a pivotal role in the development of personalized medicine (Bren, 2005; Nicholson, 2006; van der Greef et al., 2006, Kaddurah-Daouk et al., 2007, 2008). This field is based on the notion that given the uniqueness of each individual's DNA (except by twins); there seems to be a parallel uniqueness in an individual's metabolic and state that defines how one will respond to particular treatment. Indeed, a better understanding of the biochemical variation of response to medications and availability of biomarkers predictive of response would enable physicians to better select the right drug for their patients, in other words to personalized therapy. The application of metabolomics to the study of drug responses and the identification of biomarkers of responses has also evolved in the new field of pharmacometabolomics (or pharmacometabonomics). Pharmacometabolomics complements research on pharmacogenomics aimed to characterize how genetic polymorphisms affect individual responses to medications and hence, probabilities of beneficial or adverse effects. Pharmacometabolomics builds on the notion that metabolic profiling might be helpful in predicting response and side effects. By focusing on the metabolome, pharmacometabolomics is sensitive to both the genetic and environmental influences that determine the basal metabolic fingerprint of an individual, as these will also influence the outcome of a pharmacological intervention. Notably, with funding from National Institute of General Medical Sciences (NIGMS), we have established a national network called "Metabolomics Network for Drug Response Phenotype" to start to explore applications of metabolomics in understanding pathways implicated in variation to drug response for drugs such as, Statins used for cardiovascular health and SSRIs (selective serotonin reuptake inhibitors) commonly used for the treatment of depression; and potentially, to identify biomarkers of treatment response (Kaddurah-Daouk et al., 2008).

Maximizing the potential for biomarker identification using metabolomics

Using nonaffected relatives. A limitation in the studies aiming to identify biomarkers in CNS disorders relates to difficulties in the identification of individuals prior to the development of their illness or early in the course of their disease. Likewise, in many cases it is not easy to recruit sufficient numbers of medication naïve or unmedicated patients. Given the pivotal role of genetics in many of the illnesses affecting the CNS, it is possible to conduct studies comparing nonaffected relatives of patients with the illness and healthy controls. Finding biochemical abnormalities in nonaffected relatives could be helpful in the identification of biomarkers assessing the risk to develop the illness.

Focusing on longitudinal and larger studies. Current metabolomics research only illustrates the first steps towards the delineation of a metabolic signatures and biomarkers for the illnesses discussed in this review. Longitudinal studies are needed to confirm and expand on these initial findings. Moreover, preliminary metabolomics data so far are derived from only a small patient population using a couple of metabolomics platforms that captures a subset of the metabolome. Although this work exemplifies the potential for metabolomics in the study of several neuropsychiatric disorders, future studies will have larger samples and will be prospective in nature. Important consideration will continue being given to carefully matching patients and controls; and taken into account the possible confounding effects of other medications and disease. Replication and validation studies will continue to be needed in independent sets of patients and controls.

Standardization of protocols for experiment design, analysis and publication. At least two facts suggest that future studies will benefit from following research guidelines established by researchers working in the field: 1) the metabolome is highly dynamic and can be affected by a multitude of environmental factors; and 2) as described above, there are several platforms suitable for assaying the metabolome and analyzing data outputs. Addressing these t{es_def} LOGOS/Gulliver/print/elseviertree_print_gray.tifwo issues, major initiatives [e.g., Metabolomics Standards Initiative (Sansone et al., 2007); full issue of Metabolomics Journal 2007 and Standard Metabolic Reporting Structures Group (Lindon et al., 2005)] have focused on outlining guidelines that cover many important aspects in metabolic studies and reporting of metabolomics findings including collection of a biological samples (focusing on accounting for potential confounders such as fasting vs. nonfasting status in subjects), the analysis of material from that sample and chemometric and statistical approaches, and retrieval of information from the sample data. This will help create a common language among researchers in the field.

Connecting central and peripheral changes in the study of CNS disorders. Most of the studies described in this review applied diverse metabolomic platforms and assayed blood samples (plasma or serum). There are some advantages and limitations related to the use of peripheral samples. On one hand, the use of blood samples is practical given the accessibility of human plasma and the vast medical laboratory infrastructure already in place for its analysis, this is likely to remain the preferred diagnostic material for the foreseeable future. Thus, the identification of peripheral metabolomic signatures of neuropsychiatric illnesses is likely to have more potential for translation into the clinical realm. Importantly it is critical in future studies to verify that there are disease related signatures, that maybe subrogates of changes in the brain, and hence could provide clinical information and clues about disease pathogenesis (Kaddurah-Daouk et al., 2009).

On the other hand, there is a limited amount of research correlating findings seen peripherally and centrally (brain tissue or cerebrospinal fluid—CSF). However, an obvious challenge to conducting metabolomic studies linking peripheral and central, is the limited access to brain tissue. CSF can be collected following a lumbar puncture, and despite that this fluid is commonly used as a proxy for brain changes in CNS disorders (Raedler and Wiedemann, 2006), the need for special training for collection and the risk associated to the procedure, have limited the amount of studies conducted using this resource. Nevertheless, some evidence seems to suggest that central (CSF) changes in potential biomarkers might be correlated with changes in the periphery (blood; plasma or serum). For instance measurement of inflammatory markers in paired plasma/CSF samples of healthy human volunteers revealed a correlation between central and peripheral levels (Maier et al., 2005). This also seems to be the case for some metabolites. Plasma free tryptophan is clearly correlated with brain tryptophan concentration. Indeed, it has been suggested that plasma free tryptophan concentration provides an index of CSF tryptophan and 5-HT turnover in the brain (Curzon, 1979).

Likewise, CSF and serum/plasma concentrations of vitamin biomarkers are significantly correlated. Strikingly, the correlation between serum and CSF-folate can be as high 0.69 (Obeid et al., 2007). Another example is the levels of the side chain oxidized oxysterol 24Shydroxycholesterol, a potential maker of brain injury, which formed almost exclusively in the brain; and whose levels in plasma and CSF are highly correlated (Leoni et al., 2003).

Nonetheless, limited amount of evidence also suggests that abnormalities in the blood level of certain metabolites (plasma or serum) might not be correlated with central abnormalities (Levine et al., 2005). Thus, additional research using metabolomics-based approaches is needed to define metabolites whose central and peripheral levels are linked and how these correlations are influence by neuropsychiatric illnesses. Comparative studies in CSF (or post-mortem brain tissue itself) and blood could help map central and peripheral changes in neuropsychiatric disorders, enabling a more accessible way for biomarker development in blood but ensuring that these peripheral biomarkers are reflective of central changes. Given the importance of this issue and the limited amount of data, currently we are evaluating the concurrent use of postmortem brain tissue and CSF for metabolomic analysis. These types of studies are critical milestones to be reached in the developing field of metabolomics.

Indeed, the process of linking the metabolic profiles defined on blood and specimens derived from invasive sampling (e.g., CSF) is critical to identify 'bridging biomarkers' of disease, as well as both efficacy and toxicity medication. The latter will further ease the management of clinical trials. This approach conforms well to FDA's Critical Path Initiative that requests enhanced tools and biomarkers for the improvement of clinical trial design and throughput (http://www.fda.gov/oc/initiatives/criticalpath/whitepaper.html).

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, among several others all capable of accurately measuring hundreds or thousands of small molecules in biological samples, promises to substantially advance our understanding of disease pathophysiology and making possible the discovery of biomarkers for multiple disorders.

The application of metabolomics technologies to the study of neuropsychiatric disorders will enable simultaneous measurement of many metabolites in key interacting pathways. From these studies numerous new biomarkers will emerge. Measured collectively these biomarkers might provide highly relevant clinical information. In-depth knowledge of metabolic perturbations linking neuropsychiatric disorders, multiple biochemical pathways, and treatment effect–response, should provide valuable insights into disease pathophysiology and could provide novel approach for therapeutic monitoring and outcome.

Given that metabolomics has the potential to map early biochemical changes in disease and might provide an opportunity to develop predictive biomarkers that can trigger earlier interventions. Likewise, metabolomics could provide the means to sub-classify diseases, better design of clinical trials based on sub-classification of patients, early monitoring of drug effects in each patient, and timely mapping of the beneficial and side effects of drugs (pharmacometabolomics). Indeed, the potential to use biomarkers for identifying patients that are more likely to benefit or experience an adverse reaction in response to a given therapy, and thereby better match patients with therapies, is anticipated to have a major effect on both clinical practice and the development of new drugs and diagnostics (Trusheim et al., 2007).

There can be little doubt that the addition of metabolomic analyses to genomic, transcriptomic and proteomic assays will greatly enhance our understanding of mechanisms underlying the pathogenesis of illnesses and drug effects. Multiomics approaches based on the analysis of different body fluids and tissues with various profiling platforms promise to provide deeper insights into CNS disorders. Different profiling platforms can capture dynamic alterations, their response to treatment and the contribution of environmental factors to the onset. Therefore, biomarker discovery experiments based on profiling approaches facilitated by recent technical development are likely to make a great contribution to uncovering disease mechanisms in complex psychiatric disorders. Serious consideration should be given to the concurrent analysis of global metabolic changes peripherally (blood) and centrally (CSF) to establish how closely abnormalities measurable in the blood are correlated to changes in the brain. Collectively, these technologies offer great promise for the identification of clinically relevant biomarkers for neurological and psychiatric conditions.

Disclosures

Dr. Kaddurah-Daouk is equity holder in Metabolon Inc., a biotechnology company in the metabolomics domain, and she also holds IP interest in this field. M.P.Q. does not have anything to disclose.

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