Mass Spectrometry Strategies for Clinical Metabolomics and Lipidomics in Psychiatry, Neurology, and Neuro-Oncology

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Metabolomics research has the potential to provide biomarkers for the detection of disease, for subtyping complex disease populations, for monitoring disease progression and therapy, and for defining new molecular targets for therapeutic intervention. These potentials are far from being realized because of a number of technical, conceptual, financial, and bioinformatics issues. Mass spectrometry provides analytical platforms that address the technical barriers to success in metabolomics research; however, the limited commercial availability of analytical and stable isotope standards has created a bottleneck for the absolute quantitation of a number of metabolites. Conceptual and financial factors contribute to the generation of statistically under-powered clinical studies, whereas bioinformatics issues result in the publication of a large number of unidentified metabolites. The path forward in this field involves targeted metabolomics analyses of large control and patient populations to define both the normal range of a defined metabolite and the potential heterogeneity (eg, bimodal) in complex patient populations. This approach requires that metabolomics research groups, in addition to developing a number of analytical platforms, build sufficient chemistry resources to supply the analytical standards required for absolute metabolite quantitation. Examples of metabolomics evaluations of sulfur amino-acid metabolism in psychiatry, neurology, and neuro-oncology and of lipidomics in neurology will be reviewed.

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INTRODUCTION

Metabolomics, including the subfield of lipidomics, involve the analysis of endogenous biomolecules, generally <2000 Da, in complex biological matrices (Nicholson *et al*, 1999). The metabolome consists of a diverse array of biomolecules that are the ultimate products of transcription, translation, and protein activities (Figure 1). As a result of the chemical diversity of these metabolites, no single analytical platform can sample the entire metabolome/lipidome. Mass spectrometric approaches are some of the most robust analytical platforms that provide both structural and sensitive quantitative data for complex biological samples (Koal and Deigner, 2010; Dudley *et al*, 2010; Mishur and Rea, 2012). Mass spectrometry is being

used to analyze the metabolome (Dudley *et al*, 2010), lipidome (Postle, 2012), proteome (Al-Ayadhi and Halepoto, 2013), microRNAs (Izumi *et al*, 2012), epigenetic methylation and acetylation of histones (Britton *et al*, 2011), and DNA methylation (Hu *et al*, 2012). In this review, the focus will be metabolomics and lipidomics platforms and their utility in neurology, neuro-oncology, and psychiatry. Specifically, we will focus on sulfur amino-acid metabolism as an example of metabolomics studies and on plasmalogens as an example of lipidomics studies in Alzheimer's disease.

HISTORICAL BACKGROUND

Mass spectrometry platforms are utilized for the structural elucidation of unidentified biomolecules and to accurately quantitate biomolecules based upon mass selective detection. Advances in methods for the introduction of complex biological extracts into a mass spectrometer, which is under high vacuum, combined with the development of high-resolution and tandem mass spectrometry systems have

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Figure 1. Overview of omics technologies in which mass spectrometry is being utilized.

provided the mass spectrometry platforms used today for metabolomics research. The introduction in 1978 of the first commercial gas chromatograph-quadrupole mass spectrometer with a four-channel PROMIM unit for mass fragmentographic quantitation resulted in a rapid expansion of the uses of gas chromatography-mass spectrometry (GC-MS) in the neurosciences. During the 1970s and 1980s, the laboratories of Erminio Costa, Richard Wyatt, and other early visionaries trained a new generation of scientists in the uses of GC-MS in neuroscience research. A number of general principles were generated during these two decades of research utilizing GC-MS for quantitation of amino acids, biogenic amines, acetylcholine, and psychoactive drugs (see Summary Box: Basic Concepts of Targeted Metabolomics).

Although the majority of studies during this period focused on the targeted analysis of up to 10 metabolites, the first large-scale metabolomics study was published in 1978 (Gates *et al*, 1978). This study characterized over 150 urinary organic acids in a single GC-MS analysis, using the oxime-TMS derivatization procedure that remains popular today for non-targeted GC-MS metabolomics studies.

The next major advance that dominated the 1990s was the introduction of electrospray ionization (Fenn *et al*, 1989) for liquid chromatography tandem mass spectrometry (LC-ESI-MS/MS), with triple-quadrupole instruments dominating the field. This technology had the advantage of not requiring sample derivatization and the ability to analyze polar and large-molecular-weight biomolecules that could not be analyzed by GC-MS. This MS platform soon became a major methodology for drug pharmacokinetic and neurochemical analyses. The only disadvantage to LC-MS and LC-MS/MS is the inferior chromatography relative to gas chromatography.

Basic Concepts of Targeted Metabolomics

- Stable isotope internal standards supply superior accuracy and reproducibility relative to structural analogs or external standards (Ciccimaro and Blair, 2010).
- Metabolite steady-state levels are not reflective of metabolite dynamics for a number of neurochemicals, such that stable isotope precursor labeling to determine turnover/flux dynamics is required. This is clearly the case for acetylcholine (Wood and Péloquin, 1982) and GABA (Wood *et al*, 1988) turnover in the CNS.
- Measurements of the steady-state levels of metabolites generated by enzymes in confined compartments can provide valuable data regarding neurotransmitter release. Examples of this are 3-methoxytyramine as a biomarker of dopamine release and normetanephrine as a biomarker of norepinephrine release (Wood *et al*, 1987). In addition, metabolite dynamics can be assessed by monitoring their accumulation after inhibition of degradation. For example, measurement of the accumulation of 3-methoxytyramine, a metabolite of released DA, after inhibition of its metabolism with a monoamine oxidase inhibitor, provides accurate dynamics of the accumulation of this metabolite (Wood *et al*, 1987). Alternately, measurement of metabolite disappearance after inhibition of synthesis can be monitored.
- Terminal metabolites that are exported out of a compartment for excretion represent useful biomarkers of the dynamics of a defined metabolic pathway. For example, modified nucleosides originating from RNA metabolism are not reutilized for RNA synthesis but are excreted unchanged. These are valuable biomarkers of RNA modifications and metabolism (Bullinger *et al*, 2007).
- Selected ion monitoring (SIM) using chemical ionization, rather than classical electron impact (EI), is superior for quantitation (Wood *et al*, 2006), as this softer ionization results in the bulk of the ion current residing in the $[M+H]^+$ ion in positive chemical ionization (PCI) or in the $[M-H]^-$ ion in negative chemical ionization (NCI). In addition, NCI is generally associated with lower background current from the biological matrix, resulting in 10- to 1000-fold increases in sensitivity compared with EI or PCI (Wood, 1982).
- Tandem mass spectrometry (GC-MS/MS and LC-MS/MS) provides superior specificity in the analysis of complex biological matrices (McLafferty, 1981). Precursor (Q1)/product (Q3) analyses in tandem instruments provide higher levels of specificity and decreased matrix backgrounds relative to GC-MS or LC-MS (Wood and Rao, 1989). However, these analyses predominantly involve tandem quadrupole instruments with unit mass resolution, such that the specificity of product analyses requires rigorous validation with each biological matrix investigated.
- Electrospray (ESI) has dramatically increased our ability to analyze lipids, proteins, nucleic acids, and small molecules (Fenn *et al*, 1989). Coupled with high-resolution mass spectrometry, this ionization method is valuable for 'shotgun' analyses of complex biological samples with no chromatography (Schuhmann *et al*, 2012).

In the 2000s, technical advances led to the development of high-resolution mass spectrometry systems. The first commercial Fourier transform ion cyclotron resonance (Marshall *et al*, 1998), orbitrap (Hu *et al*, 2005), and time-of-flight (Emary *et al*, 1990) mass spectrometers, coupled with HPLC, were introduced into the marketplace. Unit mass resolution, as is obtained with quadrupoles, was replaced by high-resolution (> 140 000) mass analyses, a very advantageous feature for the analysis of complex biological matrices.

Mass Spectrometry and the 'Omics' Technologies

The aforementioned advances in mass spectrometry (MS) strategies have broadened the scope for metabolomics analyses of human diseases and disease models. The 'omics' technologies utilizing MS include epigenomics, metabolomics, lipidomics, and proteomics. The highest value asset of these approaches is that they can readily be applied to patient samples, thus bypassing many of the assumptions

that are intrinsic to all animal models of human disease. In the case of the neurosciences, these technologies can be applied to biofluids (the cerebrospinal fluid, plasma, saliva, urine), brain dialysates, tissue biopsies, post-mortem brain analyses, and human cell lines. In metabolomics research, there is increasing usage of plasma, saliva, and lymphoblasts derived from specific patient populations. In all of these sampling paradigms, a fixed snapshot of a metabolite's steady-state level can be obtained readily.

In clinical studies, a specific patient population is compared with appropriately matched controls in an effort to define previously unrecognized associations between metabolites and clinical end points. Preliminary efforts to define such biomarkers generally utilize non-targeted analyses, with the wealth of data obtained being subjected to extensive deconvolution to identify specific metabolites. The theoretical construct behind this approach is that the analyses are unbiased and will sample a large portion of the metabolome; however, these assumptions have a number of limitations that should not be overlooked:

1. *Bias.* Although non-targeted analytical approaches are termed 'non-biased', they are in fact biased to detect molecules that are present in a sample at high concentrations and biomolecules that ionize more efficiently than competing analytes.
2. *Trace Metabolites.* Non-targeted analyses often do not possess the sensitivity required to detect/monitor trace metabolites. Targeted metabolomics studies utilizing stable isotope internal standards are the superior approaches for monitoring trace but critical metabolites.
3. *Quantitation.* Non-targeted analyses provide relative levels of metabolites, whereas targeted methods generate absolute values. The trade-off is that, in non-targeted analyses, analytical standards are not required, whereas in targeted analyses they are critical. This is the most serious limitation for targeted metabolomics in that there are a large number of metabolites for which analytic standards are not commercially available and even fewer stable isotope internal standards are available. The perfect stable isotope internal standards are ^{13}C , ^{15}N , ^{34}S , and ^{18}O . Deuterated (^2H) internal standards are often used because of their lower cost and greater availability; however, they suffer from differences in retention times in chromatographic systems and from differential solubility in solvents, and in some cases deuterium exchange can occur. Stable isotope internal standards are superior to structural analogs in correcting for ionization efficiency, shifts in retention times, and for sample losses in extraction, transfers, and derivatization (Ciccimaro and Blair, 2010).
4. *Platform Limitations.* No single analytical platform, targeted or non-targeted, is capable of sampling the entire metabolome/lipidome. Multiple platforms are required and demand validation for different biological matrices if reproducible data are to be published. This validation is essential, such that reported metabolites are not artifacts of sample preparation or sample derivatization as has occurred, for example, with a number of reports of untargeted metabolomics studies utilizing the oxime-TMS derivatization protocol for GC-MS. In our laboratory, we utilize GC-MS/MS (Thermo Quantum triple quadrupole) for small molecules that can be volatilized by sample derivatization and nano-LC high-resolution MS (Thermo Q-Exactive orbitrap) for highly charged and high-molecular-weight biomolecules. High-resolution MS is also utilized in which sample interferences cannot be resolved by GC-MS/MS and for direct infusion ('shotgun') analyses.
5. *Matrix Effects.* Co-eluting materials can act to mask the true levels of a metabolite during the ionization process in the MS source, thereby biasing non-targeted analyses. This technical problem can be significantly minimized by the use of stable isotope internal standards in targeted analyses. Nano-LC-MS also offers a potential solution to this issue with non-targeted analyses, as the small droplets produced by the reduced flow rates result in reduced ion suppression from matrix components (Emmett *et al*, 1995; Valaskovic *et al*, 2006).
6. *Unidentified Metabolites.* There is an ever-increasing accumulation of non-identified metabolites in publications of non-targeted research studies. This is an alarming trend in that there is minimal follow-up on the identification of these metabolites.
7. *Identified but not Validated Metabolites.* Unfortunately, there is also an increasing number of metabolomics publications in which metabolites are identified by database searches of recorded masses but which are not validated with analytical standards. Further, follow-up with expanded investigations of ancillary metabolites associated with a given metabolite change is not undertaken. This lack of scientific rigor does not provide the foundation required for translational research.

Direct Infusion ('Shotgun') Analyses

The increased specificity provided by tandem MS and high-resolution MS has stimulated a new trend of constant infusion MS with no chromatography, an approach that has also been termed 'shotgun' MS. This now has also been extended to targeted studies utilizing constant infusion tandem MS multiple reaction monitoring (CI-MRM) of lipids (Han, 2005) and metabolites (Sun *et al*, 2007). These approaches are useful in a number of bioanalytical situations but demand increased vigilance in demonstrating specificity, as these methods cannot distinguish between confounding geometric isomers (eg, L-serine and D-serine), isobars (eg bis(monoacylglycero)phosphate and phosphatidylglycerol), and structural isomers (eg, alanine, β -alanine, and sarcosine).

Targeted Metabolomics Platforms: Steady-State Measurements

Targeted metabolomics assay platforms are extremely useful for hypothesis testing. These experiments require

extensive planning to define the key metabolites in biochemical pathways of interest. Rate-limiting precursors, end-product pools, critical intermediates, and potential alternate precursors or products need to be incorporated into an assay platform. As no single analytical platform can sample all metabolites, this generally requires the design of multiple platforms that provide the greatest selectivity and sensitivity for specific chemotypes. Attempting to sample a broad scope of the metabolome with a single platform inevitably results in limitations for the quantitation of some metabolites and the risk of publishing incorrect data. In the design of targeted metabolomics platforms, the use of transcriptomics and proteomics findings, along with previous findings from non-targeted studies, greatly improves the utility of individualized assay platforms. Intelligent design of targeted assay platforms allows investigators to rationally interrogate defined biochemical pathways in-depth.

Biofluid Compartments

Biopsy and post-mortem tissues are often utilized to generate metabolomics profiles that can define strategies for sampling patient biofluids. In the case of brain studies, biopsy samples are mainly limited to cancer patients. Autopsy tissues are valuable resources when the post-mortem sampling time, storage conditions, and patient clinical information are all accurately recorded.

The optimal biofluid for metabolomics studies of neurological or psychiatric disorders is the cerebrospinal fluid (CSF), which is in intimate contact with brain structures. However, CSF studies are complicated in that they generally involve sampling of the spinal CSF, which is far removed from rostral brain structures where it is formed, and also by the dynamics of CSF export to the bloodstream.

Brain microdialysis is also used to perfuse local brain areas and obtain a profile of localized metabolism (Bianchi *et al*, 2004; Wibom *et al*, 2010). However, CSF collection and brain microdialysis are both invasive procedures. Plasma is the alternate biofluid of choice, as it is more easily sampled and interacts with all tissues, providing an integrated output of changes in the homeostasis of this central compartment. Saliva metabolomics is also an area of increasing investigation because of the ease of obtaining this biofluid (Zhang *et al*, 2009). Investigation of patient plasma cell populations (erythrocytes, platelets, and lymphocytes) also provides useful information of cellular metabolism in relation to plasma measurements:

1. **Erythrocytes:** This is a circulating cell population that lacks organelles such that all proteins have to be imported. A number of transporter functions can be characterized in erythrocytes: for example, erythrocytes accumulate ergothioneine via the SLC22A4 transporter (Gründemann *et al*, 2005); erythrocytes are also useful for assessing the glutathione status as has been done in metabolic studies of schizophrenia (Altuntas *et al*, 2000).

2. **Platelets:** Platelets synthesize a large number of complex mediators that can be quantitated via metabolomic approaches. These cells also possess a large array of transporters that can be assessed, as has been done for the dopamine transporter (Frankhauser *et al*, 2006)
3. **Lymphocytes:** Mediators of immune function can be monitored in this complex cell population. Lymphocytes have been used to assess cellular glutathione status in autism (Suh *et al*, 2008) and mechanisms of DNA methylation in schizophrenia (Zhubia *et al*, 2009).

A further major resource is the availability of lymphoblasts from different disease populations (Coriell Cell Repository; Autism Genetics Resource Exchange). In addition to steady-state measurements of metabolites, transporter function and metabolic flux rates can be monitored with these cells in culture (Wood *et al*, 2011).

Although flux rates can be determined in biofluids via stable isotope precursor labeling to evaluate precursor-product relationships (Creek *et al*, 2012), such clinical studies are rarely conducted at this time. Alternate approaches are to measure levels of rate-limiting precursors in defined pathways as well as stable end-product pools. Measurements of precursor/product ratios provide valuable indices of metabolic dynamics/perturbations (Krone *et al*, 2010), and in cases of differential compartmentation of the precursor and product pools further information regarding the balance between product synthesis and transport rates can be obtained.

Translational Research: Moving Metabolomics Tools to the Clinic

The metabolome is the ultimate product of gene, mRNA, and protein activities (Figure 1). As such, metabolomics has the potential for disease diagnosis, for stratification of patients in a heterogeneous patient population, for monitoring therapeutic efficacy and disease progression, and for defining new therapeutic targets (Nordström and Lewensohn, 2010; Abu-Asab *et al*, 2011). All of these are important properties for neurological and psychiatric diseases, almost all of which are multifactorial conditions not involving a single-gene mutation.

Despite great expectations for translational research in metabolomics, only a limited number of biomarkers have been validated via this approach to date. A number of factors have contributed to this void:

- A large number of metabolomics investigators are oblivious to the complicated issue of heterogeneity in patient populations (Abu-Asab *et al*, 2011). This heterogeneity demands that the normal range for metabolites be established as a part of a comprehensive metabolomics study. Subsequent subtyping of patients within a given disease diagnosis will ultimately provide superior diagnostic tools to support personalized patient care.

- The vast majority of published metabolomics studies are statistically under-powered, limiting the ability to draw firm conclusions. There are numerous reports of patient populations with an N of 20, which is entirely insufficient to evaluate heterogeneous patient groups.
- There is an alarming trend to present large heat maps of metabolomics data without reporting the essential/critical metabolites that are changed and the magnitude of their perturbation. This is analogous to a fisherman returning home and presenting the fish echogram but not presenting the fish he/she caught.

Familial vs Sporadic Disease Populations

Patients with neurological disorders are predominantly those with sporadic disease. However, there are also smaller numbers of familial disease populations, as is observed in Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS). The intuitive assumption is that heterogeneity is less in a familial disease population. This has in fact been clearly validated with metabolomics studies of ALS-cerebrospinal fluid in sporadic and familial patients (Wuolikainen *et al*, 2011). However, heterogeneity in both familial and sporadic patient populations involves a number of factors that need to be considered in the analysis of metabolomics data sets:

- Differing rates of disease progression
- Environmental and lifestyle factors
- Differing phenotypic profiles of gastrointestinal microflora
- Co-existing pathologies
- Potential of alternate pathways to result in a final common pathology

Tiered Analyses of Biological Samples

In our studies of clinical samples, we utilize a tiered approach to identify points of interest in biochemical dysfunction and rapidly generate quantitative data. First we obtain broad-range, high-resolution (140 000 @ 200), negative and positive ion spectra (200 to 2000 daltons) with direct infusion ESI (Schumann *et al*, 2012). For these studies, we analyze both methyl-tert butyl ether (Wood *et al*, 2010) and acetonitrile/methanol/formic acid extracts (Wood *et al*, 2011), with each extraction solution containing a number of stable isotope internal standards across the analysis range of molecular weights. When biochemical differences are detected in a defined patient population, we next expand the analysis of that pathway and interconnected pathways and implement chromatographic methods to validate the observations. Once a biochemical change is better characterized, we next evaluate circulating blood cells (erythrocytes and platelets), and, if available, we utilize patient lymphoblasts for precursor-labeling studies. This tiered and integrated approach rapidly yields data that

define the potential etiology and consequences of detected metabolic changes and define which analytical standards are required to purchase, thereby containing operational costs.

Metabolomics in Psychiatric Disorders: Sulfur Amino-Acid (SAA) Metabolism in Autism and Schizophrenia

The NIMH Research Domain Criteria (RDoC) initiative has been formulated to advance research focused on defining the etiologies of mental health disorders (Morris and Cuthbert, 2012). As mental illness is considered to be the result of dysfunctional brain circuits, metabolomics has considerable value in contributing to this initiative. Non-targeted metabolomics may reveal unexpected biochemical observations, whereas targeted metabolomics platforms, designed to investigate focused hypotheses of neuronal dysfunction, are more likely to generate the data required to discount or validate hypothetical constructs of biochemical deficits or excesses in mental illness.

In multifactorial diseases, a patient's phenotype is dependent upon metabolic dysfunctions in multiple pathways; therefore, a given metabolic pathway may be involved in more than one disease population and may demonstrate heterogeneous expression within a given disease population. To exemplify this approach, we will review the current status of sulfur amino-acid metabolomics in schizophrenia and autism, two psychiatric neurodevelopmental disorders that are characterized by significant heterogeneity in phenotypic expression. A key principle of modern metabolomics is that, if a significant perturbation in a metabolic pathway is detected in a non-targeted analysis, then other metabolites in that pathway, or in alternate pathways where metabolites can be diverted, should also be detected in a more targeted metabolomics analysis. In the case of thiol pathways, the interrelationships of many complex and compartmentalized metabolites make this targeted analysis difficult but relevant, as thiols subserve a diverse array of metabolic and epigenetic functions.

In both patient populations, sulfur amino-acid metabolism is altered in a significant subset of patients. These observations are sometimes considered disturbing; however, such a view is not appropriate as this type of heterogeneity is consistent with the multifactorial basis of these diseases.

As presented in Figure 2, SAA metabolism is involved in a diverse array of biochemical pathways that regulate the following:

1. Endogenous antioxidant pools of glutathione (GSH).
2. Epigenetic methylation of DNA and methylation of numerous substrates. S-adenosylmethionine (SAM) is the methyl donor for a diverse array of methyltransferases.
3. Polyamine synthesis, which requires SAM also as an aminopropyl donor.

Figure 2. Schematic presentation of thiol metabolic pathways. CoA, coenzyme A; Moco, molybdopterin cofactor; SAM, S-adenosylmethionine; SAMI, decarboxylated S-adenosylmethionine.

4. The synthesis of 3-amino-3-carboxypropyl, which is involved in the post-translational modification of histidine in protein synthesis of elongation factor 2 and in the synthesis of wybutosine, a tRNA tricyclic nucleotide Lin (2011).
5. Sulfation pathways involving 3'-phosphoadenosine-5'-phosphosulfate (PAPS)-dependent sulfonation of sphingolipids, cholesterol, steroids, neurotransmitters, bile acids, peptides, proteins, and xenobiotics.
6. Enzymes dependent on molybdopterin cofactor (Moco). Cysteine metabolism by cysteine desulfurase (EC 2.8.1.7) is a critical step in the biosynthesis of Moco (Rouault, 2012), in which molybdenum is covalently bound to the dithiolate moiety of molybdopterin (Havemayer *et al*, 2011). There are a number of Moco-dependent enzymes that could affect neuronal function in schizophrenia and in autism. These include mitochondrial amidoxime-reducing component (mARC), an enzyme complex dependent upon molybdopterin cofactor, sulfite oxidase (EC 1.8.2.1, 1.8.3.1), and aldehyde oxidase (EC 1.2.3.1). mARC also is responsible for the reduction of N^ω-hydroxy-arginine to arginine (Kotthaus *et al*, 2011).

Metabolomics evaluations of plasma in schizophrenia (Wood and Wood, 2013) and autism have demonstrated a number of common decrements in sulfur amino acids, including a decreased antioxidant capacity (decreased glutathione). However, the unique differences in SAA metabolism include increased methionine in schizophrenia and decreased inorganic sulfate in autism (Table 1).

The increases in methionine provide the capacity to synthesize greater amounts of SAM, which in turn can lead to altered methylation status of a number of metabolites and macromolecules like DNA. In this regard, increased levels of SAM have been measured in the prefrontal cortex in schizophrenia (Vuksan-Ćusa *et al*, 2011), a brain area characterized by abnormal GABAergic neuronal development, potentially as a result of GABAergic promoter hypermethylation (Costa *et al*, 2009). Pathways dependent

upon aminopropyl or 3-amino-3-carboxypropyl donation via SAM-dependent mechanisms remain to be explored but could be responsible for a number of complex alterations in genetic regulation in schizophrenia.

In addition to its role in glutathione synthesis, cysteine is oxidized to form sulfate, the essential sulfur function required for the synthesis of 3'-phosphoadenosine-5'-phosphosulfate (PAPS), the major sulfur donor in sulfation reactions (Figure 2). Decreases in circulating sulfate levels in autism (Waring and Klovra, 2000; Geier *et al*, 2009; Adams *et al*, 2011; Kern *et al*, 2011; Bowling *et al*, 2012) are further reflected by decreased levels of dehydroepiandrosterone sulfate (DHEA-S) in autistic adults (Strous *et al*, 2005) and decreased ability to sulfonate acetaminophen in children (Alberti *et al*, 1999). More in-depth evaluation of sulfonation mechanisms and metabolites is clearly needed in autistic individuals, as this potentially includes steroids, neurotransmitters, bile acids, peptides, and xenobiotics.

In summary, a number of alterations in sulfur amino-acid metabolism have been reported for both autism and schizophrenia (Table 1). The data are sufficient to warrant implementation of focused metabolomics platforms to evaluate these complex changes in larger cohorts of patients.

Metabolomics in Neurology: SAA Metabolism in Amyotrophic Lateral Sclerosis (ALS)

There have been a limited number of metabolomics studies in ALS. In a non-targeted metabolomics evaluation of ALS plasma, decrements in catechol sulfate and 4-vinylphenol sulfate were measured (Lawton *et al*, 2012). These are microbial-host metabolites in that catechol and vinylphenol are microbial metabolites from gastrointestinal flora, although the sulfation of these metabolites is performed in the human liver. These data suggest that, as in autism, decreased sulfation capacity may occur in ALS. However, in contrast to autism, plasma inorganic sulfate is not decreased but may be slightly elevated (Pean *et al*, 1994; Woolsey, 2008). Plasma cysteine (Pean *et al*, 1994; Woolsey, 2008) and pantothenic acid (Lawton *et al*, 2012) also appear to be elevated in ALS, which are further indicators of abnormal SAA metabolism in this devastating disorder.

Non-targeted metabolomics evaluations of ALS cerebrospinal fluid (CSF) further support abnormal SAA metabolism in ALS, with decrements in both methionine and putrescine being reported (Wuolikainen *et al*, 2011). These data are suggestive of abnormal SAM function in ALS, a suggestion that can be examined using targeted SAA metabolomics platforms.

In summary, as in autism and schizophrenia, alterations in sulfur amino-acid metabolism in ALS suggest that targeted metabolomics studies should now follow preliminary non-targeted findings.

Metabolomics in Neurology: Plasmalogens in Alzheimer's Disease (AD)

In the search for biomarkers of AD, the focus has been extensively confined to the evaluation of proteins in plaques

TABLE 1 Plasma Sulfur Amino-Acid Metabolomics in Autism Spectrum Disorders (ASD) and in Schizophrenia Patients (SP)

Metabolite	Pathway	Change	Reference
Methionine	Methionine cycle	ASD: ↓ SP: ↑	James <i>et al</i> , 2004; Geier <i>et al</i> , 2009; Pastural <i>et al</i> , 2009; Paşca <i>et al</i> , 2009 Bjerkenstedt <i>et al</i> , 1985; Yang <i>et al</i> , 2013; Orešič <i>et al</i> , (2011)
Homocysteine	Methionine cycle	SP: ↑	Bjerkenstedt <i>et al</i> , 1985; Regland, 2005; Muntjewerff <i>et al</i> , 2006; Kim and Moon, 2016; Vuksan-Ćusa <i>et al</i> , 2011
Cysteine	Trans-Sulfuration	ASD: ↓ SP: ↓	James <i>et al</i> , 2004; Geier and Geier, 2006; Pastural <i>et al</i> , 2009; Paşca <i>et al</i> , 2009; Geier <i>et al</i> , 2009 Dietrich-Muszalska <i>et al</i> , 2012; Gysin <i>et al</i> , 2011
Cystine	Cysteine dimer	SP: ↓	Orešič <i>et al</i> , (2011)
Ornithine	Polyamine precursor	SP: ↑	Orešič <i>et al</i> , (2011); He <i>et al</i> , 2010
Inorganic sulfate	Inorganic sulfur metabolism	ASD: ↓	Waring and Klovra, 2000; Geier <i>et al</i> , 2009; Adams <i>et al</i> , 2011; Kern <i>et al</i> , 2011; Bowling <i>et al</i> , 2012
Glutathione (GSH)	Antioxidant	ASD: ↓ SP: ↓	James <i>et al</i> , 2004; Geier and Geier, 2006; Pastural <i>et al</i> , 2009; Geier <i>et al</i> , 2009; Adams <i>et al</i> , 2011; Kern <i>et al</i> , 2011 Dietrich-Muszalska <i>et al</i> , 2012
Cysteinylglycine	GSH metabolism	SP: ↓	Dietrich-Muszalska <i>et al</i> , 2012
Taurine	Taurine biosynthesis	ASD: ↓	Geier <i>et al</i> , 2009; Adams <i>et al</i> , 2011

Up arrows indicate increases and down arrows indicate decreases.

and tangles, hallmark features of AD pathology at autopsy. However, these proteins accumulate in the brain with normal aging and are present at levels comparable to those in AD patients, in non-agenarians who demonstrate minimal cognitive deficit (Erten-Lyons *et al*, 2009; Maarouf *et al*, 2011). These factors render these proteins and their peptide metabolites of limited value in the diagnosis of AD (Bazenet and Lovestone, 2012).

Similarly, metabolomics studies have not generated reliable biomarkers of AD. In contrast, a number of lipidomics alterations in AD have been demonstrated in post-mortem brain and in the plasma of AD patients (Florent-Bécharde *et al*, (2009); Astarita *et al*, 2010; Wood, 2012). The most robust alterations are decrements in brain sulfatides (Han *et al*, 2002) and decreases in ethanolamine plasmalogens (PlsEtn) in both brain (Ginsberg *et al*, 1995; Han *et al*, 2001; Han 2005) and plasma (Goodenowe *et al*, 2007; Wood *et al*, 2010). These lipid classes are complex structural lipids that also serve as reservoirs for lipid mediators of signal transduction.

Sulfatides and white matter ethanolamine plasmalogens (eg, PlsEtn18:0/18:1) are decreased early in the disease process, supporting MRI imaging studies that have demonstrated fiber tract disruption in mild cognitive impairment (MCI) and in the early phases of AD (Stricker *et al*, 2009). In contrast, gray matter ethanolamine plasmalogens (eg PlsEtn18:0/22:6) decrease in a disease severity-dependent manner. Decreases in plasmalogen synthesis have been demonstrated in both the AD liver (Astarita *et al*, 2010) and brain (Kou *et al*, 2011; Grimm *et al*, 2011).

Decreases in AD cortical sulfatides are paralleled by large increases in cortical (Han *et al*, 2002, Cutler *et al*, 2004; He *et al*, 2010), CSF (Satoi *et al*, 2005), and plasma (Han *et al*, 2011) ceramides, which are precursors/degradation products of sulfatides. In contrast, another study has reported no differences in plasma ceramide levels in AD (Mielke *et al*, 2010) but decreased plasma levels in MCI. Immunohistochemistry studies suggest that the increases in cortical

ceramides are mainly in astrocytes (Satoi *et al*, 2005). The potential signaling effects of alterations in brain ceramides in AD remain to be determined.

In summary, brain glycerophospholipid and sphingolipid metabolisms are dramatically affected in AD white matter early in the disease process. Further, decrements in myelin sulfatides and PlsEtn support imaging studies, demonstrating dysmyelination in MCI and AD patients. The utility of measuring decrements in plasma PlsEtn as a biomarker of AD requires further validation in larger cohorts of AD patients.

Metabolomics in Neuro-Oncology: SAA Metabolism in Glioblastoma

Glioblastoma is a devastating cancer with incredibly aggressive growth rates and an associated poor prognosis. Although a number of gene changes have been reported in glioblastoma, there have been limited metabolomics studies conducted. However, there are a number of consistent changes in SAA metabolism that may contribute to tumor progression. For example, hypotaurine and its metabolic end product taurine are elevated in glioblastoma microdialysates in GBM patients (Bianchi *et al*, 2004, Wibom *et al*, 2010), suggesting increased synthesis and release of taurine by GBM cells. This conclusion is also congruent with observations of decreased blood levels of microRNAs (Roth *et al*, 2011) that regulate cysteamine dioxygenase (EC 1.13.11.19; cysteamine→hypotaurine), the rate-limiting enzyme involved in the synthesis of hypotaurine.

Further evidence of abnormal sulfur amino-acid metabolism includes increased cysteine and decreased cystathionine in tumor microdialysates in GBM patients (Wibom *et al*, 2010). Decrements in blood levels of microRNAs (Roth *et al*, 2011) regulating cystathionine γ -lyase (EC 4.4.1.1; cystathionine→cysteine) also are consistent with these observations.

S-methylcysteine metabolism has not been studied extensively, but this SAA is avidly accumulated by glioblastoma tumors (Deng *et al*, 2011) and is increased in tumor microdialysates (Wibom *et al*, 2010). The metabolic sources of S-methylcysteine remain to be defined, but alterations in DNA repair by o-6-methylguanine-DNA methyltransferase (EC 2.1.1.63; DNA-6-o-methylguanine + protein-cysteine → DNA + protein S-methylcysteine) is one potential source, suggesting that this may represent a unique biomarker of dysregulation of DNA repair mechanisms in GBM.

In summary, sulfur amino-acid metabolism is dysfunctional in GBM patients and more in-depth studies of SAA pathways are warranted.

Overview

Advancements in mass spectrometry have revolutionized our abilities to analyze complex biological matrices. The pitfalls regarding the specificity of colorimetric and ELISA assays and the use of non-selective detectors in chromatographic systems have been overcome by mass selective detection and quantitation of biomolecules. Although there is currently a rapid expansion of metabolomics data in the literature, based upon mass spectrometric techniques, the next critical step is to move this wealth of information into focused translational research studies such that new clinical tools are realized within the next 10 years. Funding agencies are aggressively seeking translational research proposals in metabolomics. The complexity that these agencies, as well as potential investigators, have to consider is that biomarker discovery and validation (ie, a validated assay for reporting absolute patient values) requires a multidisciplinary team to achieve success. The analytical team includes synthetic organic chemists (analytical standards), analytical chemists, biochemists, statisticians, and regulatory expertise. The clinical team requires clinical researchers, practicing physicians, phlebotomists, nurses, research assistants, and their office staff. Additionally, access to multiple clinical sites is required to attain sufficient numbers of subjects to define potential subsets of patients in a multifactorial disease. In essence, a great deal of planning, collaboration, and expertise will need to be coordinated to advance metabolomics findings into clinical practice.

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