J Neuroimmune Pharmacol (2015) 10:391–395
DOI 10.1007/s11481-015-9621-1

COMMENTARY

The Role of Metabolomics in Brain Metabolism Research

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Received: 19 June 2015 / Accepted: 19 June 2015 / Published online: 23 July 2015

Abstract

This special edition of the Journal of Neuroimmune Pharmacology focuses on the leading edge of metabolomics in brain metabolism research. The topics covered include a metabolomic field overview and the challenges in neuroscience metabolomics. The workflow and utility of different analytical platforms to profile complex biological matrices that include biofluids, brain tissue and cells, are shown in several case studies. These studies demonstrate how global and targeted metabolite profiling can be applied to distinguish disease stages and to understand the effects of drug action on the central nervous system (CNS). Finally, we discuss the importance of metabolomics to advance the understanding of brain function that includes ligand-receptor interactions and new insights into the mechanisms of CNS disorders.

Keywords Metabolomics · Brain energy metabolism · Central nervous system disorders · Mass spectrometry · Nuclear magnetic resonance spectroscopy

Metabolomics represents a final piece of the 'omic puzzle in systems biology. The metabolome is defined as a complete set of metabolites (or *low-molecular-weight biomolecules)* that provide biologically relevant endpoints of metabolic processes encompassing the products of interaction between gene expression, protein expression and the cellular environment (Patti et al. 2012; Zamboni et al. 2015). Advances in genomics provide the link between human disease and its genome origins. Metabolomics characterizes genetic risk factors for disease by its abilities to reveal changes that take place as a net result of interactions between the genome and the environment and as such bridges the gap between genotype and phenotype (Kaddurah-Daouk et al. 2008) (Fig. 1). Therefore metabolomics, as an ultimate counterpart to genomics, transcriptomics and proteomics, could have considerable impact on our understanding of the cellular and molecular bases for disease. The rapidly growing interest to explore the metabolome is led by the advancements in analytical technology, the

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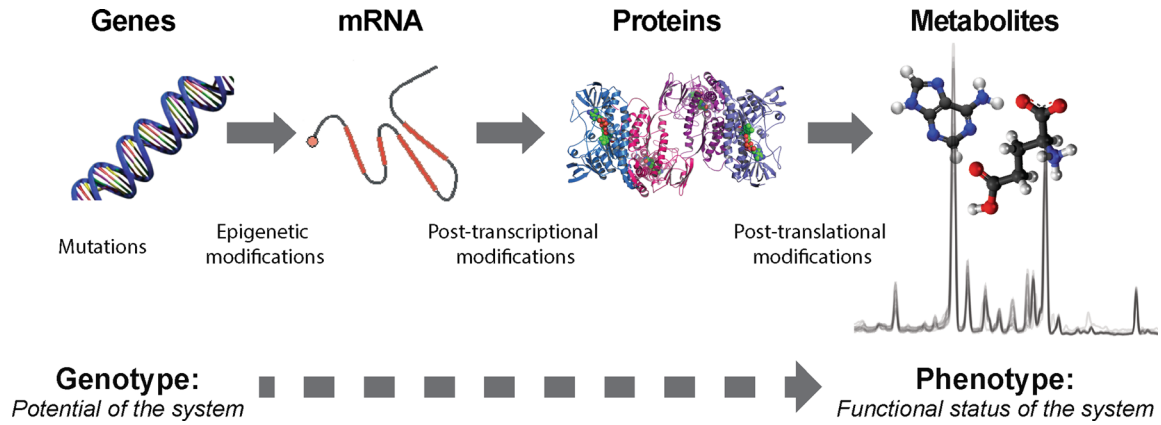

Fig. 1 Central dogma of molecular biology and systems biology. Metabolites are biologically relevant endpoints that encompass the downstream products of cellular activity. They serve as a direct read out of the biochemical activity closely associated with phenotype of the biological system

development of metabolite databases, and computational tools that enable the generation and analysis of big data sets (Benton et al. 2015; Johnson et al. 2015a; Rinehart et al. 2014; Tautenhahn et al. 2012) (Fig. 2).

Brain function has a high metabolic cost, reaching 20 % of the whole-body energy consumption in humans (Mink et al. 1981). Energy demands have to be met to insure finely tuned signaling activity and cognitive function. Recent evolutionary biology findings suggest a massive increase in the expression of genes involved in energy production in the human cortex (when compared to other nonhuman primates) and have stimulated rethinking of brain energy metabolism and its role in signaling activity and brain function (Caceres et al. 2003; Magistretti and Allaman 2015). Alterations in central carbon metabolism (i.e., glycolysis, oxidative phosphorylation, pentose phosphate pathway) in addition to signal transmission play a key role in the pathogenesis of neurodegenerative (Alzheimer's and Parkinson's diseases, Huntington's disease) and psychiatric (depression, schizophrenia, bipolar disease) CNS disorders. Small molecules rigorously mediate metabolic processes and signaling pathways. These include, but are not limited to, energy currency metabolites, neurotransmitters, secondary messengers and cell membrane constituents. Among different molecular assays, noninvasive neuroimaging techniques like positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have been widely used to study changes in brain metabolism through the measurements of cerebral blood flow, glucose utilization and oxygen consumption (Lin and Rothman 2014; Magistretti and Allaman 2015). In addition, high-field magnetic resonance spectroscopy (MRS) has permitted the visualization of metabolite distributions and metabolic flux analysis in vivo by the introduction of stable isotope-labeled compounds (Duarte et al. 2012; Lin and Rothman 2014). However, these techniques are mostly a

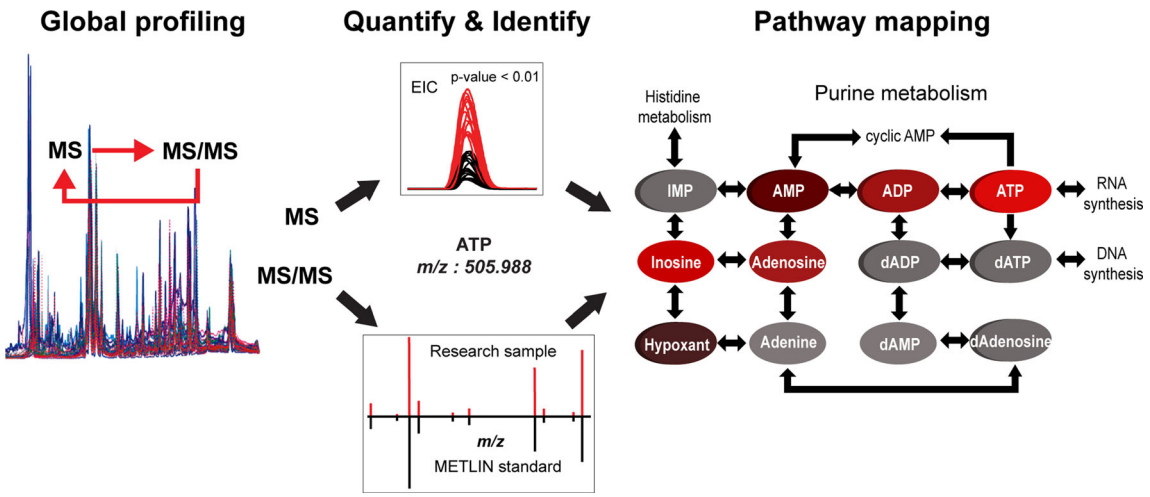

Fig. 2 Accelerated global profiling by simultaneous quantification and identification of metabolites, followed by pathway mapping. The advances in mass spectrometers enable the sequential collection of high quality MS (for comparative quantitative analysis of different groups of samples) and MS/MS data (for MS/MS matching to facilitate metabolite identification) in a single run. ATP was identified and mapped onto purine metabolism pathway among other dysregulated metabolites (*red color* tone – level of significance)

priori hypothesis-based and limit neurochemical profiling to a small subset of known highly abundant metabolites, such as glucose, lactate, N-acetyl aspartate (NAA), myo-Inostiol, creatine, choline, glutamate and glutamine.

Global metabolomics, as an alternative screening approach complements neuroimaging techniques by providing unbiased monitoring of a broad range of changes in brain metabolism, including low abundant and trace metabolites, from organismal (systemic blood analysis) and whole-organ level down to regional, cellular and sub-cellular level. In this context metabolomics can provide powerful tools to measure early biochemical changes and indicate metabolic pathway shifts associated with CNS disorders (Kaddurah-Daouk et al. 2008; Zamboni et al. 2015). This more comprehensive overview of changes will favor the understanding of mechanisms of specific disorders, the identification of biomarkers for early diagnosis of disease, deciphering and monitoring of the disease progression and/or drug response and ultimately translation of findings to clinic by defining new molecular targets for therapeutic intervention (Chen-Plotkin 2015; Wood 2014).

Brain metabolomics is particularly challenging due to the intrinsic inaccessibility (encapsulation within the blood–brain-barrier-BBB) of the brain and the high-energy demand necessary to maintain tissue function. Different analytical platforms, NMR, GC/MS and LC/MS-based, have been used to perform the metabolite profiling of biofluids (the cerebrospinal fluid, plasma, saliva, urine), brain tissue (from biopsies or post-mortem brain analysis) and brain cell lines. Several studies have shown that the metabolite biomarkers can be detected in easily accessible biofluids, however it is not clear to which extent the biofluid's content reflects the tissue activity due to the limited passage of many metabolites across the BBB (Griffin and Salek 2007). Microdialysis of metabolites from a brain tissue represents a compelling innovative strategy to assess the metabolite concentrations directly from the brain interstitial fluid, as opposed to measuring the concentrations in the blood plasma or serum (Kao et al. 2015). When the brain tissue is available from animal models, the intelligent experimental design with a highlight on an adequate metabolism quenching strategy (i.e., focused beam microwave irradiation - FBMI, funnel freezing) is essential for brain sample preparation (Epstein et al. 2013; Griffin and Salek 2007; Ivanisevic et al. 2014). Human brain tissue is difficult to procure, especially in the case of healthy control samples, and the post-mortem brain studies are further complicated through metabolic alterations that may occur in hypoxic conditions during the postmortem delays. When planning the experimental design in brain metabolomics, the high complexity of brain is also worth considering, including regional and cellular heterogeneity with distinctive metabolic profiles, like predominant glycolytic profile of astrocytes vs. oxidative profile of neurons (Magistretti and Allaman 2015). Therefore, regional brain profiling and comprehensive studies of different cell types are strongly encouraged in order to characterize their complementarity in brain function. Subregional and even cellular and subcellular resolution can be achieved by mass spectrometry imaging (i.e., nanostructure surface based – NIMS (Kurczy et al. 2015), secondary ion based – SIMS (Kurczy et al. 2010)) thus adding information about spatial distribution of metabolites that is obscured by the gross analysis of the whole (Ivanisevic et al. 2014) (Fig. 3).

The papers from this thematic issue cover several case studies where different analytical platforms and metabolomic approaches were applied to characterize the signatures of CNS disorders in humans, to distinguish neurochemical changes in genetically modified animal models, to investigate antimetabolites and drug response for treatement of neuropathic pain and schizoprenia, and to better define the role of GABA receptors. Drs. Laetitia Davidovic and Marc-Emmanuel Dumas discuss the advancements in the field of metabolomics that allowed for global metabolite profiling. They highlight the fact that the reports of global metabolite profiling have moved forward the characterization of CNS disease signatures showing that brain

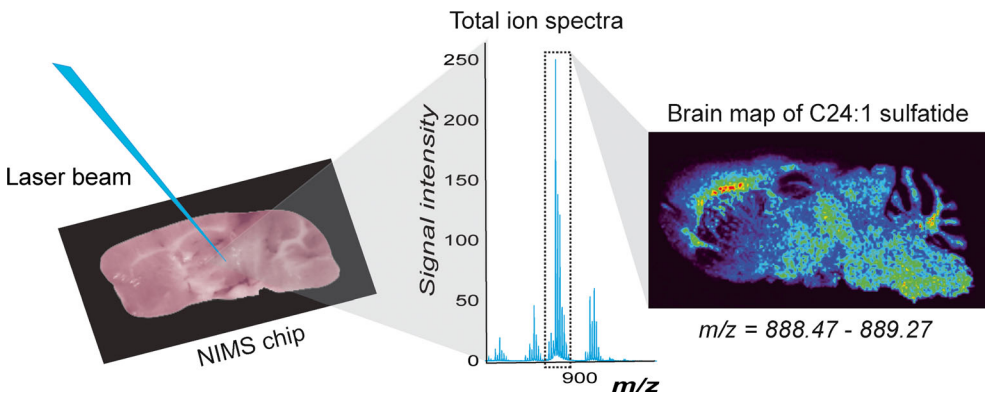

Fig. 3 Nanostructure Imaging Mass Spectrometry (NIMS) of mouse brain. Extracted brain map shows the *white-gray* matter distribution of one brain sulfatide. Image was acquired from a 2 uM brain section that was mounted on etched silicon chip, coated with perfluorinated-amino initiator, prior to imaging using laser desorption ionization technique in negative ionization mode. Data were acquired at 50 uM-spatial resolution of a laser beam

metabolism disorders are not only related to altered signal transduction but also the significant variations in central carbon pathways (Dumas and Davidovic 2015). Following this comprehensive review that focuses on key studies in the field of brain metabolomics, Dr. Joseph L. McClay and his collaborators present a story of global, murine brain tissue metabolomics to decipher the response and mechanism of action of an antipsychotic drug in the treatment of schizophrenia. They further demonstrate the power of global metabolomic screening to reveal the disruption of sphingolipid metabolism during chronic haloperidol administration (McClay et al. 2015). They also confirm the impairment of NAAG signaling in antipsychotic drug mechanisms of action. In a similar research line, Dr. Caroline H. Johnson and her collaborators explored the effects of two different therapeutics for neuropathic pain relief (Johnson et al. 2015b). In addition Dr. Alex Mountfort Dickens and his collaborators demonstrate the sensitivity of biofluid metabolomic analysis to discriminate between progressive disease stages and validate the changes that were conserved in humans and the mouse model (Dickens Mountfort et al. 2015). Finally, Dr. Caroline Rae and her team present the targeted NMR-based pharmacometabolomic approach that revealed the distinct metabolic activity of GABAρ receptors implying their specific physiological function (Rae et al. 2015).

To conclude, with regards to challenges encountered in the application of large-scale, high-throughput global metabolomic approaches, it is important to note the value of targeted validation, as a foundation toward translational research (Fig. 4). Although the snapshots of biofluid or tissue metabostasis (metabolite homeostasis) can be obtained readily using the global approach, the limitations of this approach (i.e., bias due to matrix effects, ionization efficiency and metabolite identification bottleneck) have to be addressed and the results validated via a targeted approach. In-depth investigation of findings from an untargeted study should be followed up through the targeted study of the identified affected and interconnected biochemical pathways (Wood 2014). The integration of findings from metabolomics along with those from other omic technologies and non-invasive in vivo imaging techniques (i.e., PET, fMRI, MRS), followed up by stringent targeted investigation of altered biochemical pathways in complex disease states, like many neurodegenerative and psychiatric diseases, will provide valuable insights into molecular mechanisms of disease as well as potential biomarkers for disease diagnosis. The means to study the variation in heterogeneous patient metabotypes (e.g., with regards to drug response) are furthermore opening the road toward personalized medicine.

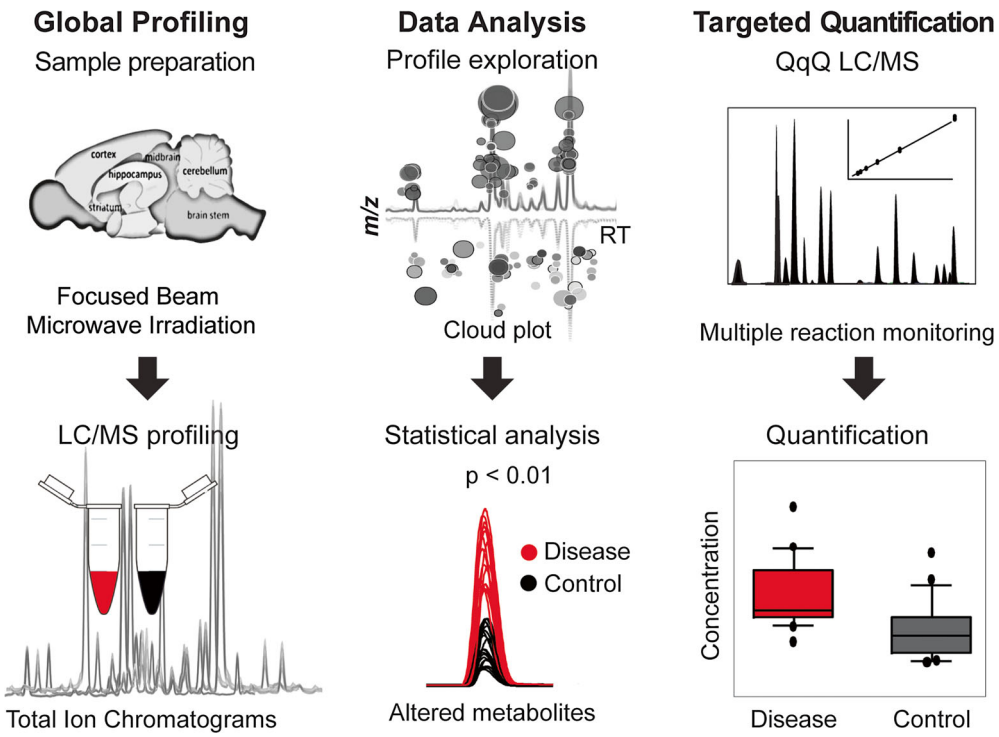

Fig. 4 Metabolomic workflow. Global profiling summarizes the experimental design with respect to metabolism quenching and global LC/MS profiling of different sample groups. LC/MS data acquisition is followed by retention time correction for chromatogram alignment and visualization of dysregulated metabolite features. Metabolite features whose levels were significantly changed in disease vs. control samples are than filtered out and identified by MS/MS matching. The identified metabolites are quantified by targeted MRM analysis using standard compounds

Acknowledgments We thank the contributors for this thematic edition, as well as Dr. Howard Gendelman, Editor-in-Chief of this journal and Reed Felderman, communication specialist, for their initiative and support in assembling this edition.

Funding This study was funded by XCMS Metabolomic Data Technology- R01 GM114368-02, R01 CA170737-02 (NIH), and 2P01DA028555-06.

Conflict of Interest Authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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