Review Article

Title:

Metabolic profiling and phenotyping of central nervous system diseases: metabolites bring insights into brain dysfunctions

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Keywords: Metabonomics/metabolomics/metabolic profiling, nuclear magnetic resonance, mass spectrometry, systems biology, biomarker, CNS disease

Abbreviations:

¹H NMR, proton nuclear magnetic resonance;
AD, Alzheimer's disease;
ALS, amyotrophic lateral sclerosis;
ASD, autism spectrum disorder;
BCAA, branched chain amino acids;
BP, bipolar disorder;
CNS, central nervous system;
CSF, cerebrospinal fluid;
DA, discriminant analysis.
DMA, dimethylamine;
FXS, Fragile X Syndrome;
GABA, γ-aminobutyric acid;
GC/LC, gas/liquid chromatography;
HR, high resolution
MAS, magic angle spinning;
MDD, major depressive disorder;
MS, mass spectrometry;
NAA, *N*-acetyl-aspartate;
O, orthogonal
PCA, principal component analysis;
PD, Parkinson's disease;
PIP, phosphatidyl-inositol-phosphate;
PIP2, phosphatidyl-inositol-diphosphate;
PLS, partial least squares;
SCA3, spinocerebellar ataxia 3;
SCFA, short chain fatty acids;
SCZ, schizophrenia;
TCA, tricarboxylic acid;
TMA, trimethylamine;
TMAO, trimethylamine-*N*-oxide;
UPLC, ultra performance liquid chromatography

Abstract

Metabolic phenotyping corresponds to the large-scale quantitative and qualitative analysis of the metabolome ie. the low-molecular weight <1KDa fraction in biological samples, and provides a key opportunity to advance neurosciences. Proton nuclear magnetic resonance and mass spectrometry are the main analytical platforms used for metabolic profiling, enabling detection and quantitation of a wide range of compounds of particular neuro-pharmacological and physiological relevance, including neurotransmitters, secondary messengers, structural lipids, as well as their precursors and, intermediates and degradation products. Metabolic profiling is therefore particularly indicated for the study of central nervous system by probing metabolic and neurochemical profiles of the healthy or diseased brain, in preclinical models or in human samples. In this review, we introduce the analytical and statistical requirements for metabolic profiling. Then, we focus on key studies in the field of metabolic profiling applied to the characterization of genetically modified animal models and human samples of central nervous system disorders. We highlight the potential of metabolic profiling for pharmacological and physiological evaluation, diagnosis and drug therapy monitoring of patients affected by brain disorders. Finally, we discuss the current challenges in the field, including the development of systems biology and pharmacology strategies improving our understanding of metabolic signatures and mechanisms of central nervous system diseases.

Introduction

While the complexity of the central nervous system presents unique challenges for developing novel therapies, the diagnosis of CNS disease currently relies on compiling and grading a bunch of clinical symptoms. This is particularly valid when considering diseases of unknown etiology such as Alzheimer's disease, schizophrenia (SCZ) or autism-spectrum disorders (ASD). One limitation of the currently available diagnosis tools is that, in most cases, the disease has to be well established for a clear diagnosis, even though early therapeutic intervention is crucial for improving prognosis of CNS diseases. There is therefore an urgent need for the development of biomarkers that can help anticipate diagnosis even before clinical signs become apparent. This will allow a more targeted and effective treatment and also to monitor treatment efficacy.

Metabolites are both intermediates and endpoints of biological processes. Their identity and abundance directly reflect biological perturbations originating not only from collective internal variations in the genome, transcriptome and proteome, but also from environmental cues (diet, lifestyle, drug intake and toxicological exposure) (Nicholson et al. 2002). Therefore, an emerging field of investigation of the central nervous system lies in the study and characterization of the metabolome: the entire complement of low-molecular weight chemicals smaller than 1 KDa present in a given sample. Among known metabolites of relevance in the context of CNS study lie notably energetic substrates, neurotransmitters, neurochemicals and structural lipids. Large-scale analysis of the metabolome is achieved through the use of metabolomics, metabonomics or metabolic profiling. Metabolomics was originally defined as the detailed qualitative and quantitative analysis of the metabolites present in complex biological samples (Oliver et al. 1998), whilst metabonomics sought the measurement of the global metabolic response of living systems to pathological stimuli or genetic manipulation (Nicholson et al. 2002; Nicholson et al. 1999). Nowadays the nuances between these two terms have faded and the term metabolic profiling is widely used. Unlike genomics, transcriptomics or proteomics approaches that focus primarily on DNA-encoded information, metabolic profiling provides robust and quantifiable metabolic phenotypes called metabotypes (Gavaghan et al. 2000). Metabotypes can be seen as the intermediary metabolic phenotypes existing between DNA variations and clinical disease phenotypes observed in animal models or clinical samples ((Dumas 2012), Fig. 1). Metabolic profiling strategies deliver unique metabolic signatures that are characteristic of the conditions under scrutiny. The metabolic signature can be established in a variety of biological matrices.

Alterations of normal brain functions can directly impact CSF or plasma composition in which metabolites are in dynamic equilibrium with intracellular and intra-tissular metabolites. In addition, metabolites in biofluids can be used to trace chemical imbalances at the brain level in the case of CSF, or at the systemic level in the case of blood and urine. This is key to identifying circulating and excreted markers related to CNS disease (i.e. "distant markers"), which can be useful for disease diagnosis, prognosis and long-term treatment efficacy monitoring purposes. In the last decade, studying the metabolome of brain tissue, cerebrospinal fluid (CSF), plasma, serum or urine of animal models and patients has yielded to the identification of metabolic signature specific of CNS disorders (Fig. 1).

In this review, we will first describe the two main analytical techniques used for metabolic profiling of CNS conditions: proton nuclear magnetic resonance (^1H -NMR) spectroscopy and mass spectrometry (MS). We will also discuss the importance of chemometrics to model metabolic changes in the brain and the systems biology approaches currently in development for integration of metabolic data. Then, we will highlight the major findings using metabolic profiling of CNS disorders and finally present the current challenges in the field.

METHODOLOGIES FOR METABOLIC PROFILING AND PHENOTYPING

A glimpse on the metabolite subsets routinely detected by metabolic profiling and phenotyping

Around 3,000 metabolites are reported in textbooks but this number is largely underestimated, as many lower abundance secondary metabolites remain to be identified and characterized. Several metabolites are of particular pharmacological relevance to study in the context of CNS disorders, as up to 550 metabolites impact human pharmacological targets (Pawson et al. 2014). Table 1 mentions metabolites that behave as neurotransmitters or signaling molecules and their cognate receptors in brain. Most neurotransmitters can be quantified in brain tissue or CSF: glutamate, γ -aminobutyric acid (GABA), *N*-acetyl-aspartyl-glutamate (NAAG), acetylcholine, taurine, aspartate, glycine, serotonin, dopamine as well as their precursors: acetate, choline/phosphatidylcholine, glutamine, *N*-acetyl-aspartate (NAA), tryptophan, kynurenines, phenylalanine. Metabolites related to energy metabolism are also quantifiable: tricarboxylic acid (TCA) cycle intermediates (acetate, pyruvate, citrate, α -ketoglutarate), glycolysis (glucose, pyruvate), ketone bodies (acetone, acetoacetate, β -hydroxybutyrate) or anaerobic metabolism (lactate). Metabolic profiling can also analyse osmolytes, such as taurine and NAA, playing critical roles in the control of cell volume and fluid balance in the brain. Markers of neuronal health such as NAA and of astroglial health such as second messenger isomer *myo*-inositol can also be quantified. Lipidomics, a specific branch of metabolomics focusing on the study of the lipid fraction, has been applied to brain research (Piomelli et al. 2007). Neuronal lipidomics covers not only structural lipids (*e.g.* glycerol, glycerophosphocholine, phosphocholine, ethanolamine) but also signaling lipids (*e.g.* ceramides and sphingosines), phosphatidylinositol (PI) secondary messengers, G-protein coupled receptor agonists (*e.g.* endocannabinoids) or activators of nuclear receptors (*e.g.* retinoic acid). Glutathione and lipid-oxidized species resulting from radical attack of reactive oxygen species can also be detected, giving access to the oxidative stress status of brain samples. Finally, metabolites synthesized by gut microbiota or derived from microbial-mammalian co-metabolism can be observed in various biofluids (for review, see Russell et al. 2013). These microbiota-related metabolites include: amines (dimethylamine; trimethylamine, TMA; trimethylamine-*N*-oxide, TMAO, detected by NMR and MS), phenylacetylglutamine (PAG), hippurate or short chain fatty acids (acetate, propionate and butyrate, typically by GC-MS), indoles, aromatic acids and cresols, typically detected by NMR and quantified by LC-MS (Russell et al. 2013). The use of cross-cutting metabolomics technologies has resulted in several studies on the role of gut microbial metabolites on the CNS and initiated a paradigm shift in neurosciences, as bacterial metabolites could affect immunity and behavior (Hsiao et al. 2013). In support of this, a study showed that the neurotransmitter GABA can be synthesized by bacterial isolates from the human intestine (Barrett et al. 2012). In addition, microbiota appears essential to modulate plasmatic levels of the

serotonin precursor tryptophan and its depletion was shown to directly impact CNS serotonergic neurotransmission (Clarke et al. 2013).

¹H-NMR-based and MS-based metabolomics

Proton nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) are the key analytical methods currently used for metabolic profiling ((Dunn et al. 2011; Nicholson et al. 2002), Fig. 1). ¹H-NMR has been predominantly used for CNS metabolite profiling. However, NMR and MS detect a partially overlapping range of metabolites with different sensitivity and sensibility. Therefore, a combined platform analysis is recommended to increase metabolic coverage.

When set in a magnetic field, the nuclei of a molecule resonate at a specific frequency when tipped by a radiofrequency (Beckonert et al. 2010; Beckonert et al. 2007). This resonance frequency is determined both by the magnetic field intensity and the electronic environment of the nuclei and can be measured by an NMR spectrometer. A unique electronic environment surrounds each atom of a molecule is surrounded by, therefore, each recorded resonance can be assigned to a specific nucleus. The natural abundant proton (¹H) is the preferred nucleus for NMR-based metabolic profiling, although ³¹P, ¹³C and ¹⁵N can also be used. High Resolution (HR) ¹H-NMR provides excellent spectral resolution and reproducibility and enables detection of metabolites above the micromolar range. Owing to the rapidity of data acquisition (10 min./sample) and its relatively low cost per sample, ¹H-NMR is particularly convenient for high-throughput analysis of liquid samples (tissue extracts, urine, plasma, CSF). Various publications present standardized protocols for optimal sampling and NMR data acquisition in liquid phase using ¹H-NMR (Beckonert et al. 2007) (Fig. 1). The methanol chloroform extraction enables the simultaneous extraction of both lipids and aqueous metabolites from a single sample, making it well suited for NMR investigations of brain (Le Belle et al. 2002). One advantage of NMR is that liquid samples can be analyzed following minimal sample preparation limited to the addition of internal standards (Beckonert et al. 2010; Beckonert et al. 2007): tetramethylsilane for samples in organic solvents or trimethylsilyl propionate for aqueous samples. These deuterated standards are used for calibrating the chemical shifts (δ , expressed in units of parts per million, ppm). δ corresponds to the variation in resonance frequency of each proton as compared to the standard (Fig. 1). The development of magic-angle-spinning (MAS) ¹H-NMR has allowed the acquisition of highly resolved spectra directly on intact tissues, such as brain tissues or biopsies (Blaise et al. 2007; Cheng et al. 1997; Davidovic et al. 2011; Garrod et al. 1999; Tsang et al. 2005). Detailed protocols for analysis of tissues and biopsies in solid phase using HR MAS ¹H-NMR are available (Beckonert et al. 2010), with specific guidelines concerning brain tissues (Tsang et al. 2005). ¹H HR-MAS NMR is less reproducible compared to conventional liquid ¹H-NMR, but bypasses the extraction step that may introduce sample handling and extraction yields variations. NMR is extremely reproducible over 5 orders of magnitude and operates in the low μ M range, accessing a large number of metabolic intermediates and endpoints.

Over the last decade, the use of mass spectrometry (MS) for metabolic profiling has been impelled by improvements in both the separation methods prior to MS analysis and the sensitivity of detectors. Time-of-flight and quadrupole time-of-flight (ToF and qToF) detectors are now of sufficient sensitivity to allow detection in the picomolar range. The type of chromatography used as a separative step prior to MS orientates the detection of specific subclasses of metabolites (polar versus non-polar, high or low molecular weight, etc...). Gas

chromatography-MS (GC-MS) detects naturally or chemically-derivatized polar metabolites. This includes amino acids, organic acids, amines and amides within a 18-350 Da molecular weight range (*e.g.* ammonium to cholesterol) (Dunn et al. 2011). Conversely, ultra-high-performance-liquid-chromatography-MS (UPLC-MS) does not require derivatization of compounds and separates nonpolar metabolites within a 50-1,500 Da range. This notably comprises high-molecular-weight lipid species (*e.g.* triglycerides and phospholipids) and nonpolar amino acids (*e.g.* tryptophan, the branched chain amino acids: valine, leucine, isoleucine) (Dunn et al. 2011). Multi-dimensional mass spectrometry-based shotgun lipidomics (MDMS-SL) uses a targeted platform for specific study of lipids (Han et al. 2011). Capillary electrophoresis-mass spectrometry (CE-MS) detects accurately ionic and highly polar metabolites that may not be easily detected by either LC or GC-MS methods (Ramautar et al. 2009). Liquid chromatography coupled with electrochemical colorimetric array detection (LCECA) separates metabolites based on their oxidation potential and was used to detect metabolites involved in tryptophan, purine and tyrosine pathways (Bird et al. 2012; Kristal et al. 2007).

After the separation step, the sample is ionized and ions are separated by the mass spectrometer according to their mass-to-charge (m/z) ratio. In GC-MS, ionization is usually obtained by electron impact ionization, which fragments the analyte, while LC-MS will use electrospray ionization in which the analyte is sprayed in droplets of solvent. The signals corresponding to the analytes are sensed by detectors and processed into a spectrum of the m/z ratio of the particles present (Dunn et al. 2011). One of the strengths of mass spectrometry is its sensitivity, however, the dynamic range of the MS detectors (10^3 or 10^4) is often compensated by the sources (10^2 to 10^3).

On the NMR or MS spectrum, each metabolite is registered by a unique combination of peaks (chemical shift δ for NMR and m/z ratio for MS, Fig. 1b), providing a fingerprint specific to each metabolite and giving access to qualitative data about metabolite identities. There is however a bottleneck for structural identification in untargeted metabolomics: metabolites with similar structures lead to signals overlapping at the same resonance in the NMR spectrum, which can only be resolved by using multidimensional NMR, typically ^1H - ^1H and ^1H - ^{13}C NMR. Likewise, several structures with the same molecular mass will result in the same m/z ratio in the MS spectrum, which requires high-resolution MS or MS/MS to resolve ambiguities. NMR and MS peak intensities are linearly related to the relative abundance of the metabolite in the sample, providing quantitative data. The next section introduces the statistical methods used in the metabolomics field to extract biological information from NMR or MS spectra.

Multivariate analysis of spectral data.

Biological samples display a high biochemical complexity related to the coexistence of hundreds or thousands of distinct metabolites. As a result, NMR and MS acquisitions generate a vast amount of data, typically stored in megavariable matrices (n individuals $\ll$ k variables) (Fig. 1) (Fonville et al. 2010). Data analysis is a crucial step to reduce data matrices and facilitate spectral interpretation to identify markers predicting the class of the sample (*e.g.* case vs. control) for a given input (*e.g.* metabolic profile, Fig. 1) (Cloarec et al. 2005; Crockford et al. 2006; Fonville et al. 2010).

To analyze metabolomics data, many statistical users draw upon Principal Component Analysis (PCA), a method originally described by Pearson in the 20's. PCA is an unsupervised statistical method used to visualize differences, trends and relationships between samples and variables. Avoiding *a priori* classification of the

samples, PCA models the directions of greatest variation in data, which are named principal components (PC). PCA scores describe total variation between individuals and allow detection of clusters within the total variance of the dataset and strong outliers, *i.e.* individuals in the sample strongly deviating from the rest of the dataset. PCA loadings describe the importance of each variable responsible for variations between samples (Fonville et al. 2010). Often in the metabolomics field, PCA will not have sufficient discriminatory potential and will be used to deplete the datasets from outliers prior to a supervised analysis that will be used to tackle subtle metabolic variations.

As a supervised statistical method, Partial Least Square Discriminant Analysis (PLS-DA) integrates information about the class of the samples (*e.g.* disease vs. control, untreated vs. treated, treatment dose). This information is implemented in the model through a dummy Y matrix that is added to the X matrix of spectral data (Fonville et al. 2010). PLS-DA will model the relationships between the variables (metabolite peaks) and the class of the samples, with PLS scores providing information about class separation (*e.g.* diseased or healthy class; Fig. 1) and PLS loadings informing on variables responsible for separation (*e.g.* metabolites; Fig. 1). By removing structured noise from data, the development of Orthogonal PLS (O-PLS) has considerably improved spectral interpretation and is now widely used (Fonville et al. 2010).

Finally, PCA and PLS models are adequately validated at the statistical level in terms of: i) goodness-of-fit to the existing data (R^2 statistic) and ii) predictive ability for new data (Q^2 statistic, root mean square of error of prediction, RMSEP). Both R^2 and Q^2 are routinely used in PLS modeling, whereas RMSEP tends to be used for other methods (Fonville et al. 2010). A typical 7-round cross-validation is applied to prevent overfitting, with 1/7th of the samples being excluded from fitting the mathematical model in each round, then being predicted. In the PLS framework the R^2Y parameter estimates the goodness-of-fit of the model, representing the fraction of explained Y-variation, whereas Q^2Y summarises the predictive ability of the model, *ie* the fraction of predicted variance derived from cross validation. This led to the implementation of several “rules of thumb”, for instance considering that a model with a $Q^2Y > 0.4$ is reliable. To provide more systematic assessment of multivariate predictive significance, random permutation tests were introduced to further validate supervised models. Permutation testing is typically implemented by removing the dependence between explicative metabolome data (X) and class membership random relabeling of the metabolomic data and refitting supervised PLS models. After 200-10,000 iterations of the random permutation process, the empirical probability (*ie*, empirical *p*-value) that the observed separation might be due to chance is calculated by comparing the model’s original Q^2Y to the population of Q^2Y fitted during the random permutation process.

To illustrate the use of PLS-DA, we provide as an example the metabolic profiling of brain tissues from the *Fmr1*-KO mouse model of Fragile X Syndrome (FXS), the most common inherited form of intellectual disability and first genetic cause of autism. In this study, cortex, cerebellum, hippocampus and striatum metabolites from *Fmr1*-KO and WT mice were profiled using HR-MAS 1H NMR (Davidovic et al. 2011). Supervised O-PLS-DA analyses generated a three-dimensional score plot (Fig. 2A). Each position on the plot represents the metabolomic fingerprint derived from the NMR spectrum of an individual sample. We have shown that control wild type (WT) brain samples segregate in distinct peripheral regions of the model. This strikingly illustrates the anatomical and functional differences between each brain region that is directly reflected in their distinct metabolic content (Fig. 2A). Conversely, *Fmr1*-KO mouse brain regions all tend to project towards distinct areas of the model,

indicating a characteristic metabolic signature in KO samples, distinct from the WT samples (Fig. 2A). The O-PLS coefficients are represented as a pseudo-spectrum to highlight the spectral regions directly interpretable as metabolites, most responsible for discrimination between *Fmr1*-KO and WT cortical samples (Fig. 2B). On the plot, the colour scale indicates the intensity of the correlation to the KO or WT class. This enabled to show that *Fmr1*-deficiency is significantly associated in the cortex with a decrease in GABA, glutamate, glutamine, *N*-acetyl-aspartate and lactate and an increase in acetoacetate and lipid-oxidized species (mainly carbonyls, such as CH₃-CH₂-CH₂-CO- and -CH₂-CO-) (Fig. 2B). For the cortical model, the predictive ability was of 61% ($Q^2_{Yhat} = 0.61$). This means that based on the metabolic profile of a given sample, the model was able to predict its WT or KO class in 61% cases.

Pathway-mapping of metabolic signatures

A key perspective in metabolic phenotyping of CNS diseases remains the interpretation of metabolic signatures: a single metabolite is unlikely to be a biomarker of a CNS disease, while combinations of altered metabolites form a metabolic signature of the disease. Systems biology approaches were introduced to highlight the metabolic pathways that are significantly enriched in the metabolic signature (Pontoizeau et al. 2011; Xia and Wishart 2010). In metabolite-set enrichment analyses (MSEA), the complex metabolic pattern is mapped onto existing metabolic pathways compiled in publicly available databases (Figure 3A). For example, the KEGG pathway database is widely used by the metabolic profiling community and integrates metabolic, genomic, chemical and systemic functional information (Kanehisa et al. 2012). The metabolic pattern is then repeatedly tested for association with each pathway in the database, using enrichment tests such as Chi² test in the case of a qualitative overrepresentation analysis or for a quantitative enrichment analysis (Pontoizeau et al. 2011; Xia and Wishart 2010). The result of the analysis is finally displayed in a summary plot, as obtained using free online resources such as the MSEA webserver (www.msea.ca) (Xia and Wishart 2010). For instance, MSEA was used to highlight significant alterations of D-glutamate and D-glutamine metabolism and Glutathione metabolism, (i.e. oxidative stress response) in the cortex of Fragile X Syndrome mouse model (Fig. 2C).

KEY METABOLIC MARKERS ASSOCIATED WITH CNS DISEASES

Over the last decade, there has been a marked increase in the number of studies characterizing the neurochemical and metabolic profile of murine models and clinical samples of neurological disorders. To summarize these numerous findings, we constructed a synoptic table, providing an overview of the key studies using metabolic profiling approaches to characterize animal models or human samples with a specific focus on three classes of CNS diseases: neurodegenerative, neurodevelopmental and psychiatric disorders (Tab. 2). We then highlight common metabolic pathways affected in CNS diseases by performing MSEAs for the various metabolic signatures identified in brain or CSF samples of each disease (Tab. 3).

Neurodegenerative diseases

Studies in preclinical models. Initial studies using metabolic profiling to study CNS diseases examined brain extracts from two mouse models of Neuronal Ceroid Lipofuscinosis (NCL), the most prevalent form of paediatric

neurodegeneration, associated with the *CLN3* (MIM204200) and *CLN8* (MIM 600143) *loci* (Griffin et al. 2002; Pears et al. 2005). Despite small samples size (n=5), metabolic profiling revealed that both NCL models displayed converging metabolic abnormalities. These abnormalities were even detectable at one month of age, before the animals expressed the neurological phenotypes (Griffin et al. 2002; Pears et al. 2005). MSEA highlighted alterations in D-glutamate and D-glutamine metabolism (Tab. 3). These alterations were in agreement with previously reported changes in expression of genes involved in glutamine/glutamate/GABA metabolism in the *Cln3* mouse model (Brooks et al. 2003). Analysis of the plasma from the *Mdn* model reveals up-regulation of carbonyl residues, mainly lipid oxidised species $\text{CH}_2\text{-CH}_2\text{-CH}_2$ and a decrease in lactate and glucose, indicative of increased oxidative stress and compromised energetic metabolism (Pears et al. 2005). More recently, the mouse model of PHARC (polyneuropathy, hearing loss, ataxia, retinosis pigmentosa, and cataract) disorder (MIM612674) was studied using LC MS-based metabolic profiling (Blankman et al. 2013). The disease is caused by mutations in the poorly characterized serine hydrolase alpha/beta-hydrolase domain-containing (ABHD)12 enzyme. Profiling of brain lipids from the *Abhd12* KO mouse revealed a massive increase in a rare set of very long chain lysophosphatidylserines lipids (7 up-regulated species) previously reported as pro-inflammatory lipids behaving as Toll-like receptor 2 activators. This increase appears from 2 months of age and is concomitant with microglial activation coupled to auditory and motor defects that resemble the behavioral phenotypes of human PHARC patients. These studies illustrate how metabolic profiling can be used to better understand the molecular basis of human disorders in mouse models. Furthermore, these studies performed in rodent models strikingly highlight that metabolic abnormalities are detectable even before neurological or cognitive defects arise, and across various biological matrices such as brain tissues or biofluids.

Alzheimer's disease (AD, MIM104300). AD represents the most common form of progressive dementia characterized by accumulation of neurotoxic plaques (accumulation of abnormally folded beta amyloid peptide) and tangles (aggregates of the microtubule-associated protein Tau) in the brain of patients (Frost et al. 2014; Karran et al. 2011). Metabolic profiling of AD preclinical and clinical samples has received considerable attention from the scientific community over the past years (Tab. 2).

Metabotyping of the brain of two mouse models of AD, CRND8 and Tg2576 mice, identified common markers, with noticeable decreases in glutamate, *N*-acetylaspartate, creatine, gamma-aminobutyric acid and phosphocholine (Lalande et al. 2014; Salek et al. 2010). Our MSEA reveals that the metabolism of glutamate is significantly affected (Tab. 3). Lipid profiling identified a complementary set of dysregulated metabolites in the CRND8 animal model (Lin et al. 2013). Notably, alterations in eicosanoids (including 7 prostaglandins) as part of arachidonic acid metabolism were observed, suggestive of the neuroinflammation that is also observed in AD patients (Latta et al. 2014). However, these preclinical findings await replication in patient samples since only one study profiled the metabolome of *post mortem* cortical samples from AD. The approach reached 94% accuracy for disease prediction, however, no metabolite assignment was performed (Graham et al. 2013).

Studies using different metabolomic platforms identified common AD markers in cerebrospinal fluid from patients. The metabolism of catecholaminergic neurotransmitters (dopamine, epinephrine and norepinephrine) is tightly linked to phenylalanine and tyrosine metabolism and MSEA (Tab. 3) indicates convergent variations in these pathways (Czech et al. 2012). Importantly, the severity of the disease was correlated with decreased

noradrenaline (Kaddurah-Daouk et al. 2011b) and increased levels of vanillylmandelic acid, a degradation product of xanthine and dopamine (Kaddurah-Daouk et al. 2013b). Also, MSEA (Tab. 3) highlighted that two independent studies indicated alterations in methionine metabolism (Czech et al. 2012; Ibanez et al. 2012). Finally, three studies performed on independent cohorts identified a common lipid signature in serum of AD patients characterized by a decrease in several lipid classes, notably sphingomyelins (Han et al. 2011; Oresic et al. 2011). One study even showed that sphingomyelins and ceramides species levels correlated with cognitive performance in AD patients (Han et al. 2011). More recently, a study profiled the plasma lipid profile of a cohort of cognitively normal elderly adults and followed the same individuals four years, at a time by which some individuals had developed AD (Kanekiyo et al. 2014). The authors identified a metabolic signature of ten plasmatic lipids that were predictive of the occurrence of mild cognitive impairment or Alzheimer's disease within a 2–3 year timeframe with over 90% accuracy. These data support the involvement of dyslipidemia in AD and are in line with the abundant literature on the APOE4 variant as a major risk allele for AD that leads to disruption in sterol and sphingolipid metabolism (Kanekiyo et al. 2014).

Huntington's disease (HD, MIM143100). HD is an autosomal dominant progressive neurodegenerative disorder caused by abnormal trinucleotidic repeats expansions in the *Huntingtin1 (HD1)* gene leading to motor impairment, cognitive decline and dementia. HD is studied in various mouse models and the 3-nitropropionic acid intoxication rat model in which neurodegeneration of striatal spiny neurons mimics the human disease. Brains of the R6/2 HD mouse model and the rat-treatment model display common metabolic anomalies (Tsang et al. 2009; Tsang et al. 2006) (Tab. 2). Strikingly, in both models, GABA, choline and NAA are decreased and MSEA predicts significant striatal alterations in glutamate metabolism as detected by MSEA (Tab. 3). These metabolic results would need to be further confirmed in *post mortem* samples of HD patients. Urine and plasma of R6/2 mice display metabolic variations associated with altered energy metabolism, notably in glucose levels (Tsang et al. 2006). Importantly, plasmatic hyperglycemia is observed in the two models and seems indicative of defective systemic glucose metabolism, reflecting metabolic modifications in peripheral tissues in addition to specific neurodegenerative changes. Strikingly, similar hyperglycemia was obtained when comparing plasmas from asymptomatic and early symptomatic humans and mice, indicating a clear translation of plasmatic metabolic biomarkers (Tsang et al. 2009; Tsang et al. 2006; Underwood et al. 2006). An increased level of glucose in plasma is in line with previous hypothesis that HD is associated with diabetes (Farrer 1985). Also, increased markers of lipids beta-oxidation (glycerol and dethylen glycol) and alterations in lactate levels are compatible with a systemic deregulation of energy expenditure, which may be related to cachexia symptoms observed in HD patients (Underwood et al. 2006). A study identified branched chain amino acids (valine, leucine, isoleucine) as plasmatic markers of HD, whose levels correlate with disease progression and abnormal triplet repeat expansion size in the *HD1* gene (Mochel et al. 2007). These data collectively suggest that systemic deregulations of glucose, neurotransmitters and BCAA metabolism contribute to HD and that the corresponding metabolites represent potential clinical biomarkers that could be useful to monitor disease progression.

Parkinson's Disease (PD, MIM168600). PD is the second most common neurodegenerative disorder after Alzheimer's disease, resulting from the neurodegeneration of dopaminergic neurons in *substantia nigra*. PD is

associated with motor problems (ataxia, tremor) and dementia. PD is mostly idiopathic but can also be of genetic origin. Two metabolic profiling studies of PD patients reveal decreased plasmatic levels of the antioxidant uric acid, the final product of purine metabolism (Bogdanov et al. 2008; Johansen et al. 2009). One study showed that levels of uric acid are negatively associated with PD and disease progression (Bogdanov et al. 2008; Johansen et al. 2009). Also levels of glutathione were increased strongly reinforcing the contribution of oxidative stress to HD (Bogdanov et al. 2008; Johansen et al. 2009). Based on the plasmatic variations of 9 metabolites mostly involved in purine metabolism, one study could predict whether the PD was secondary to a genetic mutation or of idiopathic origin (Johansen et al. 2009). The model built reached 85.7% sensitivity and 80% specificity, suggesting the possibility to develop future PD diagnosis approaches based on plasma metabolic profiling.

Motor neuron diseases. Motor neuron diseases form a heterogeneous set of neurodegenerative disorders affecting both upper and lower motor neurons, which trigger progressive muscle atrophy. The most common forms of MND are amyotrophic lateral sclerosis (ALS, MIM105400) and spinocerebellar ataxia (SCA). Various brain regions of the mouse model of the motor neuron disease Spinocerebellar Ataxia 3 (SCA3, MIM109150) were profiled (Griffin et al. 2004). This study showed that SCA3 cerebellum is the most affected region, paralleling the strong cerebellar deficits observed in SCA3 patients (Seidel et al. 2012). SCA3 cerebellum displays altered glutamate metabolism as detected by MSEA (Tab. 3) and these alterations precede detection of neurological deficits. These data now await further confirmation in clinical samples of SCA patients.

A study involved 95 MND-affected patients, 85% being affected by ALS, and identified a metabolic signature in CSF that could predict MND with 78.9% sensitivity and 76.5% specificity (Blasco et al. 2014). MSEA revealed that the discriminating metabolites were related to branched chain amino acids metabolism, as well as phenylalanine and tyrosine metabolism (Tab. 3). One study studied the CSF of ALS patients (Blasco et al. 2013) and identified alterations in pyruvate and ketone body metabolism as revealed by MSEA (Tab. 3). Interestingly, these results found considerable echo as another study that highlighted systemic disruption of energy metabolism in ALS, with altered plasmatic levels of acetate, acetone and beta-hydroxybutyrate (Kumar et al. 2010).

Psychiatric conditions

Depression. The etiology and pathophysiology of Major Depressive Disorder (MDD, unipolar depression, MIM608516) and bipolar disorder (BD, MIM125480), two major chronic and debilitating psychiatric conditions, remain mostly unknown despite the identification of many genetic and epigenetic factors that may predispose to the development of these diseases. Metabolic profiling of samples from patients affected by these disorders has contributed to a better understanding of the globally deregulated neuronal pathways. A study on prefrontal cortex *post mortem* samples from bipolar patients reports specific metabolic changes associated with notable increases in glutamate, *myo*-inositol and creatine (Lan et al., 2009). Importantly, treatment of rats with the mood-stabilizing agent lithium decreases the glutamine/glutamate ratio, *myo*-inositol and creatine levels in the brain (McLoughlin et al. 2009), strongly suggesting that lithium treatment reverses the metabolic signature in BD patients. The prefrontal cortex of the chronic unpredictable mild stress (CUMS) depression rat model displayed lower levels of isoleucine and glycerol in combination with higher levels of beta-alanine and NAA (Chen et al.

2014), indicative of Alanine metabolism dysregulations (Tab. 3). Metabolic profiling of CSF samples reveals that remitted MDD patients display a metabolic signature significantly different from the controls or currently depressed patients (Kaddurah-Daouk et al. 2012). The absence of a significant metabolic signature in currently depressed MDD patients might originate from the high inter-individual clinical heterogeneity in these patients, combined with small sample size (Kaddurah-Daouk et al. 2012). However, this study identified that in depressed MDD patients, lower levels of serotonin precursor tryptophan and higher levels of both 5-hydroxyindoleacetate (a serotonin catabolite) and 4-hydroxy-3-methoxyphenyl glycol (a dopamine catabolite) are correlated with more severe symptoms of anxiety (Kaddurah-Daouk et al. 2012). Systemic biomarkers of depressive disorders were identified in serum samples from bipolar patients (Sussulini et al. 2009) and MDD patients (Paige et al. 2007). These studies revealed specific metabolic signatures involving variations in lipids, neurotransmitters and energy-related metabolites (lactate, acetate for BD and 3-hydroxybutanoic acid for MDD). Further studies using bigger cohorts may contribute to the development of metabolomics-based biomarkers for future diagnosis of various forms of depressive disorders.

Schizophrenia (SCZ, MIM181500). SCZ is a common disorder with a lifetime prevalence of approximately 1%. The disease is characterized by a myriad of symptoms: psychotic symptoms (hallucination and delusion) and severe cognitive symptoms (inappropriate emotional responses, disordered thinking and concentration, erratic behavior) as well as social and occupational deterioration (Fornito and Bullmore 2014). In a seminal study, the prefrontal cortex of schizophrenic patients presented deficiencies in D-glutamine and D-glutamate metabolism (MSEA, Tab. 3) and more generally in neurotransmitters biosynthesis (Prabakaran et al. 2004). This was accompanied by a strong increase in lactate and in lipids, suggesting alterations in pyruvate metabolism (MSEA, Tab. 3) in the schizophrenic brain (Prabakaran et al. 2004). Another study also detected alterations in pyruvate metabolism in the CSF of SCZ patients, with a specific increase in glucose coupled to decreased signals for lactate and acetate (Holmes et al. 2006). To further substantiate the relevance of studying CSF metabolic profiles to predict disease, metabolic profiling of patients prodromal for psychosis -which is usually considered as the early clinical stage prior to onset of psychosis and schizophrenia- showed that 36% of these patients displayed metabolic profiles characteristic of schizophrenia patients (Huang et al. 2007). This suggests that detectable metabolic alterations precede the occurrence of overt psychosis, making it possible to identify prodromal markers.

Systemic markers were identified in the plasma of drug-naïve SCZ patients, highlighting a decrease in polyunsaturated fatty acids PUFAs (n3, n6 and n9 fatty acid families), in energy substrates (lactate, acetoacetate, glucose) and uric acid (Cai et al. 2012). This hypo-uremia was also detected in the plasma of an independent cohort of SCZ patients (Yao et al. 2010a; Yao et al. 2010b). Uric acid being the main plasmatic antioxidant, decreased levels in biofluids of SCZ patients is indicative of increased susceptibility to oxidative stress, corroborating several studies in animal models and patients (Emiliani et al. 2014). Finally, several gut microbial metabolites such as hippurate and trimethylamine presented altered levels in the urine of SCZ patients (Cai et al. 2012). This suggests that deregulations of the gut microbiome may participate to SCZ pathophysiology and to other neurological conditions (Collins et al. 2012; Cryan and Dinan 2012).

The metabolic signature of antipsychotic treatment, the principal neuroleptic drugs used to treat schizophrenia

was also studied. In rat brain, most of the antipsychotics and mood stabilizing drugs induce an increase in NAA and modulated glucose metabolism, neurotransmitters (notably glutamate) and secondary messengers inositols (McLoughlin et al. 2009), these pathways being affected in the brain (Prabakaran et al. 2004) and CSF (Holmes et al. 2006; Huang et al. 2007) of SCZ patients (Tab. 2). Metabolic profiling of SCZ patients before and after antipsychotic treatment was performed in brain samples, CSF and plasma. Metabolic markers were identified in *post mortem* brain tissue from drug-naïve schizophrenic patients, corresponding to amino acid and glutamine metabolism normalized by lifetime antipsychotic treatment (Chan et al. 2011). One of the key findings is that patients responding to atypical antipsychotics (50% of patients) present a normalization of their CSF metabolic profiles well before clinical improvements were observed (Chan et al. 2011; Holmes et al. 2006). Conversely, no normalization of the CSF metabolic signature was observed in patients in whom treatment had not been initiated at the first psychotic episode (Holmes et al. 2006). This study highlights at the metabolic level the importance of early therapeutic intervention for schizophrenic patients. In two independent studies using different metabolomics platforms plasmatic metabolic signature of schizophrenia was normalized upon treatment with the antipsychotic risperidone (Cai et al. 2012; Yao et al. 2010a). One study studied in details the plasmatic lipidic profiles of SCZ patients before and after 3 weeks treatment with the antipsychotics olanzapine, risperidone or aripiprazole (Kaddurah-Daouk et al. 2007). Olanzapine and risperidone were shown to have strong modifying effects on plasmatic lipid composition, with notable increases in triacylglycerol and decrease in fatty acids (Kaddurah-Daouk et al. 2007). This is consistent with the strong metabolic effects reported for these two drugs (Ballon et al. 2014).

These promising results would need to be confirmed in larger cohorts in order to derive markers of diagnosis and monitoring of treatment efficacy in plasma or urine of SCZ patients.

Neurodevelopmental disorders

Autism spectrum disorders (ASD, MIM209850). ASD constitute a family of complex neurodevelopmental disorders. ASD patients display impairments in social interactions and communication, restricted interests and increased anxiety. ASD originates from a combination of genetic and environmental factors. Notably, strong correlations between maternal infection during pregnancy and ASD incidence have been described (Knuesel et al. 2014). The maternal immune activation (MIA) mouse model of environmentally-triggered ASD is obtained by poly(I:C)s injections in pregnant mice that induce an IL6-mediated maternal immune response affecting neurodevelopment of progeny, mimicking *in utero* viral infection (Meyer 2014). The plasma of this MIA model was studied using metabolomics (Hsiao et al. 2013). This study showed that levels of the microbial-mammalian co-metabolite 4-ethylphenylsulfate (4-EPS) were increased 40 times in the plasma of MIA mouse model of ASD (Hsiao et al. 2013). This finding is particularly relevant as 4-EPS was shown to induce anxiety-related behavioral anomalies in wild type mice (Hsiao et al. 2013). Moreover, a probiotic bacteria, *Bacteroides fragilis*, restores 4-EPS levels to normal and reverse anxiety-linked behaviors in MIA mice (Hsiao et al. 2013). This study illustrates how metabolic profiling contributes to elucidate the molecular trigger of altered behavior in MIA ASD and strengthen the link between altered microbiota and ASD.

This link is also supported by the identification of several altered urinary metabolites of microbial origin in ASD patients. Notably, variations in the microbial-mammalian co-metabolites: dimethylamine, hippurate and

phenylacetylglutamine were observed in one cohort of ASD patients (Yap et al. 2010). Variations in the urinary levels of microbial-mammalian co-metabolites were also identified in an independent cohort of ASD patients: hippurate, one of its precursors 3-hydroxyphenylacetate and co-metabolite 3-hydroxyhippurate, as well as indole-3-acetate (Emond et al. 2013). These studies performed in ASD mouse models and clinical samples strongly support a recent theory involving gut microbial deregulations in neurological conditions (Collins et al. 2012; Cryan and Dinan 2012).

Down syndrome (DS, MIM190685). DS is the most frequent genetic cause of intellectual deficiency caused by triplication of chromosome 21. Serum metabolic profiling of first-trimester pregnant women identified novel markers for the prediction of fetal DS (Bahado-Singh et al. 2013) earlier than regular tests and without amniocentesis. An OPLS-DA model derived from serum levels of 3-hydroxybutyrate, 3-hydroxyisovalerate and 2-hydroxybutyrate reached 75% sensitivity and 86.2% predictivity. Metabolic variations in DS placenta and foetus contribute to the specific serum metabolic signature observed in pregnant mothers. Some of these metabolites are known to be associated with oxidative stress (2-hydroxybutyrate), poor myelination and neurotoxicity (3-hydroxyisovalerate and 3-hydroxybutyrate), which are phenotypic characteristics associated with DS.

Rett Syndrome (RS, MIM 312750). RS is the major genetic cause of profound intellectual disability in females, due to the inactivation of the X-linked *MECP2* gene encoding the transcription factor methyl cytosine phosphate dinucleotide guanine binding protein (Weng et al. 2011). ¹H-NMR-based metabolic profiling of brain extracts of the *Mecp2*-null mouse reveals alterations in D-glutamine and glutamate metabolism ((Viola et al. 2007), MSEA Tab. 3).

Fragile X Syndrome (FXS, MIM300624). FXS is the most common form of inherited intellectual disability and the most frequent identified genetic cause for autism-spectrum disorders since 15-20% of FXS patients meet the criteria for ASD (Penagarikano et al. 2007). A comprehensive metabolomic study on various brain regions of the FXS mouse model, the *Fmr1*-knockout mouse (Mientjes et al. 2006), has revealed region-specific metabolic signature, with cerebellum and cortex being the most affected regions (Davidovic et al. 2011). This study indicated that various neurotransmitters, including GABA, glutamate, taurine and acetylcholine and their precursors are perturbed in the FXS mouse model. MSEA analysis revealed that D-glutamine and D-glutamate metabolism as well as arginine and proline metabolism were significantly affected ((Davidovic et al. 2011), Tab. 1). Also, increased levels of lipid-oxidised species were detected in the cortex of *Fmr1*-KO mice (Davidovic et al. 2011), indicative of increased oxidative stress, in line with previous observations (Bechara et al. 2009; de Diego-Otero et al. 2009). Moreover, this metabolic profile has striking overlap with the patterns detected in the mouse model of RS (Viola et al. 2007), another syndrome leading to intellectual disability.

IDENTIFICATION OF COMMON METABOLIC MARKERS OF CNS DISEASES

The rise in the number of CNS-related metabolomic studies provides a unique opportunity to cross-examine the metabolic signatures associated to the above-mentioned disorders and converge towards the identification of

common markers, strongly suggesting that common mechanisms can directly contribute to co-morbid pathologies with different clinical manifestations (Tab. 2&3).

CNS perturbations directly detected in the brain. NAA is the second most abundant molecule in the brain after glutamate (Moffett et al. 2007) and its levels are decreased in a number of CNS conditions (Tab.2). NAA is a neuronal osmolyte, as well as a precursor for both lipid and myelin synthesis in oligodendrocytes and a precursor of the neurotransmitter *N*-acetylaspartylglutamate (NAAG). MSEA of the metabolic signatures determined in various neurological conditions (Tab. 3) reveals terms linked to biosynthesis of amino-acid neurotransmitters such as “D-glutamine and D-glutamate metabolism”, “Alanine, aspartate and glutamate metabolism” or “Glycine, serine and threonine metabolism” are recurrently enriched in the metabolic signature of CNS disorders (Tab. 3). Deregulations in brain energy metabolism are consistently observed in CNS conditions, as revealed by MSEA of metabolic signatures which highlights alterations in energetic substrates (Tab. 3) As an example, lactate levels are affected in pathologies as diverse as AD, HD, PD, SCA, CLN, BD, SCZ or FXS (Tab. 2). Lactate is normally a breakdown product of glycolysis, however, several studies now suggest that lactate is metabolized by the intact brain in an activity-dependent manner and has a direct neuroprotective effect (Wyss et al. 2011). MSEA of CSF metabolic signatures of ALS and schizophrenia present the terms “Gluconeogenesis” or “Pyruvate metabolism” (Tab. 3). Brain energy supply is required not only for basal cellular metabolism which results in ATP fuelling for basic biological reactions, but also for the synthesis and degradation of neurotransmitters, preservation of membrane ionic gradients and maintenance of secondary messengers cascades and may have drastic consequences on neuronal functions as hypothesized for schizophrenia (Prabakaran et al. 2004) and Fragile X Syndrome (Davidovic et al. 2011).

Systemic metabolic dysregulations in CNS disorders. Given that metabolic signatures for various CNS diseases partially overlap (Tab. 2), relying on a single biomarker to predict a specific CNS disease is not sufficient. One may more rely on combinations of biomarkers, which may define altered metabolic pathways and will be more precise than a single biomarker to detect and monitor the disease. Metabolomic models for prediction of the diseases are built on several metabolites, illustrating the necessity to use global metabolic signatures that guarantee the specificity of the diagnosis. Importantly, systemic biomarkers detectable in urine, plasma or CSF would be the most relevant for translation to human and several studies demonstrate the great potential of metabolic not only for diagnosis but also for treatment assessment. As such, pilot studies on CSF of schizophrenic and depressed patients reveal that metabotyping predicts the disease status for a given sample, as well as treatment outcome (Holmes et al. 2006; Huang et al. 2007; Kaddurah-Daouk et al. 2011a; Kaddurah-Daouk et al. 2007; Lan et al. 2009; Prabakaran et al. 2004). This is particularly relevant for psychiatric conditions of unknown etiology since, up to date, diagnosis rely on psychocognitive tests and no biochemical or genetic test is available for these diseases. By systematic cross-examination of metabolic pathway enrichment in brain-related metabolic phenotyping studies, it is anticipated that metabolomics will lead to a shift from disease-based classifications towards mechanism or pathway-based mechanisms.

FUTURE CHALLENGES AND UNDDRESSED QUESTIONS.

Have covered several aspects of modern high-throughput metabolic phenotyping in the CNS field, it is anticipated that many basic neuroscience findings will be made with these technologies. However this is a fast-evolving field and several unaddressed questions represent future challenges.

Cell type and organelle-specific metabolomics. So far, metabolic profiling studies have focused on global changes in CNS-specific metabolites (brain, CSF) or systemic markers (plasma, urine) to predict diseases. One current challenge in the metabolomics field would be to move to the study of cell-type specific metabolic variations. For example, primary cultures of neurons or astrocytes of animal models of CNS diseases could be used to detect cell-type specific metabolic variations which could help determine whether the global brain changes originate more from one cell type or another and help refine more targeted treatments. A recent study combined whole-cell patch clamp recording and capillary electrophoresis (CE)-mass spectrometry (MS)-based metabolomics to gather information at the single-cell level on *ex vivo* rat brain thalamic slices (Aerts et al. 2014). Patch-clamp recording yielded information on the electrophysiological properties of the selected cell. The patch-clamp pipette was then used to retrieve a portion of the cytoplasm of the recorded cells, providing sufficient substrate for metabolomic analysis using CE-MS. Combining morphological, electrophysiological data and neurochemical of each recorded cell profile enabled to accurately determine their very nature: glutamatergic or GABAergic inhibitory neurons or astrocytes. This study shows that adjacent thalamic nuclei produce dramatically different neurotransmitter profiles, reflecting their unique neuronal populations. The current development of induced pluripotent cells (iPS cells) from CNS disease-affected patient's fibroblasts, that have the capacity for differentiation into specific neuronal or glial lineages offers great promises in the field (Orlacchio et al. 2010). Such approach would also provide insights in the neuron/glial cell co-metabolome. Importantly, the development of high-throughput methodologies for metabolomic analysis would enable systematic drug screening on neuronal or glial cultures systems, as performed on cancer cell lines and primary cultures (Tiziani et al. 2011). In addition, metabolic profiling will provide information on cell-specific secondary effects of drugs and help refine treatments. Another challenge lies in the metabolic profiling of smaller cellular compartments via organelle metabolomics. Nerve terminals preparations such as synaptoneurosomes could be analyzed to determine the specific levels of neurotransmitters in these structures in CNS pathological conditions. Also, the study of membrane-enriched preparation could lead to the accurate determination of the lipids composition with different electrical properties.

Pharmacometabonomics. Another key perspective has been introduced with the accurate prediction of the response to a treatment based on predose metabolic profiling (Clayton et al. 2009; Clayton et al. 2006). This personalized medicine approach was coined "pharmacometabonomics" and opens new avenues for metabolic research in CNS diseases. Following the first studies, which focussed on predicting the response of rats (Clayton et al. 2006) and humans (Clayton et al. 2009) to a sub-toxic dose of acetaminophen using naïve (pre-dose) urine samples, several papers were subsequently published in various contexts, and in particular, in the management of Major Depressive Depression (MDD) (Kaddurah-Daouk et al. 2013a; Kaddurah-Daouk et al. 2011a). In the latest double-blind pharmacometabolomic study (Kaddurah-Daouk et al. 2013a), the authors tested whether the

baseline metabolic profile of a patient with MDD can predict patient response to treatment with the antidepressant sertraline over 1- to 4- weeks. In this study, Kaddurah-Daouk and co-workers identified that lower plasma levels of branched chain amino acids (BCAAs) correlate with positive treatment outcomes. These results on BCAAs in CNS disorders are very timely as Newgard and co-workers determined that supplementation in BCAA was associated with anxiety-like phenotypes in the mouse (Coppola et al. 2013). Remarkably, Novarino *et al.* demonstrated that mutations in branched chain amino acid keto dehydrogenase kinase gene (*BCKDK*) were associated with a particular form of heritable autism with epilepsy (Novarino et al. 2012). These three studies suggest that BCAAs (and potentially other metabolites) play a prominent role in CNS disorders and that metabolic profiles can predict treatment outcomes in the clinical setting. These recent developments in pharmacometabonomics also open new perspectives in patient stratification for CNS disorders. Future studies should involve larger cohorts of patients to identify common and specific prognostic biomarkers for each class of drugs. CNS diseases clinical practice is compromised by noncompliance of patients to treatment mostly because of non-responsiveness or side effects. In this context, metabolomics could be a valuable tool to stratify patients into responding and non-responding groups to determine the best treatment based on predose metabolic profiling. Such approach would directly improve personalized and optimized treatment for patients and therefore maximize treatment outcome.

Genetic determinants of metabolotypes. A new research area in metabolomics corresponds to the identification of genetic determinants for metabolic phenotypes (*i.e.*, metabolotypes). By acquiring genome-wide genotyping and metabolic phenotyping data in parallel, it is now possible to determine quantitative trait loci for metabolic traits (mQTL, *i.e.* polymorphisms determining metabolite abundance) in genetic intercrosses in rodents or segregating human populations (Dumas et al. 2007; Suhre et al. 2011a; Suhre et al. 2011b). Using a similar approach, brain metabolic profiles could be correlated to DNA polymorphisms and haplotypes to identify brain-specific mQTLs, which should result in a better understanding of the genetic regulation of brain metabolotypes; at least in animal models. The affordability of next generation sequencing methods in combination with corresponding metabolomic data, would enable in the near future mQTL applications to highlight susceptibility SNPs and CNVs for neurological diseases of complex etiology, such as schizophrenia, bipolar disorder or autism-spectrum disorders. An integrated metabolomic and genomic approach was used to study selective serotonin reuptake inhibitor (SRI) treatment outcome in patients with major depressive disorder (MDD) (Ji et al. 2011). This study shows that glycine levels are negatively correlated to SRI treatment outcome, indicating that discrete sequence variations in genes encoding enzymes involved in glycine metabolism might contribute to the metabolomic findings. In a subsequent genome-wide association study (GWAS), the same team identified SNPs for six genes encoding enzymes in glycine biosynthesis were selectively associated to SRI response: for instance SNP rs10975641 in the glycine decarboxylase (GLDC) gene was associated with treatment outcome phenotypes (Ji et al. 2012). These two studies illustrate that GWAS identify SNPs and candidate genes validating metabolic markers and pathways previously identified by metabolomics. It is also anticipated that with the availability of DNA methylation chips, epigenome-wide association studies between “-C-phosphate-G-” (CpG) methylation sites and metabolomic traits will shed a new light on the epigenetic regulation of metabolic patterns in CNS diseases.

Integration of biological and signalling networks regulating brain metabolotypes. Metabolite-set enrichment analysis algorithms represent a real progress in terms of unbiased assessment of the coherence of metabolic signatures at the pathway level, which is not always the case, as exemplified in Tab. 2 presenting metabolic signatures, which do not yield significant enrichment in metabolic pathways (Tab. 3). MSEA approaches remain limited because (i) they test metabolite for membership in metabolic pathways using arbitrary pathway definitions. (ii) they do not provide any understanding of the regulation of metabolic patterns, for instance how disease causing genes (if known) mechanistically contribute to the observed metabolic signature. In order to address these challenges, we used interactome-based systems biology strategies, by coupling protein-protein interaction networks and signaling/transduction pathways to metabolic pathways (Davidovic et al. 2011). A novel alternative strategy, integrated metabolome and interactome mapping” (iMIM) was devised and implemented (Figure 3A). Using iMIM, metabolic phenotypes are directly mapped onto a compilation of protein-protein interaction (ppi), metabolite-enzyme interactions and ligand-receptor interactions networks extracted from literature and databases. Since the human interactome is estimated to have 650,000 ppi (Stumpf et al. 2008), the analysis of the topology of the resulting network has become necessary to identify key proteins regulating the metabolic signatures associated with disease-causing genes. To identify key proteins (*i.e.* the busiest proteins) in the interaction network, shortest paths between causal genes and the downstream metabolic consequences are first computed, then network statistics such as the ‘pivotal betweenness’ (Davidovic et al. 2011; Freeman 1977) are used to highlight the busiest intersections in the network (*i.e.*, the proteins shared by several shortest paths). These central proteins correspond to key regulators relaying the signal between the causal genes and the metabolites. Using iMIM approach, we identified the regulatory proteins controlling the metabolic signature of Fragile X Syndrome in the brain, and identified links with Alzheimer’s disease and oxidative stress (Davidovic et al. 2011). It is anticipated that new developments and refinements in the iMIM methodology will consistently improve the reliability and the predictive power of the output predictions. Notably, one perspective involves the introduction of weighting factors integrating other “omics” data in the metabolic network. For example, taking into account variations in transcriptomic or proteomic profiles could help refining the bioinformatic predictions of key proteins and key pathways and increase biological relevance towards the studied disease. Also, interactome-mapping of metabolic phenotypes could be easily amenable to the study of monogenic disorders or polygenic disorders and should help understanding metabolomic signatures of CNS conditions.

Conclusions and perspectives

The study of brain metabolism and CNS diseases has recently witnessed a series of shifts, related to the development of state-of-the-art methods in spectrometry, statistics and systems biology. Metabolomics has now been used for over fifteen years in CNS research and has enabled key advances in terms of identification of CNS disease signatures, showing that brain metabolism is not restricted to neurotransmitter synthesis, and that intermediate metabolism variations contribute to generating complex brain disorder phenotypes. However, the detailed mechanistic understanding of the generation of these complex metabolic patterns remains a challenge. The integration of genetic, epigenetic, interaction and signaling networks to understand the regulation of brain metabolic biochemistry will undoubtedly lead to a better understanding of CNS disease mechanism. The translation of pharmacometabonomic approaches identifying predictive circulating markers for treatment

efficacy into the CNS disease clinic is one of the key challenges, which could significantly influence clinical monitoring of patients and therapeutic decisions.

Acknowledgements

The authors would like to thank Mr. Frank Aguila for figure editing. LD is funded by FRAXA Research Foundation, Agence Nationale de la Recherche (ANR JCJC SVE6 MetaboXFra), Conseil Général 06, Fondation Jérôme Lejeune and the CNRS PICS program. MED and LD are funded by The Royal Society - CNRS/ International Exchange Program (IE120728) and the Coordinated Action NEURON-ERANET under European Community Framework Program grant agreement (291840) which funded the μ NeuroINF project. The authors declare no conflict of interest.

Figures and tables

Figure 1. Typical metabolic phenotyping workflow

A. For metabolic profiling of CNS conditions, human cohorts need to be adequately powered (typically $n=30$ to 1,000 individuals), whereas for animal models $n=5-10$ per condition should be sufficient. **B.** Biofluids (urine, plasma, CSF, saliva) or intact brain samples or extracts are collected in a standardized manner (biopsies, *post mortem samples*, surgery resection). **C.** Spectral data are acquired on each sample using Nuclear Magnetic Resonance or Mass Spectrometry, followed by computer-assisted data import, preprocessing and databasing, resulting in a megavariable matrix summarizing the metabolite peaks (variables, 1 to k) for each sample (individuals, 1 to n). **D.** Extensive pattern recognition multivariate statistical analysis of spectral data enables classification and/or prediction of diseased and control samples based on statistical scores (left graph). Model loadings are derived to determine the metabolites whose variations maximize the discrimination between diseased and healthy individuals. Orthogonal Partial Least Square Discriminant Analysis (O-PLS-DA) models can be represented as a pseudo-spectrum (right graph). Correlation to the class of the sample (diseased or healthy) is given by color-scaled covariance values. Positive model coefficients correspond to higher metabolite concentrations in the diseased condition, whereas negative model coefficients are associated with higher metabolite concentrations in healthy condition. Significantly affected metabolites represent candidate biomarkers of the disease, here $m1$ to $m3$.

Figure 2. Metabolomics in CNS research by example: the metabolic signature of Fragile X Syndrome in the brain of its mouse model.

A. Following initial metabolic profiling of four brain anatomical regions (cortex, cerebellum, striatum and hippocampus) by ^1H HR-MAS NMR spectroscopy, the metabolic variation related to brain region and FXS disease status was analysed using orthogonal partial least square discriminant analysis. The 3D O-PLS-DA score plot shows an efficient segregation of the eight groups of brain samples, based on anatomical region and disease status. **B.** To highlight the metabolites responsible for the FXS signature in cortical samples, the O-PLS-DA model loadings were represented as a pseudo-spectrum plot. Positive model coefficients correspond to higher metabolite concentrations in KO animals, whereas negative model coefficients are associated with higher metabolite concentrations in WT animals. Adapted from (Davidovic et al. 2011).

Figure 3. Systems biology strategies to integrate metabolomic data.

A. Metabolite-Set Enrichment Analysis. Metabolites that are significantly affected by a disease form a complex metabolic pattern, which can be difficult to interpret. Using previous knowledge about metabolic pathways compiled in a database, the complex metabolic pattern is then mapped onto the metabolic pathways. The metabolic pattern is repeatedly tested for association with each pathway in the database, using enrichment tests such as χ^2 test in the case of a qualitative overrepresentation analysis, or for a quantitative enrichment analysis (Pontoizeau et al. 2011; Xia and Wishart 2010). The result of the analysis is finally displayed in a summary plot, as obtained using free online resources such as the MSEA webserver (www.msea.ca, (Xia and Wishart 2010)). **B.** Integrated Metabolome and Interactome Mapping. : a strategy to navigate the underlying

signalling networks and understand metabolic signatures. Disease-causing candidate genes are put into relation with patterns of significant metabolites via protein-protein and metabolite-protein interactions. The iMIM network helps visualizing the physical molecular interactions between any given causal gene and any given downstream metabolite through multiple paths, involving one or several intermediary proteins when the gene does not encode an enzyme, a transporter, a receptor, or more generally, a protein binding metabolites. This visualization is implemented using graph models in which the nodes correspond to biomolecular entities: genes, proteins and metabolites, whereas their physical interactions (binding, metabolic reactions) are encoded by the edges. In iMIM, network metrics are then used to define shortest paths and pivotal proteins between metabolites and disease-causing genes. One strategy to efficiently rank these proteins is to use the betweenness ($b(v)$, (Freeman 1977)). The betweenness corresponds to a measure of the proportion of the shortest paths going through a given node. Proteins with high $b(v)$ are predicted to be pivotal proteins in the network. Finally, interactome-wide permutation tests are performed in order to assess the statistical significance of the resulting network.

Table 1. Examples of pharmacologically active metabolites and their cognate receptors of relevance in CNS disorders.

Each metabolite is provided with database identifiers to enable direct consultation of several metabolic databases: KEGG (<http://www.genome.jp/kegg/compound/>), Human Metabolome Database (HMDB: <http://www.hmdb.ca/>) or PubChem (<http://pubchem.ncbi.nlm.nih.gov/>) to retrieve information on structure or biosynthesis of the compound of interest. Lists of genes encoding the different receptors are provided to illustrate the diversity of the receptors that can accommodate a single ligand.

Table 2. Synoptic of findings from key metabolomic studies defining metabolic signatures of CNS diseases.

Table 3. Metabolic pathway enrichment in brain and CSF metabolic signatures of CNS diseases.

A pathway meta-analysis was performed using metabolite lists compiled in Table 1 by metabolite set enrichment analysis (MSEA) based on the webserver www.msea.ca (Xia and Wishart 2010) to highlight significantly affected metabolic pathways in each CNS condition. Only pathways showing significant enrichment are presented.

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	Metabolite	KEGG ID	HMDB ID	PubChem ID	Receptor official human gene symbol	Receptor class
Neurotransmitters	5-Hydroxytryptamine	C00780	HMDB00259	5202	HTR1A/B/D/E/F, HTR2A/B/C, HTR3A/B/C/D/E, HTR4, HTR5A, HTR6, HTR7	5-Hydroxytryptamine receptors
	Acetylcholine	C01996	HMDB00895	187	CHRM1, CHRM2, CHRM3, CHRM4, CHRM5, CHRNA1/2/3/4/5/6/7/9/10, CHRNB1/2/3/4, CHRND, CHRNE, CHRNG	Acetylcholine receptors, Muscarinic Acetylcholine receptors, Nicotinic
	Adenosine	C00212	HMDB00050	60961	ADORA1, ADORA2A/B, ADORA3	Adenosine receptors
	Adrenaline	C00788	HMDB00068	5816	ADRA1A/B/D, ADRA2A/B/C, ADRB1/2/3	Adrenergic receptors
	Noradrenaline	C00547	HMDB00216	439260		
	Dopamine	C03758	HMDB00073	681	DRD1/2/3/4/5	Dopamine receptors
	GABA	C00334	HMDB00112	119	GABRA1/2/3/4/5/6, GABRB1/2/3, GABRG1/2/3, GABRD, GABRE, GABRQ GABBR1/2 GABRR1/2/3	GABA _A receptors GABA _A receptors GABA _B receptors
	Glutamate	C00217	HMDB03339	23327	GRIA1/2/3/4, GRIK1/2/3/4/5, GRIN1/2A/2B/2C/2D/3A/3B GRM1/2/3/4/5/6/7/8	Glutamate receptors, Ionotropic Glutamate receptors, Metabotropic
	Glycine	C00037	HMDB00123	750	GLRA1/2/3/4, GLRB	Glycine receptors
Phosphates	Melatonin	C01598	HMDB01389	896	MTNR1A/AB	Melatonin receptors
	ATP	C00002	HMDB00538	5957	P2RX1/2/3/4/5/6/7	P2X receptors, transmitter-gated channels
	ADP	C00008	HMDB01341	6022	P2YR1/12/13	P2Y receptors, G-protein-coupled
	ATP	C00002	HMDB00538	5957	P2RY2/11	
	UDP	C00015	HMDB00295	6031	P2RY6	
	UTP	C00075	HMDB00285	6133	P2RY2/4	
	UDP-glucose	C00029	HMDB00286	53477679	P2RY14	
Lipids	Inositol triphosphate	C00081	HMDB00189	8583	ITPR1/2/3	Inositol triphosphate receptors
	Leukotriene B4	C02165	HMDB01085	5280492	LTB4R1/R2	Leukotriene receptors
	Lysophosphatidic acid	C00681	HMDB07853	3950	LPR1/2/3/4/5/6	Lysophospholipid receptors
	Sphingosine-1-phosphate	C06124	HMDB00277	5353956	S1PR1/2/3/4/5	
	Leukotriene B4	C02165	HMDB01085	5280492	PPARA	Peroxisome proliferator activated receptor α
	5-Hydroxyeicosatetraenoic acid	C04805	HMDB11134	5280733		
	Unsaturated fatty acids	FA0103	N/A	N/A		
	Prostaglandin I2	C01312	HMDB01335	5282411	PPARD	Peroxisome proliferator activated receptor δ
	Unsaturated fatty acids	FA0103	N/A	N/A		
	5-Hydroxyeicosatetraenoic acid	C04805	HMDB11134	5280733	PPARG	Peroxisome proliferator activated receptor γ
	9-Hydroxyoctadecadienoic acid	C14767	HMDB10223	5282945		
	13-Hydroxyoctadecadienoic acid	C14762	HMDB06939	6443013		
	Prostaglandin J2	C05957	HMDB02710	5280884		
	Prostaglandin D	C00925	HMDB00693	53477714	PTGDR/DR2	Prostanoid receptors
	Prostaglandin E	C00584	HMDB01220	5280360	PTGER1/ER2/ER3/ER4	
	Prostaglandin F1	C05442	HMDB00937	5280794	PTGFR	
	Arachidonylethanolamine	C10203	HMDB32696	5281706	CNR1/2	Cannabinoid receptors
	2-Arachidonoylglycerol	C10521	HMDB34127	5281804		

	Disease	Samples details	Reference		Number of samples	Analytical platform	Metabolites
NEURODEGENERATIVE DISORDERS	Neuronal Ceroid Lipofuscinoses	<i>Mdn</i> mouse model	Griffin et al. 2002	brain/plasma	n=5 per group	HR ¹ H-NMR	<i>Brain</i> UP: beta-hydroxybutyrate, taurine, glutamate, CH2CH2CH2 lipids DOWN: creatine, glutamine, gamma-aminobutyric acid <i>Plasma</i> UP: CH2CH2CH2 lipids DOWN: lactate, CH3-CH2 lipids, glucose
		<i>Cln3</i> mouse model	Pears et al., 2005	brain	n=5 per group	HR ¹ H-NMR	UP: glutamate, myo-inositol, creatine DOWN: gamma-aminobutyric acid, lactate, taurine, N-acetyl-aspartate
		Cln3 mouse model brain	Griffin et al., 2005	brain	n=5 per group	HR ¹ H-NMR	UP: glutamate, myo-inositol, creatine DOWN: gamma-aminobutyric acid, lactate, taurine, N-acetyl-aspartate
	PHARC	<i>Abhd12</i> mouse model	Blankman et al., 2013	brain	n=3-5 per group	LC MS	phosphatidylserine (2 sp. up; 2 sp. down), lysophosphatidylserine (7 sp. up), phosphatidylglycerol (2 sp. up; 1 sp. down), lysophosphatidylinositol (1 sp. up; 1 sp. down)
	Alzheimer's Disease	CRND8 mouse model	Salek et al. 2010	brain	n=5 per group	HR ¹ H-NMR	UP: lactate, aspartate, glycine, alanine, leucine, isoleucine, valine, fatty acids DOWN: N-acetylaspartate, glutamate, glutamine, taurine, gamma-aminobutyric acid, choline, phosphocholine, creatine, phosphocreatine, succinate
		CRND8 mouse model	Lin et al., 2013	brain	n=6 per group	LC-MS	hippurate, salicylurate, prostaglandin E1/E3/B1/H2/FF2beta/A2/F1alpha and derivatives, 16-hydroxy-2,12-dimethoxypicrasa-2,12-diene-1,11-dione, 5-hydroxyeicosatetraenoic acid, leukotriene A4, 6-phospho-D-gluconate, 9-oxononanoic acid, androsterone, glucuronide, mannitol, 11beta-17-Dihydroxy-6alpha-methylpregn-4-ene-3,20-dione, dehydromomifoliol, D-glucuronate, oleandolide,15S-hydroxy-11-oxo-5Z,9Z,13E,17Z-prostatetraenoic acid, annabidiolic acid, 4-heptyloxybenzoic acid, 5-oxo-1,2-campholide
		Tg2576 mouse model	Lalande et al., 2014	brain	n=5 per group	HR 1H-NMR	DOWN: glutamate, N-acetylaspartate, myo-inositol, creatine, gamma-aminobutyric acid, phosphocholine
		human <i>post mortem</i>	Graham et al., 2013	brain	n=15 controls n=15 patients	UPLC-QTOF-MS	Unassigned metabolites
		human <i>post mortem</i>	Kaddurah-Daouk et al. 2011	CSF	n=15 controls n=15 patients	LCECA HPLC	UP: 5-hydroxytryptophan, methoxy-hydroxyphenyl-glycol, methoxy-hydroxymandelate DOWN: norepinephrine, 3-methoxytyramine, alpha tocopherol, ascorbate
		human	Kaddurah-Daouk et al., 2013	CSF	n=38 controls n=40 patients	LCECA HPLC	UP: methionine, 5-hydroxyindoleacetic acid, vanillylmandelic acid, xanthosine, glutathione DOWN: glutathione/methionine, 5-hydroxyindoleacetic acid/5-hydroxytryptophan
		human	Ibanez et al. 2012	CSF	n=85 patients	CE-MS	UP: choline, valine, serine DOWN: carnitine
		human	Czech et al., 2012	CSF	n=51 controls n=79 patients	GC-MS LC-MS/MS	cysteine, tyrosine, phenylalanine, methionine, serine, pyruvate, taurine, creatinine, cortisol, dopamine, uridine
		human	Oresic et al., 2011	plasma	n=46 controls n=47 patients	UPLC-MS/GCxGC-MS	DOWN: ether phospholipids, phosphatidylcholines, sphingomyelins, sterols
		human	Han et al., 2011	plasma	n=26 controls n=26 patients	MDMS-SL	UP: ceramide (65 sp.) DOWN: sphingomyelin (33 sp.)
		human	Mapstone et al., 2014	plasma	n=525 participants	MDMS-SL	serotonin, phenylalanine, proline, lysine, phosphatidylcholine (10 sp.), taurine, acylcarnitine
	Huntington's Disease	R6/2 mouse model	Tsang et al. 2006b	brain/plasma/urine	n=10 per group	HR/HR-MAS ¹ H-NMR	<i>Brain</i> UP: creatine, glutamine, lactate DOWN: acetate, N-acetyl-aspartate, gamma-aminobutyric acid, choline <i>Plasma</i> UP: glucose, unsaturated lipids, creatine DOWN: glutamate <i>Urine</i> UP: glucose, acetate, citrate, glycine, 2-oxoglutarate, succinate trimethylamine N oxide DOWN: alpha-ketoisocaproate, dimethylglycine, lactate, spermine, trimethylamine
		3-Nitropropionic acid rat model	Tsang et al. 2009	brain	n=5 per group	HR-MAS ¹ H-NMR	UP: succinate DOWN: taurine, gamma-aminobutyric acid, creatine, N-acetyl-aspartate, choline
		HD-N171-N82Q mouse	Underwood et al., 2005	plasma	n=10 per group	GC-TOF-MS	glycerol, glucose, monosaccharides, lactate, urea, malonate, valine, pyroglutamate
		human	Underwood et al., 2005	plasma	n=20 controls n=30 patients	GC-TOF-MS	glycerol, monosaccharides, lactate, alanine, leucine, 2-amino-N-butyrate, ethylene glycol, alpha-hydroxybutyric acid, valine, malonate, urea
		human	Mochel et al., 2010	plasma	n=32 samples	HR ¹ H-NMR	DOWN: valine, leucine, isoleucine
	Parkinson's Disease	human	Bogdanov et al. 2008	plasma	n=25 controls n=66 patients	LCECA HPLC	UP: glutathione, 2-hydroxy-2-deoxyguanosine DOWN: uric acid
		human	Johansen et al., 2009	plasma	n=15 controls n=41 patients	LCECA HPLC	DOWN: hypoxanthine, uric acid, hypoxanthine/xanthosine, xanthosine/xanthine, hypoxanthine/uric acid
MOTOR NEURON DISEASES	Spinocerebellar Ataxia	<i>M/MI</i> mouse model	Griffin et al. 2004	brain	n=5 per group	HR/HR-MAS ¹ H-NMR	UP: glutamine DOWN: gamma-aminobutyric acid, choline, phosphocholine, lactate
	Amyotrophic Lateral Sclerosis	human	Blasco et al., 2013	CSF	n=128 controls n=66 patients	LC MS	UP: pyruvate, ascorbate, acetone DOWN: acetate
		human	Kumar et al., 2010	plasma	n=30 controls n=25 controls	HR ¹ H-NMR	UP: glutamate, acetate, acetone, beta-hydroxybutyrate, formate DOWN: glutamine, histamine, N-acetyl derivatives
	Motor Neuron Disease	human	Blasco et al., 2014	CSF	n=86 controls n=95 patients	HR ¹ H-NMR	2-hydroxy-isovaleric acid, valine, isoleucine, 2-oxo-3-methyl isovaleric acid, 2-oxoisovaleric acid, isobutyric acid, threonine, tyrosine, phenylalanine, 1-methyl-histidine, histidine
PSYCHIATRIC CONDITIONS	Major Depressive Disorder	CUMS model	Chen et al., 2014	brain	n=8 per group	GC-MS	UP: N-acetylaspartate, β-alanine DOWN: isoleucine, glycerol
		human	Kaddurah-Daouk et al. 2012	CSF	n=18 controls n=14 depressed n=14 remitted	LCECA HPLC	UP: 5-hydroxyindoleacetic acid, tyrosine/hydroxyphenyllactate, methionine, glutathione/methionine, xanthine/homovanillic acid, homovanillic acid/5-hydroxyindoleacetic acid DOWN: 5-hydroxyindoleacetic acid/tryptophane, 5-hydroxyindoleacetic acid/kynurenine, xanthine methionine, homovanillic acid
		MDD rat models	Shi et al., 2013	plasma	n=9 per group	HR ¹ H-NMR	<i>CUMS model</i> UP: trimethylamine, aspartic acid, glutamate, acetoacetic acid, N-acetyl glycoproteins, β-alanine, lactate, leucine, isoleucine, lipids DOWN: proline, β-hydroxybutyrate, valine; <i>FST-1d model</i> DOWN: trimethylamine; <i>FST-14d model</i> UP: α-glucose, β-glucose, β-hydroxybutyrate, valine, lipids
		human	Paige et al., 2007	plasma	n=9 controls n=10 patients	GC-MS	DOWN: gamma-aminobutyric acid, stearate, 3-hydroxybutanoic acid, glycerol
	Bipolar Disorder	human <i>post mortem</i>	Lan et al., 2009	brain	n=10 controls n=10 patients	HR-MAS ¹ H-NMR	UP: lactate, phosphocholine, creatine, myo-inositol, glutamate DOWN: polyunsaturated lipids
		human <i>post mortem</i>	Schwarz et al., 2008	brain	n=15 controls n=15 patients	UPLC-MS	<i>Grey matter</i> : phosphatidylcholines (12 sp.), palmitic acid, heptadecanoic acid, oleic acid <i>White matter</i> : phosphatidylcholines (12 sp.), linoleic acid, oleic acid, ceramides (3 sp.) <i>Red blood cells</i> : stearic acid, linoleic acid
		human	Sussulini et al., 2012	plasma	n=25 controls n=25 patients	HR ¹ H-NMR	acetate, valine, proline, glutamate, glutamine, asparagine, lysine, choline, arginine, proline, myo-inositol, arginine, lipids (VLDL), lipoproteins, lactate, acetate
	Schizophrenia	phencyclidine rat model	Weisseling et al., 2013	brain	n=8 per group	HR ¹ H-NMR	DOWN: choline, glutamine, glutamate, glycine, taurine
		human <i>post mortem</i>	Prabakaran et al. 2004	brain	n=10 controls n=10 patients	HR-MAS ¹ H-NMR	<i>Prefrontal cortex</i> UP: lipid, taurine, glutamate/glutamine, lactate DOWN: adenosine, uridine, myo-inositol, phosphocholine, acetate, gamma-aminobutyric acid
		human <i>post mortem</i>	Schwarz et al., 2008	brain/red blood cells	n=15 controls n=15 patients	UPLC-MS	<i>Grey matter</i> : phosphatidylcholines (10 sp.), palmitic acid, stearic acid <i>White matter</i> : phosphatidylcholines (6 sp.), palmitic acid, stearic acid, heptadecanoic acid, oleic acid, ceramides (3 sp.) <i>Red blood cells</i> : DOWN: stearic acid, linoleic acid, arachidonic acid, ceramide (1 sp.), palmitic acid, oleic acid
		human <i>post mortem</i>	Chan et al., 2011	brain	n=10 controls n=10 patients	LC-MS	<i>Prefrontal cortex</i> UP: glutamine, creatine DOWN: valine, leucine, isoleucine, alanine
		human	Holmes et al. 2006a	CSF	n=70 controls n=37 (cohort 1) n=17 (cohort 2) patients	HR ¹ H-NMR	UP: glucose DOWN: acetate, lactate, glutamine (pH shift), alanine (pH shift)
		human	Cai et al. 2012	plasma/urine	n=11 controls n=11 patients	UPLC-MS/ HR ¹ H-NMR	<i>Plasma</i> UP: lactate, alanine, glycine, lysophosphatidylcholine (4 sp.) DOWN: lipoproteins, 3-hydroxybutyrate, phosphatidylcholine (1 sp.), acetoacetate, glucose, LDL/VLDL/HDL, uric acid, unsaturated fatty acids <i>Urine</i> UP: glycine, valine, glucose, uric acid, pregnanediol DOWN: creatine, creatinine, taurine, hippurate, trimethylamine-N-oxide, citrate, alpha-ketoglutarate
		human	Yao et al., 2010a, 2010b	plasma	n=30 controls n=25 patients	LCECA HPLC	UP: xanthosine/guanine, N-acetylserotonin, N-acetylserotonin/tryptophane, melatonin/serotonin DOWN: guanine/guanosine, uric acid/guanosine, uric acid/xanthosine, melatonin/N-acetylserotonin
NEURODEVELOPMENTAL DISORDERS	Rett Syndrome	<i>Mecp2</i> -KO mouse model	Viola et al., 2007	brain	n=4 per group	HR ¹ H-NMR	UP: glutamine DOWN: myo-inositol, phosphocholine/ glycerophosphocholine, glutamine/glutamate
	Fragile X Syndrome	<i>Fmr1</i> -KO mouse model	Davidovic et al., 2011	brain	n=10 per group	HR-MAS ¹ H-NMR	UP: acetotacetate, CH2-CO/CH2-CH2-CH2-CO, taurine, creatine, beta-amino butyrat DOWN: lactate, glutamine, gamma-aminobutyric acid, N-acetyl-aspartate, glutamate, myo-inositol, aspartate, acetate, N,N,N-trimethyl-lysine, alanine, kynurenine, N-alpha-acetylornithine, adenine, inosine, purine, ethanolamine
	Autism spectrum disorders	maternal immune activation ASD mouse model	Hsiao et al., 2013	plasma	n=10/group	GC/LC-MS	UP: 4-ethylphenylsulfate, ribose DOWN: 3-methyl-2-oxovalerate, 4-methyl-oxopentanoate, glycyvaline, equolsulfate, eicosenoate, stearate, 13-hydroxyoctadecadienoic acid, 9-hydroxyoctadecadienoic acid, octadecanedioate, 12-hydroxyeicosatetraenoic acid, 1-palmitoylplasmenylethanolamine, 1-stearoylglycerophosphoinositol
		human	Yap et al., 2010	urine	n=34 controls n=39 patients	HR ¹ H-NMR	UP: acetate, dimethylamine,succinate, taurine, N-methyl nicotinic acid, N-methyl nicotinamide, N-methyl-2-pyridone-5-carboxamide DOWN: glutamate, hippurate, phenylacetylglutamine
		human	Emond et al., 2013	urine	n=24 controls n=26 patients	GC-MS	UP: succinate, glycolate DOWN: hippurate, 3-hydroxyphenylacetate, vanillylhydracrylate, 3-hydroxy-hippurate, p-hydroxy mandelate, 1H-indole-3-acetate, palmitate, stearate, 3-methyladipate
	Down Syndrome	human	Bahado-Singh et al. 2013	1 st trimester pregnant woman plasma	n=60 controls n=30 patients	HR ¹ H-NMR	UP: 2-/3-hydroxybutyrate, 2-hydroxyisovalerate, acetamide acetone, carnitine, lactate, pyruvate, L-methylhistidine DOWN: dimethylamine, methionine

			Neurodegenerative disorders				Psychiatric disorders		Neurodevelopmental disorders
	Enriched metabolic pathway	SMPDB or KEGG ID	AD	HD	NCL	MND	MDD	SCZ	FXS
Amino acids	Alanine metabolism	SMP00055				✓	✓	✓	
	Alanine, aspartate and glutamate metabolism	map00250	✓						✓
	Arginine and proline metabolism	SMP00020	✓		✓				✓
	Cysteine metabolism	SMP00013				✓			
	Glycine, serine and threonine metabolism	map00260	✓						
	Methionine metabolism	SMP00033	✓					✓	
	Valine, leucine, isoleucine degradation	SMP00032	✓			✓		✓	
	Histidine metabolism	SMP00044				✓			
Neurotransmitters	D-Glutamine and D-glutamate metabolism	map00471	✓		✓				✓
	Glutamate metabolism	SMP00072		✓		✓		✓	
	Catecholamine biosynthesis	SMP00012	✓						
	Tyrosine metabolism	SMP00006	✓						
	Phenylalanine and tyrosine metabolism	SMP00008	✓			✓			
	Tryptophan metabolism	SMP00063	✓				✓		
	Taurine and hypotaurine metabolism	SMP00021		✓					
Energy substrates	Gluconeogenesis	SMP00128						✓	
	Ketone body metabolism	SMP00071				✓			
	Pyruvate metabolism	SMP00060				✓		✓	
	Urea cycle	SMP00059						✓	
	Pyrimidine metabolism	SMP00046						✓	
Other	Aminoacyl-tRNA biosynthesis	map00970	✓						
	Ammonia recycling	SMP00009	✓					✓	
	Betaine metabolism	SMP00123	✓						

CNS CONDITION

human cohort murine model

SAMPLES COLLECTION

biofluids

- urine
- plasma
- CSF
- saliva

brain samples

- intact
- extracts

NMR / MS DATA ACQUISITION

data import
preprocessing
databasing

MULTIVARIATE STATISTICAL ANALYSIS

scores

loadings

DISEASE PREDICTION

CANDIDATE BIOMARKERS

A METABOLITE SET ENRICHMENT ANALYSIS

PATHWAY ENRICHMENT TESTING

	metabolites in P1	
	present	absent
# mets in pattern	10	2
# mets in network	12	24

Chi-test & associated p-value P derivation

B INTEGRATED METABOLOME AND INTERACTOME MAPPING

NETWORK METRICS

- Derivation of pivotal betweenness for each protein : $b(v)$

$$b(v) = \frac{1}{n*(n-1)} * \sum_{i,j,v \in v} \frac{sp_{ij}(v)}{sp_{ij}}$$

- Interactome-wide permutation testing