Metabolomics of Human Brain Aging and Age-Related Neurodegenerative Diseases

Mariona Jové, PhD, Manuel Portero-Otin, PhD, MD, Alba Naudi, PhD, Isidre Ferrer, PhD, MD, and Reinald Pamplona, PhD, MD

Abstract

Neurons in the mature human central nervous system (CNS) perform a wide range of motor, sensory, regulatory, behavioral, and cognitive functions. Such diverse functional output requires a great diversity of CNS neuronal and non-neuronal populations. Metabolomics encompasses the study of the complete set of metabolites/low-molecular-weight intermediates (metabolome), which are context-dependent and vary according to the physiology, developmental state, or pathologic state of the cell, tissue, organ, or organism. Therefore, the use of metabolomics can help to unravel the diversity—and to disclose the specificity—of metabolic traits and their alterations in the brain and in fluids such as cerebrospinal fluid and plasma, thus helping to uncover potential biomarkers of aging and neurodegenerative diseases. Here, we review the current applications of metabolomics in studies of CNS aging and certain age-related neurodegenerative diseases such as Alzheimer disease, Parkinson disease, and amyotrophic lateral sclerosis. Neurometabolomics will increase knowledge of the physiologic and pathologic functions of neural cells and will place the concept of selective neuronal vulnerability in a metabolic context.

Key Words: Alzheimer disease, Amyotrophic lateral sclerosis, Bioenergetics, Metabolomics, Oxidative stress, Parkinson disease, Selective neuronal vulnerability.

INTRODUCTION

Neurons in the human central nervous system (CNS) perform a wide range of motor, sensory, regulatory, behavioral, and cognitive functions that are dependent on the complex organization of groups of cell populations that are composed of diverse neuronal and non-neuronal cells. Central nervous system neurons differ morphologically in size, number of dendrites, complexity of the dendritic tree, number and type of synapses, axonal length, and degree of axonal myelination, among other cellular traits; this is also true of non-neuronal cells. From a physiologic standpoint, neuronal diversity can be expressed by the chemical specificity of the neurotransmitters that they use for chemical transmission and neuromodulation of specific populations and by their electrical properties. This morphologic and functional diversity among neuronal cells suggests that although all neurons contain an identical genetic code, each neuronal type has its own genomic expression profile. In fact, approximately 80% of host genes show some cellular expression in the brain, with most genes expressed in a relatively small number of cells. Specifically, 70.5% of genes are expressed in less than 20% of total cells; the genes with the greatest percentage of expressing cells are related to cellular metabolism (1, 2). The neuronal genomic profile configures a transcriptomic and proteomic pattern that, in turn, is expressed and translated into a neuron-specific metabolomic profile (Fig. 1). Glial cells also have their own metabolomic profiles. As a result, metabolite profiling can be used not only as a source of potential biomarkers in clinical practice but also in a hypothesis-generating approach that could help counter neurodegeneration (3).

Specific regions of the CNS exhibit differential vulnerabilities to aging and various age-related neurodegenerative diseases (NDDs). Alzheimer disease (AD), Parkinson disease (PD), and amyotrophic lateral sclerosis (ALS) primarily affect defined subsets of neurons and involve characteristic ranges of molecular and pathologic features (4, 5). In addition to specific etiologic patterns, all of these diseases share aging as the main risk factor (6–8). Therefore, these age-related NDDs could be, in part, viewed as a form of accelerated aging, or at least as exhibiting cellular traits associated with aging in an exacerbated way. Hence, specific metabolomic profiles and metabolomic signatures may be useful for improving understanding of the mechanistic processes of the corresponding disease and for identifying specific metabolic markers.

To understand which mechanisms are involved in resistance/sensitivity to neuron demise and death, we need to define the molecular bases of selective neuronal vulnerability (SNV) in different cell populations under physiologic conditions. Selective vulnerability of certain glial cell populations to particular diseases (e.g. tauopathies and multiple system...
MEANING OF METABOLOMICS

The specific functions of every cell and the interactions among different cells are under strict molecular control and compose a tightly regulated metabolic program that serves general homeostasis (13). The overall network of interconnected reaction sequences that interconvert cellular metabolites constitutes cell metabolism. In view of the multiplicity of cells and cellular functions in the CNS, the complexity of tissue metabolism greatly exceeds the mere arithmetic addition of intermediates, varying according to the physiology, developmental state, or pathologic state of the cell, tissue, organ, or organism (16). Metabolomics is the systems biology science that allows monitoring of changes in the whole metabolome or is a pool of metabolites reflecting variations in genomic, transcriptomic, and proteomic fluctuations. It is estimated that the human metabolome contains approximately 41,519 metabolites (HMDB: The Human Metabolome Database; http://www.hmdb.ca) (17–19). Brain metabolites include all small molecules present in the brain and therefore represent all compounds that are involved in brain functions (i.e. bioenergetics substrates, membrane lipids, building blocks of proteins and polysaccharides, neurotransmitters, biologically active compounds, antioxidants, and intermediate products of catabolic and anabolic reactions). Recently, it has been estimated that neurons have a metabolome formed of roughly 7,000 metabolites.

Metabolomic profile can also be assessed in a variety of fluids such as cerebrospinal fluid (CSF) and plasma. Human CSF is a rich source of putative biomarkers of various neurologic diseases. Currently available metabolomics methods can routinely identify and quantify 36% of the “detectable” human CSF metabolome (20). An updated CSF metabolome database containing a set of 476 human CSF compounds, their concentrations, related literature references, and links to their known disease associations is available at the CSF metabolome database (HMDB CSF Metabolome Toolbox; http://www.csfmetabolome.ca) (21).

Challenges to Biomarker Discovery of SNV in Aging and Age-Related NDDs

Biomarkers are specific biologic compounds that possess a particular molecular feature that makes them useful for measuring the progress of physiologic or pathologic processes or for monitoring treatment. Because of the inherent difficulties in obtaining and characterizing the relevant tissues affected in CNS disorders, biomarkers are difficult to identify. Four basic challenges to biomarker identification in CNS aging and/or NDDs can be verified: 1) availability of tissue at the site of pathology; 2) poor clinical diagnostics and extent of disease progression at the time of diagnosis; 3) complexity of the brain and tissue heterogeneity; and 4) lack of functional endpoints and models for validation (22).

Many of the problems in biomarker identification in aging and NDDs are related to the acquisition and quality of the required tissues, particularly those at the actual site of pathology. Frequently, samples derived from postmortem tissues have important limitations because the agonal state and intervals between death and tissue processing hamper the preservation of a number of metabolites. In particular, the life span of certain metabolites can range from seconds to a few hours, thus reducing the potential capacities of metabolomic studies in living individuals and in experimental cellular and animal models. Because most human NDDs are exclusively human, regional or selective study of certain neuronal populations in human brains is required. For developing disease diagnostics, plasma and CSF can be more easily attained antemortem; however, for discovering etiologically relevant genes, proteins, or metabolites, the preferred biologic source are often those pathologically affected tissues, which are more difficult to obtain. Progress in overcoming the problem of tissue availability and acquisition has been achieved with advances in brain banking (23, 24). New freezing techniques and shorter postmortem intervals are making higher-quality tissue more accessible. In addition, new computerized database methods are cataloging and organizing
donor submissions in ways that maximize the amount and quality of information available to researchers.

Clinical diagnostics and stage classification of patient populations are poorly developed for most NDDs. Even in the better-case scenario of AD, a recent clinic-pathologic comparison study of more than 900 patients diagnosed at major centers in the United States found that 17% to 30% of clinical diagnoses were inconsistent with autopsy diagnoses (25). Advances in brain imaging techniques show promise for providing a more definitive antemortem diagnosis of NDDs. Currently, definitive clinical diagnoses of NDDs can only be achieved through evaluation of their respective pathologic traits within the brain at autopsy. Many of these NDDs are differentiated by a complex set of neuropathologic traits, which share a significant number of common characteristics. Thus, neuropathologic diagnosis can mainly be performed postmortem, obviating any opportunities for early therapeutic interventions. Thus, biomarker identification for early diagnostics will be crucial for improving treatment of affected individuals.

In many cases, the complexity of the CNS itself presents a major barrier to the identification of useful biomarkers. In most organs (e.g., liver, heart, and muscle), cells are more homogeneous in their phenotypes, transcriptomes, proteomes, and cellular interactions. However, in the CNS, transcriptomes, proteomes, morphologic phenotypes, and interactive connections vary widely within neurons and glia. Heterogeneity of the representative neuropathologies further confounds biomarker identification. Currently, there is interest in broad molecular profiling of specific single cells to overcome these constraints (26). However, the metabolome is very difficult to measure at the single-cell level because of rapid metabolic dynamics, the structural diversity of the molecules, and inability to amplify or tag small-molecule metabolites.

Finally, the scarcity of model systems for functional validation in NDDs makes confirmation of candidate biomarkers extremely difficult. It is important to evaluate whether changes in surrogate endpoints (observed in DNA, RNA, protein, or metabolites) have any measurable effect on the actual phenotype of a cell or animal model. Efforts to date illustrate that validation of human disease biomarkers is not sufficient when performed in vitro or rodent models, as exemplified by the apparent difficulty in mimicking the dramatic histopathology that occurs in human NDDs. This highlights the need to perform retrospective and prospective diagnostic studies to determine the accuracy, sensitivity, and specificity of any biomarker of a clinical phenotype.

**Systematic Approach to Biomarker Identification: Metabolomics Platforms**

Ideally, a systematic approach to biomarker identification will involve multiple technologies to investigate disease processes at all levels, including whole genome association studies to identify etiologic mutations or polymorphisms; expression profiling, proteomics, and metabolomics to identify expression signatures; and protein and metabolic profiles that either are specific for the disease process or provide mechanistic insights into the pathologic processes. For biomarker identification in NDDs, unique challenges necessitate the concurrent use of each of these technologies. Genomics is used to identify relevant disease genes, aberrant cellular signaling pathways, and expression signatures correlated with the disease. Proteomics is used to identify aberrant protein expression, posttranslational modification, protein interactions, and protein profiles that are specific for a particular disorder. Finally, metabolomics is implemented to identify the presence of abnormal levels of metabolites that are specific for and indicative of an underlying disease process.

The ability to simultaneously measure dynamic changes in many molecules in CNS samples has only recently become available through the use of advanced analytic technologies such as high-resolution nuclear magnetic resonance (NMR) and mass spectroscopy (MS), coupled with either high-resolution or ultrahigh-resolution liquid chromatography (LC) or gas chromatography (GC), and the development of sophisticated data analysis methods. Detailed descriptions of the analytic platforms available for multiple metabolomic applications, sample preparation and measurements, data preprocessing, data and statistical analyses, and biomarker discovery and pattern recognition in aging and NDDs are also available (27–32).

A number of early metabolomic studies used $^1$H NMR, also called magnetic resonance spectroscopy (MRS), to determine changes associated with disease progression in AD animal models and AD patients. An advantage of $^1$H MRS is the rapid detection of a relatively large number of molecules with excellent quantitative precision in a high-throughput manner. This method is also noninvasive and provides the opportunity to study metabolites in living organisms. The disadvantages of $^1$H MRS are its high cost and relatively low sensitivity. In fact, it typically detects only the most abundant metabolites contained in the analyzed sample.

Liquid chromatography electrochemistry array metabolomics platform is another method used for both targeted and nontargeted applications to detect changes in neurotransmitter pathways and pathways involved in oxidative stress. This method has high sensitivity and reproducibility but does not allow generation of structural information and has relatively low throughput.

Mass spectroscopy is the most commonly used technique for the identification and quantification of known metabolites, both for the detection of molecules with low abundance signals and for the detection of reproducible but unidentified molecules. The coupling of MS with either GC or LC has been applied successfully for targeted metabolomics to analyze changes in lipids (lipidomics) or other metabolites. These methods are also used to detect global changes in biochemical networks (i.e., nontargeted metabolomics). Compared with NMR, MS is more sensitive and allows the measurement of a broader array of metabolites. However, one of the disadvantages of MS is that it typically requires chemical manipulation to produce ionic species that are more readily separated. Currently, LC-MS represents the major instrumental technology; it also shows strong penetration in other ‘omics’ fields such as proteomics, where it continues to displace 2-dimensional gel electrophoresis.

In metabolomics, the prominence of LC-MS can be attributed mostly to a large number of accessible instruments and open-source data processing software, the wide metabolite coverage provided by LC-MS (often with high sensitivity and specificity), and the versatility of the technology (33).
The major advantages and potential benefits of adopting a metabolomic approach to metabolic profiling include: a) the possibility of identifying novel markers and gaining biochemical understanding; b) the integration of metabolomics results with other system biology approaches, such as genomic, transcriptomic, and proteomic data; and c) the description of the real-time metabolic status of the studied system. Figure 2 shows the 4 major steps in metabolomic analysis. The example is given for AD patients using LC-MS spectra of brain samples. The LC-MS platform provides total ion chromatogram data where all the ion molecules are represented (Fig. 2I). Then, using the different ion species detected in samples, a pattern recognition analyses can be performed (Fig. 2II). Multivariate statistics simplify the interpretation of variation between samples that contain thousands of variables (in this case, metabolites), reducing the variation to a 2-dimensional or 3-dimensional model. Multivariate statistics include 2 major categories: supervised and unsupervised.

Unsupervised techniques (i.e. principal component analysis [PCA] and hierarchical clustering analysis) are used to establish whether any intrinsic clustering exists within a data set without a priori knowledge of sample class. In contrast, supervised methods use the class information given for a training set of samples to optimize the separation between 2 or more sample classes. These include soft independent modeling of classification analysis and partial least discriminate analysis, among others (34). Univariate statistics is then applied to identify specific disease biomarkers. This step is particularly limited for database development (Table 1; Fig. 2III). Finally, after the potential biomarkers are defined, metabolites are quantified and validated (Fig. 2IV).

**Analytic Technologies: Current State of the Art**

Continuous developments in both LC and MS technology have led to remarkable advances in separations and in key components. Figure 2 shows the 4 major steps in metabolomic analysis using AD patients as example. **(I)** Total ion chromatogram of representative control and AD brain samples. The y axis represents mass spectra count, and the x axis represents retention time. **(II)** Pattern analysis using PCA. Principal component analysis is an unsupervised multivariate statistics method that is used to establish whether any intrinsic clustering exists within a data set without a priori knowledge of sample class. Red squares, control samples; blue squares, AD samples. **(III)** After application of univariate statistics, different metabolites arise as potential biomarkers, and the identification step is crucial. In this specific case, arachidonic acid arose as a potential biomarker. **(IV)** Finally, the potential biomarkers found in (III) are validated and quantified.
Whole metabolome analysis not possible

Metabolites present in biologic samples show diversity of chemical properties and wide concentration ranges and can derive from many different sources.

Advances in sample collection (e.g. metabolites extraction methods), advances in LC-MS separations, and advances in key operating MS characteristics such as ionization methods, sensitivity, mass accuracy, mass resolution, scan speed, and data acquisition rates

Poor compatibility of MS analyses

Problems with combining data from different MS analyses, which delay correlation of data obtained from different instruments and laboratories

Advances in software for a more efficient use of data collected by new versions of mass spectrometers and for the combination of data obtained from different instruments and laboratories

Lack of standardized protocols

Large-scale studies need to overcome the difficulty of standardizing methods and the risk of instrumental drifts such as retention time drifts caused by deterioration of the analytic column or MS detection instability caused by MS ion source contamination. In addition, it is now well understood that MS-generated data for global profiling are very complex and contain a high percentage of noise. It is also well understood that most detected features do not correspond to unique metabolites.

Advances in the specific design of appropriate metabolomics experimentation protocols, with special emphasis on the selection of controls and test groups; analytic developments for quality control and validation (use of quality control measures is necessary to assess a system’s stability and suitability)

Software data extraction

Software for data extraction is typically developed by the same instrument company and offers limited autonomy for optimization of the data extraction process. Reanalysis of the same sample set with different software settings could provide notable changes, and this could pass unnoticed. Use of open-source software allows greater flexibility but still needs advanced computing abilities.

Differences in the performance of various software (either commercial or open-source algorithms) used for data extraction should be systematically evaluated.

Identification of candidate markers (MetID)

LC-MS spectra are highly variable: Adduct and cluster formation is not controllable or predictable. Fragmentation is not reproducible even in instruments of the same type. As a result, commercial or freely available LC-MS spectral libraries still represent an aspirational, rather than a robust, solution to the identification of unknowns. Consequently, annotation of metabolites or de novo structure elucidation in complex biologic matrices requires significant effort and time.

The combination of high-resolution MS, MS-MS analyses, and advanced informatics tools are deemed as the most promising tools for MetID. For metabolite annotation (i.e. the assignment of a detected peak to a known metabolite), candidate lists are generated based on accurate mass detected. However, the process from this point is not straightforward, as the number of putative identities can be very large and diverse. As an example, an m/z value of 235 can be ascribed with 30 ppm of error to 4 different metabolites, with 50 ppm of error to 8 different metabolites, and with 100 ppm of error (thus comprising m/z from 234.9994 to 235.02) to 16 different metabolites (according to the Metlin Database). If we consider all potential chromatographic or mass spectrometric adducts, this number increases to 81 different metabolites in positive ionization and to 100 different metabolites in negative ionization. Incorporation of independent (orthogonal) data, such as retention time data, can promote MetID.

Biochemical pathway analysis and development of related tools

Even in the case where metabolites are identified, comprehension of such data is not trivial within complex metabolic pathways and fluxes.

Advances in the development of biochemical pathway analysis tools to effectively search in generated multidimensional data, exploiting existing knowledge (e.g. public databases such as KEGG and HMDB, and lipid maps) to build associations between collected signals (such initiatives include Biocyc, Metabolights, Reactome, MGI Genome, and MassTrix); incorporation of data from known biochemical pathways may contribute to MetID, identifying signals (peaks) that represent missing metabolites in half-covered pathways or known unknowns (i.e. peaks that are frequently found but not identified or peaks whose MetID has not been confirmed)

TABLE 1. Current Problems and Limitations of LC-MS–Based Metabolic Profiling

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HMDB, Human Metabolome Database; KEGG, Kyoto Encyclopedia of Genes and Genomes; MetID, metabolite identification.
the art of LC-MS–based metabolic profiling still have some limitations and problems that need to be solved (Table 1).

ENERGY METABOLISM AND DERIVED OXIDATIVE STRESS AS A DRIVING FORCE FOR SNV

The development of the use of oxygen for efficient energy generation was a driving force for the evolution of complex organisms that demand adaptive changes in structural and functional systems (35–38). Redox reactions linked with the use of oxygen are responsible for the production of reactive species (RS), which may act as second messengers in signal transduction networks; thus, RS have key functions under physiologic conditions. On the other hand, RS may also have damaging effects caused by oxidative nonenzymatic chemical modifications of cellular components (38–40). Consequently, aerobic life requires the emergence and selection of antioxidant defense systems (41), and diverse molecular and structural antioxidant defenses have evolved (38). The appearance and evolution of the CNS paralleled this physiologic evolution.

Human evolution is characterized by the rapid expansion of brain size and a tremendous increase in cognitive capabilities, leading to the emergence of unique and complex cognitive skills. These changes have long been related to changes in brain metabolism, particularly linked to an increment of energy demand (42). Large brains are metabolically expensive, and neurons are the highest energy-demanding cells. Thus, humans allocate approximately 20% of their total energy to the brain, compared with 11% to 13% in apes and 2% to 8% in other mammalian species (43). This increased metabolic demand has been associated with elevated expression of genes involved in neuronal functions and energy metabolism, leading to specific metabolic profiles (44, 45). Yet, the brain is highly vulnerable to changes in energy homeostasis and oxidative stress. In neurons, approximately 85% to 90% of cellular oxygen is consumed by the mitochondria to produce energy as adenosine triphosphate molecules. A main side effect of adenosine triphosphate production is the formation of RS. Reactive species mostly consist of reactive oxygen species (ROS) and reactive carbonyl species. Superoxide anion, the product of a 1-electron reduction of oxygen generated by mitochondrial complexes I and III (9, 10, 46), is the main ROS and precursor of other ROS (39). Although mitochondrial oxygen consumption and ROS production are independently modulated (47, 48), brain mitochondria show a high rate of ROS production compared with other tissues (at least in rodents) (43). Other sources of ROS in the CNS are α-ketoglutarate dehydrogenase, cyclooxygenase and lipoxigenase pathways, mitochondrial monoamine oxidase, catecholamine autoxidation, and plasma membrane reduced nicotinamide adenine dinucleotide phosphate oxidase, among others (39).

In addition to ROS, the oxidation of both carbohydrates and lipids (particularly polyunsaturated fatty acids) gives rise to a new generation of RS named reactive carbonyl compounds (e.g. glyoxal, methylglyoxal, malondialdehyde, and 4-hydroxynonenal) (6, 49, 50). Neural cell–derived RS induce chemical modifications in other molecules, generating oxidative damage. The targets of this damage are all cellular constituents (i.e. nucleic acids, proteins, lipids, and carbohydrates) (51–55). This oxidation-derived molecular damage leads to the formation of nonenzymatic chemical modifications such as amino acid oxidation products, advanced glycation endproducts, and advanced lipoxidation endproducts (6, 56–60). A major consequence of oxidative damage is the loss of function and structural integrity of modified biomolecules with a wide range of downstream functional consequences such as induction of cellular dysfunction and tissue damage (Table 2).

Consequently, protection against oxidative damage is pivotal for CNS function, and an array of metabolic adaptations have been adopted for this control (38). These include a) the resistance of neuronal structural components to oxidative damage. This susceptibility, defined as the ease with which macromolecules endure oxidative injury, is intrinsically linked to the specific structure or chemical composition of carbohydrates, lipids, nucleic acids, and proteins. In this scenario, the CNS is particularly susceptible to the formation of reactive carbonyl species from carbohydrates and polyunsaturated fatty acids caused by the high content of these substrates in comparison with other tissues (61, 62); b) the emergence of neuronal regulatory components of ROS generation (59, 63–70); and c) the incorporation of major molecular antioxidant defenses, which are shared by all aerobic cells and have been selected and conserved during animal evolution (39). Finally, a neuronal adaptation mechanism that deserves mention is the antioxidant response element/Nrf2 signaling pathway (71, 72), as the RS signaling cascade culminates in the nuclear translocation of, and transactivation by, the transcription factor Nrf2.

All these facts lead to the proposal that oxidative stress homeostasis is a major driving force in determining the differential vulnerability of brain cells to aging and NDDs. The relevance of oxidative stress homeostasis to neuronal survival explains how neurons are intrinsically equipped with a biochemical mechanism that couples glucose metabolism to antioxidant defense (73). Thus, neurons are programmed to metabolize glucose actively through the pentose phosphate pathway. This metabolic pathway generates reducing equivalents in the form of the cofactor NADPH(+) , which maintains the antioxidant glutathione in its reduced state. Glutathione is the most abundant nonprotein thiol to buffer oxidative stress in brain tissues, and it is crucial to maintaining overall antioxidant status. This shift of glucose to the pentose phosphate pathway occurs at the expense of a low glycolytic rate for subsequent energy generation by mitochondria. Notably, these metabolic pathways are, in turn, sources of substrates that generate reactive carbonyl species with damaging effects (74).

Given that environmental conditions across a range of physiologic and pathologic conditions may be associated with oxidative stress threats (as are phases of the life cycle), neurons are continually under pressure. Neurons may therefore be exposed to relevant physiologic costs that are mainly expressed in oxidative damage and consumption of the energy needed to keep the antioxidant defenses upregulated and to activate repair systems. The need for continuous adaptation, the presence of inherent susceptibility, and SNV may be the factors that modulate neuronal aging in a physiologic context and that trigger the development of NDDs. In our view, the
TABLE 2. Effects of Oxidative Stress at the Molecular and Cellular Levels

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<tr>
<td>Formation of nonenzymatic and irreversible chemical modifications, adducts, and cross-links (AAOPs, AGEs, and ALEs)</td>
<td>Changes (mostly loss) in hormonal and enzymatic activities</td>
<td>Antioxidant response signaling (Nrf2/ARE pathway)</td>
<td>Bioenergetic defects</td>
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<td>High resistance to proteolytic digestion</td>
<td>Alteration in receptor affinity and specificity</td>
<td>Activation of uncoupling proteins</td>
<td>Modification of tyrosine kinase receptors and signalling pathways</td>
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<td>Alteration of electrophoretic mobility</td>
<td>Altered trafficking and processing of proteins</td>
<td>Heat shock response signaling</td>
<td>Inflammatory responses through activation of the nuclear factor κB pathway</td>
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<td>Conformational changes</td>
<td>Alteration of metabolism</td>
<td>Docosanoid biosynthesis</td>
<td>Endoplasmic reticulum stress, activation of the UPR pathway, and apoptosis</td>
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<tr>
<td>Alteration in solubility</td>
<td>Inactivation of nitric oxide</td>
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<td>Neuronal death</td>
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<td>Lipid oxidation induced by its formation in lipids</td>
<td>Immunogenicity</td>
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<tr>
<td>Alterations in thermal stability</td>
<td>Enhanced DNA mutation rate</td>
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<tr>
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AAOP, amino acid oxidation product; AGE, advanced glycation endproduct; ALE, advanced lipoxidation endproduct; UPR, unfolded protein response.

study of bioenergetics and oxidative stress in NDDs could take advantage of metabolomics and complement other techniques such as proteomics. By applying new and previously described metabolomics techniques in this field, we could further study bioenergetics status under a particular pathologic condition and expand the range of oxidative stress–derived lipids and metabolites analyzed (75, 76).

DIFFERENCES AMONG CLOSELY INTERRELATED SPECIES AND INTERREGIONAL DIFFERENCES IN BRAIN METABOLISM

From the point of view of comparative physiology, the brain shows interspecies differences in oxidative stress homoeostasis (11, 77). Accordingly, brain mitochondrial ROS production differs among vertebrate species (e.g. mammals and birds) in a specific way: The longer is the maximal life span, the lower is the free radical generation (78–80). This low ROS generation shown by brains from long-lived species is associated with a compositional profile resistant to oxidative stress (73), low content/activity of antioxidants as adaptive response (79), and low oxidative damage of the cellular constituents (81–84). Consequently, the human brain, as a long-lived postmitotic tissue belonging to a long-living species, is highly efficient in lowering the steady-state level of oxidative stress, thus reducing the energy cost of maintaining a high antioxidant status; this energy could be directed to other cellular functions.

The investigation of human-specific changes in brain metabolism is a recently emerged research area that focuses on intermediate molecular phenotypes. Although metabolic pathways that are significant for brain function (e.g., energy metabolism, neurotransmitter synthesis and degradation, and protein and lipid biosyntheses) are highly conserved across diverse taxa (85), some findings suggest that brain metabolism may have experienced considerable changes in primates and, specifically, in human evolutionary lineage. The first study to address this possibility used H NMR analysis to analyze the levels of 16 metabolites in the prefrontal cortex of 12 healthy adult humans, 5 adult chimpanzees, and 6 adult rhesus macaques (86). In this study, 7 of 16 metabolites displayed statistically significant concentration differences among species. Interestingly, contrary to the view of a higher metabolic rate of the human brain, concentrations of lactate (one of the nonglucose energy metabolites used by neurons) were lower in the human brain than in the brains of chimpanzees and macaques, and concentrations of glutamate (the main brain energy metabolite and excitatory neurotransmitter) did not show differences among the species. The second (and most recent) study of the metabolic evolution of the human brain analyzed the levels of 61 characterized metabolites in the prefrontal cortex and cerebellum of 49 humans, 11 chimpanzees, and 45 rhesus macaques, using GC-MS (44). The results confirmed the clear separation of human, chimpanzee, and macaque metabolic profiles in both brain regions, according to multivariate analyses. Interestingly, the prefrontal cortex showed significantly higher human-specific metabolic changes than the cerebellum (i.e. 11 [18%] vs 3 [5%] annotated metabolites, respectively). Despite boundaries in the scope of the metabolites and brain areas examined, these studies suggest that an important fraction of the brain metabolome has diverged from closely related primate species.
There is also evidence suggesting brain cross-regional differences in the same individual, at least in the context of oxidative stress. In line with the proposed role of oxidative stress as a driving force for metabolic adaptations in SNV, interregional analyses demonstrate that there are location-specific profiles of selected oxidative stress-related variables. For example, Table 3 shows a summary of studies verifying physiologic interregional differences that are essentially expressed at the level of resistance to oxidative stress, regulatory factors of ROS generation and energy metabolism, antioxidant state, and molecular oxidative damage.

Metabolomics of 3 different mature and healthy human brain regions (i.e. entorhinal cortex, hippocampus, and frontal cortex) confirm the existence of cross-regional differences in the human brain (Fig. 3; Jové et al, unpublished results). It is clear that more studies are needed to establish a metabolomic-wide atlas of metabolites in the adult human brain and to demonstrate how metabolic profiles are transformed in different brain regions during aging, at the onset of age-related NDDs, and throughout the progression of age-related NDDs.

MODIFICATIONS IN BRAIN (AND SYSTEMIC) METABOLISM FAVOR NEURONAL VULNERABILITY IN AGING

Aging causes a multitude of detrimental changes in all animal species at all levels of biologic organization and tissues; these changes decrease maximal functional capacities and homeostasis and increase the probability of experiencing degenerative processes, eventually leading to death. All of these changes probably originate from a small number of basic causes that continuously operate throughout the life span and determine the rate of aging, which is species-, tissue-, and cell type-specific (11). Because the CNS is not an exception, neuronal and non-neuronal cells are also affected by (and adaptively respond to) aging as much as cells in other organ systems. Nevertheless, although aging impacts functions in most neural cell populations, not all groups are affected at the same time and to the same degree. This differential SNV to aging across neuronal populations is accentuated in NDDs. In fact, whether an individual succumbs to NDDs during aging, this fact does not reveal the specific mechanisms that cause a loss of particular cellular/tissue functions. Consequently, whether some key cellular or extracellular molecules are preferential targets of these nonenzymatic modifications and whether the extent of their modification is enough to explain impaired cellular and tissue functions in aging are not clear. In this regard, the determination of steady-state levels for a given marker during the aging process does not allow the identification of the putative damaged target. However, available data on redox proteomics targets during aging show a pattern partially shared by age-related NDDs (6, 106, 120-122). Notably, the general outcome of these studies is that proteins belonging to different metabolic pathways (i.e. glycolysis and energy metabolism, electron transport chain, oxidative phosphorylation and other mitochondrial components, structural components, chaperones, stress proteins, stress responses, and ubiquitin-proteasome system) are preferentially damaged and exhibit detectable age-associated increases in oxidative damage, resulting in loss of functional activity (6, 122).

Interestingly, this selectivity also affects proteins such as amyloid β and tau, α-synuclein, TDP-43 (TAR DNA-binding protein 43), and superoxide dismutase (SOD), which are often considered hallmarks of NDD. In this sense, it is important to point out that the accumulation and nonenzymatic modifications of amyloid β and tau in AD, of α-synuclein in PD, and of CuSOD/ZnSOD and TDP-43 in motor neurons in ALS occur to a lesser extent during normal aging. It is relevant to mention that these “pathogenic” proteins—and probably several proteins of different categories affected by oxidative damage—belong to the proteome of the so-called “intrinsically disordered proteins” (123). Intrinsically disordered proteins are proteins that possess no definitely ordered 3-dimensional structure; they exhibit low sequence complexity and are generally enriched in polar and charged residues (e.g. arginine and lysine) while being depleted of hydrophobic residues (other than proline). This particular amino acid sequence is especially susceptible to nonenzymatic modifications by RS (52), thus becoming proteins prone to aggregation.

If the aging process is fundamental to age-related NDDs, then interventions that slow this process are expected to also protect against NDDs. Studies of the effects of dietary restriction (a manipulation that slows the aging process and extends the average and maximal life span in worms, flies, rodents, monkeys, and humans) indicate that this might be the case (10, 11). Age-related deficits in cognitive and motor functions, defects in energy metabolism, and increases in oxidative stress and leading protein and DNA damage are reduced in animals maintained on dietary restriction (i.e. reduced...
<table>
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<tr>
<th>Species</th>
<th>Parameter</th>
<th>Regions Compared</th>
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<tr>
<td>Human</td>
<td>Polyunsaturated fatty acid content and MDA formation</td>
<td>Cortex, cerebellum, caudate, putamen (medial and lateral), globus pallidus (medial and lateral), and substantia nigra</td>
<td>(87)</td>
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<tr>
<td></td>
<td>Lipid composition (phospholipid contents and distribution, dolichol, cholesterol, and ubiquinone)</td>
<td>Frontal cortex (gray matter and white matter), nucleus caudatus, hippocampus, pons, cerebellum, and medulla oblongata</td>
<td>(88)</td>
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<td></td>
<td>Fatty acid composition of PE and PC fractions</td>
<td>Frontal gray matter, frontal white matter, hippocampus, and pons</td>
<td>(89)</td>
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<tr>
<td>Rat</td>
<td>Phospholipid fatty acid composition</td>
<td>Frontal cortex, occipital cortex, hippocampus, striatum, hypothalamus, cerebellum, and pituitary</td>
<td>(90)</td>
</tr>
<tr>
<td>Mouse</td>
<td>Fatty acid composition</td>
<td>Olfactory bulb, frontal cortex, striatum, hypothalamus, occipital cortex, thalamus, hippocampus, cerebellum, midbrain, pons medulla, and pituitary</td>
<td>(91)</td>
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<tr>
<td>Rat</td>
<td>Aminophospholipid fatty acid composition</td>
<td>Frontal cortex and hippocampus</td>
<td>(92)</td>
</tr>
<tr>
<td></td>
<td>Fatty acid composition</td>
<td>Cerebellum, medulla, hypothalamus, striatum, hippocampus, cortex, and midbrain</td>
<td>(93)</td>
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<tr>
<td>Human</td>
<td>Peroxidizability index membrane fatty acid composition</td>
<td>Frontal cortex, hippocampus, substantia nigra, medulla, and spinal cord</td>
<td>(55)</td>
</tr>
<tr>
<td>Mouse</td>
<td>Expression of the uncoupling protein UCP2</td>
<td>More than 80 brain structures</td>
<td>(94)</td>
</tr>
<tr>
<td></td>
<td>Expression of the uncoupling protein BMCP1/UCP5</td>
<td>Cortex, hippocampus, thalamus, amygdala, and hypothalamus</td>
<td>(95, 96)</td>
</tr>
<tr>
<td>Human</td>
<td>Expression of the apoptosis-inducing factor, which regulates mitochondrial complex I assembly</td>
<td>Frontal cortex, temporal cortex, and hippocampus</td>
<td>(97)</td>
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<tr>
<td>Mouse, rat, and human</td>
<td>Distribution analysis of deacetylase SIRT1</td>
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<tr>
<td>Rat</td>
<td>Mitochondrial oxidative phosphorylation, mitochondrial Ca⁺⁺ sequestration, and mitochondrial ROS production</td>
<td>Brain and spinal cord</td>
<td>(99)</td>
</tr>
<tr>
<td>Mouse</td>
<td>Protein oxidation (carbonyl content and protein-bound sulfhydryl concentration)</td>
<td>Cortex, hippocampus, striatum, midbrain, cerebellum, and hindbrain</td>
<td>(100)</td>
</tr>
<tr>
<td>Rat</td>
<td>Antioxidants (MnSOD, CuSOD/ZnSOD, GPx, and GSH), DNA repair, and DNA oxidation Proteasome subunits and proteasome-associated proteins</td>
<td>Cerebellum, caudate putamen, cortex, hippocampus, midbrain, pons, and medulla</td>
<td>(97)</td>
</tr>
<tr>
<td></td>
<td>Nucleic acid oxidation</td>
<td>Cerebellum, cerebral cortex, hippocampus, striatum, and brainstem</td>
<td>(98)</td>
</tr>
<tr>
<td>Mouse and rat</td>
<td>Protein oxidation and proteasome activities</td>
<td>Hipocampus (CA1, CA3, CA4, and dentate gyrus), cerebral cortex, and white matter</td>
<td>(98)</td>
</tr>
<tr>
<td>Mouse</td>
<td>Proteasomal activity</td>
<td>Cortex, hippocampus, and cerebellum</td>
<td>(101)</td>
</tr>
<tr>
<td></td>
<td>Mitochondrial and nuclear DNA repair capacity</td>
<td>Cortex, cerebellum, globus pallidus, striatum, and substantia nigra</td>
<td>(102)</td>
</tr>
<tr>
<td>Rat</td>
<td>Lipid peroxidation, protein carbonyls, and antioxidant status</td>
<td>Frontal cortex, hippocampus, caudate nucleus, cerebellum, and brainstem</td>
<td>(103)</td>
</tr>
<tr>
<td></td>
<td>Iron content and rates of lipid peroxidation</td>
<td>Cortex, striatum, and hippocampus</td>
<td>(104)</td>
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<tr>
<td></td>
<td>Lipid peroxidation, protein carbonyls, protein thiols, DNA oxidation, and glutathione</td>
<td>Brainstem, hypothalamus, cortex, hippocampus, cerebellum, and striatum</td>
<td>(39)</td>
</tr>
<tr>
<td>Mouse</td>
<td>Glutathione redox state</td>
<td>Cortex, striatum, and hippocampus</td>
<td>(105)</td>
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<tr>
<td></td>
<td>Mitochondrial DNA repair activities</td>
<td>Cortex, hippocampus, striatum, cerebellum, and brainstem</td>
<td>(106)</td>
</tr>
<tr>
<td>Mouse</td>
<td>Transcriptome</td>
<td>12 major brain regions</td>
<td>(1)</td>
</tr>
<tr>
<td>Human</td>
<td>Lipidome</td>
<td>146 regions</td>
<td>(2)</td>
</tr>
<tr>
<td>Mouse</td>
<td>DNA methylome</td>
<td>Brain and spinal cord</td>
<td>(108)</td>
</tr>
</tbody>
</table>

DG, dentate gyrus; GPx, glutathione peroxidase; GSH, reduced glutathione; MDA, malondialdehyde; PC, phosphatidylethanolamine; PE, phosphatidylethanolamine.
alterations of lipoprotein and energy metabolism, characterized by a relative increase in high-density lipoprotein levels and a reduction in very-low-density lipoprotein levels (140). Moreover, insulin-sensitive animals show higher levels of glucose and acetate, suggesting a dietary restriction–modulated increase in metabolic flux through the pentose phosphate pathway (140). The availability of these energy-associated metabolites in plasma can impact CNS metabolism and, consequently, its function.

SNV IN AGE-RELATED NDDS

Aging, a progressive endogenous process (11), is the main risk factor for age-related NDDs (4). Therefore, age-related NDDs share these characteristics and can be considered to be progressive, endogenous metabolic disease processes (7). The progressive character of NDDs means that the cause(s) of NDDs must be present during the whole life span or, at least, at relatively early ages. Exogenous factors, including high energy intake, dietary antioxidants, diabetes, physical and ischemic lesions, infectious agents, environmental toxins, and vascular risk factors, are not primary causes of the intrinsic NDD process; however, they may interact with endogenous causes, thereby enhancing or diminishing their effects.

As cells in the CNS experience increased amounts of oxidative stress and loss of energy homeostasis during aging (6, 12), it can be postulated that neurons selectively vulnerable to age-related NDDs are particularly sensitive to energy demands and oxidative stress. Within the complex scenario of cellular diversity and energy demands, there emerges again the concept of SNV, which is described as the endogenous differential sensitivity of neuronal populations in the CNS and their susceptibility to stresses that may cause cell damage and death (4, 5). The concept of SNV has important applications in NDDs.

We are far from a real understanding of the SNV characteristics of major NDDs, and the reasons for the dramatic demise of substantia nigra pars compacta dopaminergic neurons in PD and motor neurons in ALS, to name 2 well-known examples, are still poorly understood despite the large number of data on these particular neuron populations. We also do not know why AD is 10-fold more frequent than PD, which in turn is 10-fold more frequent than ALS (141).

Because the metabolome represents a more sensitive level of organization than the transcriptome or the proteome (16, 142–144), it is an excellent subject for investigation of SNV and NDDs. Going further in this line, the combination of different “omics” in the same tissue or biologic sample potentiates the robustness of molecular approaches to understanding SNV in NDDs.

Alzheimer Disease

The neuronal populations at high risk for AD (including entorhinal cortex, hippocampal CA1, and frontal cortex neurons) are particularly vulnerable to glucose deprivation (8, 145–148) and oxidative stress (6, 55, 58, 149–151). Bioenergetics failure and oxidative stress become the molecular substrate over which subsequent cellular dysfunction may account for neuronal damage and death in AD. These factors may act upon mitochondrial alterations, generation and accumulation of oxidatively modified cell components, loss of
Ca²⁺ homeostasis, endoplasmic reticulum stress, inflammatory responses, signal transduction defects, cytoskeletal alterations, neurotrophic support failure, hyperexcitability, and synapse loss, among others (4, 5, 7, 152, 153).

Few studies have taken advantage of the use of metabolomics in AD (Table 4). Brain concentrations of 24 metabolites were measured in TgCRND8 mice (a model of AD) using 1H NMR (170). TgCRND8 mice, which encode a mutant form of the APP 695 bearing the Swedish and Indiana mutations, develop extracellular amyloid β deposits in the brain as early as age 2 to 3 months (170). That study demonstrated a widespread metabolism perturbation with some cross-regional differences; with the use of multivariate statistics with few metabolomics variables, this created a model with 60% predictive power (170).

Metabolite profiles in blood that were potentially relevant to the pathogenesis and progression of AD were assessed in a Finnish cohort of familial AD (155). A total of 139 lipids and 544 small polar metabolites were detected, and a molecular signature comprising 3 metabolites predictive of AD progression was identified (155).

Another study was based on the premise that alterations in ceramides and sphingomyelins play a role in amyloidogenesis and inflammatory stress related to neuronal apoptosis (151). Changes in sphingomyelin and ceramide levels in plasma from 26 AD subjects were compared with those from 26 cognitively normal controls; resulting data provided new insights into the AD sphingolipidome and the potential use of metabolite signatures as a potential biomarker (154).

A metabolomics pilot study used LC coupled with colorimetric array detection to analyze 30 metabolites within the neurotransmitter pathways of dopamine and serotonin and within the pathways involved in oxidative stress from postmortem ventricular CSF of 15 AD patients and 15 nondemented subjects (156). In the same line, an interesting recent study used a nontargeted metabolomic approach based on capillary electrophoresis MS. Using multivariate statistics, this study yielded an AD progression prediction model that was able to correctly classify 97% to 100% of the samples in the diagnostic groups (157). Predictive power was confirmed in a blind small test set of 12 CSF samples, reaching 83% diagnostic accuracy (157).

Choline, dimethylarginine, arginine, valine, proline, serine, histidine, creatine, carnitine, and suberylglycine were identified as potential biomarkers of disease signatures (154).

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Finally, a large proportion of the entire polar metabolome of postmortem brain tissue from 15 AD patients and 15 healthy subjects was analyzed by combining ultra-performance LC (UPLC) quadrupole time-of-flight MS and chemometrics (158). This approach allowed the correct prediction of disease status in 94% to 97% of cases; predictive power was confirmed in a blind test set of 60 samples, reaching 100% diagnostic accuracy (158).

Parkinson Disease

Dopaminergic neurons of the substantia nigra pars compacta seem to be particularly vulnerable to mitochondrial oxidative stress, though to a lesser degree than the entorhinal cortex, hippocampus, and frontal cortex (55, 57, 126, 171–173). Dopaminergic neuron dysfunction and death are the main clinical manifestations of PD (173, 174). This is partly caused by mitochondrial complex I defects and ROS generation in PD (175). Later, several genes encoding proteins relevant to maintaining mitochondrial integrity were shown to be causative of familial PD, including DJ1, PINK1, LRRK2, HtrA2, and parkin (176–184). Several subunits of mitochondrial complex I are oxidatively damaged, functionally impaired, and misassembled in PD (185). Phosphorus and proton MRS have confirmed generalized mitochondrial dysfunction in PD (186). In addition, neurochemical studies in optimally preserved human postmortem brain tissue have shown decreased brain cortex and mitochondrial O₂ uptake and reduced complex I activity in PD (172, 187). Globally, these data again reinforce the importance of mitochondrial (dys)function, acting through loss of redox homeostasis, in SNV in PD.

In addition, deficits in glucose metabolism and oxygen consumption and subsequent dysregulation of energy metabolism have been described in dopaminergic neurons of the substantia nigra and cerebral cortex in PD (174, 188–192). Bioenergetic defects and oxidative stress can also increase neuronal vulnerability, inducing disturbances of Ca²⁺ homeostasis that lead to neuronal dysfunction and death in PD (5, 173, 193, 194).

Another research direction giving a prominent role to oxidative stress as a pathogenic pathway involved in neuronal death in PD is focused on establishing an association between levels of urate in the serum or CSF and PD progression. It is important to take into account that uric acid, considered a powerful endogenous antioxidant (195), is a product of purine metabolism, which in turn is closely linked to the pentose phosphate pathway and, consequently, to glycolysis. Thus, available evidence has shown an inverse correlation between urate concentration and clinical progression of PD, with reduced urate levels suggesting an increase in dopaminergic neurodegeneration and advanced PD symptomology (196–199). Furthermore, urate levels have been determined to be a sensitive indicator of risk for developing PD, with higher urate levels predicting a significantly lower risk of PD.

Very few studies have used metabolomics to evaluate biomarkers associated with molecular signatures and pathways involved in PD (Table 4). Two studies focused on brain tissue. In the first study, energy deregulation in cerebral tissue was examined in Park2 knockout mice and in mice subjected to the effect of CCCP, a complex I blocker, with pentose phosphate being the most affected pathway (200). The second study was a case report of the brain metabolome in a patient who had undergone PINK1 A168P/W437X mutations in comparison with idiopathic PD (162). The results showed that brain metabolomics, examined with MRS and positron emission tomography, were clearly distinguishable from those of idiopathic PD (162).

With respect to CSF metabolomics in PD, CSF samples from 48 PD subjects and 57 age-matched controls were analyzed using UPLC linked to GC-MS (161). The results showed that of 243 structurally identified metabolites, 19 compounds differentiated PD from controls at a 20% false discovery level. In PD, the concentration of 3-hydroxykynurenine (an excitotoxin) was increased by one third, and that of glutathione was decreased by 40%. Four of the 19 compounds differentiating
TABLE 4. Summary of Metabolomic Studies of Human Age-Related NDDs

<table>
<thead>
<tr>
<th>Samples Origin</th>
<th>Analytic Platforms</th>
<th>Study Subjects</th>
<th>Findings</th>
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<tr>
<td>AD</td>
<td>Plasma MDMS-SL</td>
<td>AD patients (n = 26) vs cognitively normal controls (n = 26)</td>
<td>Metabolomics identifies altered plasma sphingolipidome in AD patients.</td>
<td>(154)</td>
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<tr>
<td></td>
<td>Plasma UPLC-MS and GCxGC TOFMS</td>
<td>AD patients (n = 47), MCI patients (n = 143), and healthy control (n = 46); among MCI patients, 52 progressed to AD on follow-up</td>
<td>Metabolomics identifies hypoxia, pentose phosphate pathway, oxidative stress, and membrane lipid remodeling in progression to AD.</td>
<td>(155)</td>
</tr>
<tr>
<td></td>
<td>CSF LC-ECA</td>
<td>AD patients (n = 15) vs nondemented subjects with autopsy-confirmed diagnoses (n = 15)</td>
<td>Metabolomics shows alterations in tyrosine, tryptophan, purine, and tocopherol pathways in patients with AD.</td>
<td>(156)</td>
</tr>
<tr>
<td></td>
<td>CSF CE-MS</td>
<td>Subjects with different cognitive status related to AD progression (n = 85)</td>
<td>The prediction model used is able to correctly classify 97%–100% of the samples in the diagnostic groups. Predictive power is confirmed in a blind small test set of 12 CSF samples, reaching 83% diagnostic accuracy.</td>
<td>(157)</td>
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<tr>
<td></td>
<td>Brain UPLC quadrupole TOFMS</td>
<td>AD patients (n = 15) vs healthy age-matched controls (n = 15)</td>
<td>Metabolomics correctly predicts disease status in 94%–97% of cases. Predictive power is confirmed in a blind test set, reaching 100% diagnostic accuracy.</td>
<td>(158)</td>
</tr>
<tr>
<td>PD</td>
<td>Plasma LC-ECA</td>
<td>PD patients (n = 66) vs healthy controls (n = 25)</td>
<td>2 groups were perfectly separated.</td>
<td>(159)</td>
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<tr>
<td></td>
<td>Plasma LC-ECA</td>
<td>Idiopathic PD (n = 41), PD patients carrying the G2019S mutation in the LRRK2 gene (n = 12), healthy family members with (n = 21) or without (n = 10) mutation, and control subjects (n = 15)</td>
<td>LRRK2 patients with the G2019S mutation have unique metabolomic profiles that distinguish them from idiopathic PD patients. Asymptomatic LRRK2 carriers can be separated from gene-negative family members, raising the possibility that metabolomic profiles could be useful in predicting which LRRK2 carriers will eventually develop PD. The results also suggest that there are aberrations in the purine pathway in PD which may occur upstream of uric acid.</td>
<td>(160)</td>
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<tr>
<td></td>
<td>CSF UHPLC-MS, UHPLC/MS-MS, and GC-MS</td>
<td>PD patients (n = 48) vs healthy control subjects (n = 57)</td>
<td>Of 243 structurally identified biochemicals, 19 compounds differentiate PD from controls at a 20% false-discovery level. The Support Vector Machine classification model distinguishes between groups at 83% sensitivity and 91% specificity for learning data, and at 65% and 79% from cross-validation.</td>
<td>(161)</td>
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<tr>
<td>Brain</td>
<td>MRS</td>
<td>A patient (n = 1) with the PINK1 A168P/W437X mutation (a case report)</td>
<td>Brain metabolism is clearly different.</td>
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</tr>
<tr>
<td>ALS</td>
<td>Plasma HPLC-EC</td>
<td>Motor neuron disease patients (n = 28) vs healthy controls (n = 30)</td>
<td>Multivariate regression techniques allow discriminating between motor neuron disease patients and controls.</td>
<td>(163)</td>
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<td>Serum 1H NMR</td>
<td>ALS patients (n = 30) vs Hirayama disease patients (n = 10) and healthy subjects (n = 25)</td>
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<td>Plasma GC-MS plus UHLC/MS-MS</td>
<td>ALS patients (n = 62) vs healthy subjects (n = 69) (Study 1); ALS patients (n = 99) and healthy controls (n = 48) (Study 2)</td>
<td>23 metabolites are significantly altered in plasma from ALS patients in both studies.</td>
<td>(165)</td>
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<td>CSF 1H NMR</td>
<td>ALS patients (n = 50) vs control subjects (n = 44)</td>
<td>PCA demonstrates that the pattern of analyzed metabolites (17) discriminates between groups and that patients are accurately classified 81.6% of the time.</td>
<td>(166)</td>
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<td></td>
<td>CSF GC-TOFMS</td>
<td>ALS patients stratified according to hereditary disposition and clinical subtypes of ALS in relation to controls</td>
<td>After multivariate statistical modeling, the results show that there are significant differences in metabolome profile among patients with FALS, patients with SALS, and patients carrying a mutation in the SOD1 gene, suggesting that different subtypes of ALS may be partially dissimilar.</td>
<td>(167)</td>
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<tr>
<td></td>
<td>CSF GC-TOFMS</td>
<td>ALS patients (n = 16) with 6 different mutations in the SOD1 gene vs ALS patients without such mutations</td>
<td>After multivariate data analysis, ALS patients with the D90A SOD1 mutation appear as a different metabolite entity in the CSF.</td>
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<td></td>
<td>CSF UPLC-HRMS</td>
<td>ALS patients (n = 66) vs non-ALS patients (n = 128)</td>
<td>Metabolome analysis predicts the diagnosis of ALS in more than 80% of cases. OPLS-DA identifies 4 features that discriminate between diagnostic groups.</td>
<td>(169)</td>
</tr>
</tbody>
</table>

CE, capillary electrophoresis; FALS, familiar ALS; GCxGC, gas chromatography x gas chromatography; HPLC-EC, high-performance LC with electrochemical detection; HRMS, high-resolution MS; LC-ECA, LC with electrochemical coulometric array; MCI, mild cognitive impairment; MDMS-SL, multidimensional MS-based shotgun lipidomics; OPLS-DA, orthogonal partial least-squares discriminant analysis; SALS, sporadic ALS; TOFMS, time-of-flight MS; UHLC, ultraperformance LC; UHPLC, ultrahigh-performance LC.
PD from controls were N-acetylated amino acids, suggesting a generalized alteration in N-acetylation activity (161).

Finally, 2 metabolomic studies were carried out in plasma. In one of them, 25 controls and 66 PD patients were examined (159). Metabolomics allowed a complete separation of the 2 groups, and uric acid was significantly reduced whereas glutathione was significantly increased in PD (159). The other study was focused on identifying the plasma metabolomic profiles of patients with PD caused by G2019S LRRK2 mutations (n = 12), asymptomatic family members with (n = 21) or without (n = 10) G2019S LRRK2 mutations, patients with idiopathic PD (n = 41), and nonrelated healthy subjects (n = 15) (160). Plasma metabolomic profiles of both idiopathic PD and LRRK2 PD subjects were clearly separated from controls. In addition, LRRK2 PD patients had metabolomic profiles distinguishable from those with idiopathic PD. Finally, metabolomic profiles of LRRK2 PD patients were different from those of their family members, but there was a slight overlap between family members with and without LRRK2 mutations. Both LRRK2 and idiopathic PD patients showed significantly reduced uric acid levels. A significant decrease in hypoxanthine levels and in the ratios of major metabolites of the purine pathway was also observed in the plasma of subjects with PD (160).

**Amyotrophic Lateral Sclerosis**

Specific combinations of intrinsic neuronal vulnerabilities to oxidative stress also seem to account for the selective vulnerability of motor neurons to ALS. Thus, although motor neurons show particular resistance of cellular components to oxidative damage compared with higher regions of the CNS (55, 61, 108), metabolic and functional differences between brain and spinal cord mitochondria determined that the spinal cord had an intrinsically higher risk of oxidative damage and calcium overload than the brain (55, 61, 108, 201–206), thereby predisposing motor neurons to dysfunction (99, 207) and cell death (208–210). Furthermore, motor neurons vulnerable to ALS are particularly prone to hyperexcitation because of their low expression of γ-aminobutyric acid and glycine receptors (210). Moreover, particular motor neuron vulnerability in ALS is associated with impaired glucose metabolism (205, 211, 212) and increased oxidative damage (99, 120, 213).

To date, very few studies have used metabolomic approaches to evaluate biomarkers associated with molecular signatures and pathways involved in ALS (Table 4). These studies have focused their assessment on CSF (n = 4) and plasma (n = 3), with the aim of identifying metabolites (and metabolic pathways) that are affected in ALS. Studies based on CSF analysis demonstrate that the metabolome correctly predicts the diagnosis of ALS (166, 169) and that the metabolome can discriminate among sporadic, familial, and specific mutations in the SOD1 gene of patients with ALS (167, 168). Metabolomic studies of plasma have been performed using different platforms, including GC-MS and ultrahigh-performance LC/tandem MS (MS-MS) (165), 1H NMR (164), and high-performance LC with electrochemical detection (163). These 3 studies were able to discriminate between ALS and control subjects based on a specific set of metabolites with an abnormal profile in the pathologic condition; however, no biologic marker has yet been validated for routine clinical practice in ALS.

**SUMMARY AND FUTURE PROSPECTS**

Although generalizations should be treated with caution at this point, based on the need to replicate findings in larger populations, current evidence regarding the neurons most affected in NDDs suggests that their specific, particular SNV expresses a steady-state level of oxidative stress and loss of energy metabolism that are prone to inducing neuronal dysfunction; this is aggravated by changes produced by the aging process, leading to the persistent activation of neuronal pathways that ultimately lead to neurodegeneration.

Elucidating how individual factors in the redox and energy metabolism homeostasis network are associated with particular NDDs will require further studies, but current evidence is consistent with the existence of specific mechanistic associations among neuronal vulnerability, oxidative stress, aging, and neurodegeneration. Massive data obtained from “omics” approaches—particularly metabolomics—in the coming years will allow for the emergence of an accurate definition of the SNV of all specific regions making up the CNS and for the identification of key factors in the onset of brain aging and neurodegeneration. In addition, metabolomics may help to identify and evaluate biomarkers of NDDs that can be detected at the level of CSF or even plasma, improving diagnosis and leading to the development of potential therapeutic interventions and treatments.

Metabolomics is not primarily directed toward the causes of the disease but rather shows the final results of metabolic functions, including alterations of those altered functions. As an example, increased plasma glucose levels do not indicate the cause of diabetes mellitus but rather identify one of the endpoints of diabetes. At this time and focusing on the nervous system, metabolomics data are still scarce: in many instances, we cannot discern whether altered levels of a particular metabolite are the direct consequence of the abnormal function of a particular pathway or epiphenomena remotely linked to the cause of the disorder. Again, a combined approach of different “omics” in particular settings, accompanied by potent bioinformatics processing of rough data, will help to increase knowledge about physiologic and pathologic processes in the human brain.

Despite the potential of metabolomics in the study of the nervous system under physiologic and pathologic conditions, the present review has also revealed the tremendous lack of information regarding metabolomes in different brain regions and under different physiologic and pathologic conditions. Focused study of metabolomes at the cellular level in the future will lead to improved understanding of neuronal function in different settings. Obviously, these arguments are not limited to normal conditions; regional metabolomics and cellular metabolomics will also permit delineation of factors that sustain specific neuronal vulnerability. It should be borne in mind that available studies of metabolomics in the CSF and other fluids represent a rough approach to regional and cellular metabolomes in a group of individuals affected by a particular process and, although useful as biomarker tools, are
still far from the final goal of expanding knowledge of individual cell metabolomes and of metabolomes of selected cellular subpopulations in normal and pathologic states. It is worth stressing, however, that the half-life of a number of metabolites ranges from seconds to minutes; therefore, their identification in postmortem human brain is technically impossible. Only the profile of metabolites preserved for a relative period can be presumably recognized in postmortem human brain. Even considering this limitation, the remaining metabolites, counted in hundreds, may allow the segregation of individual cellular metabolomes in different settings.

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