



HAL
open science

Wildtype motoneurons, ALS-Linked SOD1 mutation and glutamate profoundly modify astrocyte metabolism and lactate shuttling

Sylvie Mavel, Blandine Madji Hounoum, Emmanuelle Coque, Franck Patin, Patrick Vourc'H, Sylviane Marouillat, Lydie Nadal-Desbarats, Patrick Emond, Philippe Corcia, Christian Andres, et al.

► To cite this version:

Sylvie Mavel, Blandine Madji Hounoum, Emmanuelle Coque, Franck Patin, Patrick Vourc'H, et al.. Wildtype motoneurons, ALS-Linked SOD1 mutation and glutamate profoundly modify astrocyte metabolism and lactate shuttling. *Glia*, 2017, 65 (4), pp.592 - 605. 10.1002/glia.23114 . hal-01826428

HAL Id: hal-01826428

<https://univ-tours.hal.science/hal-01826428v1>

Submitted on 15 Nov 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

RESEARCH ARTICLE

Wildtype Motoneurons, ALS-Linked SOD1 Mutation and Glutamate Profoundly Modify Astrocyte Metabolism and Lactate Shuttling

Madji Hounoum Blandine,¹ Mavel Sylvie,¹ Coque Emmanuelle,² Patin Franck,^{1,3}
 Vourc'h Patrick,^{1,3} Marouillat Sylviane,¹ Nadal-Desbarats Lydie,^{1,5} Emond Patrick,^{1,5}
 Corcia Philippe,^{1,4} Andres Christian R,^{1,3} Raoul Cédric,² and Blasco Hélène^{1,3}

The selective degeneration of motoneuron that typifies amyotrophic lateral sclerosis (ALS) implicates non-cell-autonomous effects of astrocytes. However, mechanisms underlying astrocyte-mediated neurotoxicity remain largely unknown. According to the determinant role of astrocyte metabolism in supporting neuronal function, we propose to explore the metabolic status of astrocytes exposed to ALS-associated conditions. We found a significant metabolic dysregulation including purine, pyrimidine, lysine, and glycerophospholipid metabolism pathways in astrocytes expressing an ALS-causing mutated superoxide dismutase-1 (SOD1) when co-cultured with motoneurons. SOD1 astrocytes exposed to glutamate revealed a significant modification of the astrocyte metabolic fingerprint. More importantly, we observed that SOD1 mutation and glutamate impact the cellular shuttling of lactate between astrocytes and motoneurons with a decreased in extra- and intra-cellular lactate levels in astrocytes. Based on the emergent strategy of metabolomics, this work provides novel insight for understanding metabolic dysfunction of astrocytes in ALS conditions and opens the perspective of therapeutics targets through focusing on these metabolic pathways.

Key words: metabolomics, excitotoxicity, NMR, LC-HRMS, co-culture, *in vitro* model, cross-talk

GLIA 2017;00:000–000

Introduction

Amyotrophic lateral sclerosis (ALS) is a late-onset neurodegenerative disease that predominantly affects survival of upper and lower motoneurons (Geser et al., 2008). Astrocyte and microglia activation as well as skeletal muscle atrophy are other hallmarks of the disease. Approximately 90% of ALS cases are sporadic (sALS), with the remaining 10% being inherited familial ALS (fALS). Approximately 10 to 20% (1–2% of total) of fALS cases are linked to various dominantly

inherited mutations in the gene encoding the superoxide dismutase 1 (SOD1) (Rosen, 1993). A wealth of studies have demonstrated that non-cell-autonomous effects of SOD1 contribute to the pathogenic process (Ilieva et al., 2009). Notably, a strong astroglial reaction precedes motoneuron death and research has suggested that it actively contributes to the degenerative process (Di Giorgio et al., 2007; Ilieva et al., 2009). However, little is known about mechanisms implicated in astrocyte dysfunction and subsequent motoneuron degeneration. Non-cell-autonomous toxicity of SOD1 in astrocytes

View this article online at wileyonlinelibrary.com. DOI: 10.1002/glia.23114

Published online Month 00, 2016 in Wiley Online Library (wileyonlinelibrary.com). Received Aug 17, 2016, Accepted for publication Dec 29, 2016.

Address correspondence to: Hélène Blasco pharmD PhD; Dr. Hélène BLASCO, Laboratoire de Biochimie et Biologie moléculaire, Hôpital Bretonneau, CHRU de Tours, 2, Bd Tonnellé, 37044 - Tours cedex 1, France. E-mail: helene.blasco@univ-tours.fr

From the ¹Université François-Rabelais, INSERM U930 "Imagerie et Cerveau," CHRU de Tours, Tours, France; ²The Neuroscience Institute Montpellier, INSERM U1051, Saint Eloi Hospital, Montpellier, France; ³Laboratoire de Biochimie et de Biologie Moléculaire, Hôpital Bretonneau, CHRU de Tours, Tours, France; ⁴Centre SLA, Service de Neurologie, CHRU de Tours, Tours, France; ⁵Plateforme Scientifique et Technique "Analyses des Systèmes Biologiques" PST-ASB, Université François-Rabelais, 37032 Tours Cedex 1, France.

Author Contribution: S.M., C.R., B.M., E.C., P.E., P.V., C.R.A., and H.B. conceived of the experiments and wrote the manuscript. H.B. and S.M. directed the project. B.M., F.P., S.M., E.C., and C.R. performed experiments. L.N.D. performed NMR analysis.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Additional Supporting Information may be found in the online version of this article.

implicates the release of factors or cell-contact mechanisms that can trigger death of motoneurons (Aebischer et al., 2011; Almad et al., 2016; Bowerman et al., 2015; Rojas et al., 2014). Astrocytes also play a crucial role in the survival and function of motoneurons in numerous ways, such as maintenance of synaptic glutamate homeostasis and modulation of neuronal susceptibility to glutamate excitotoxicity, or lactate-mediated metabolic coupling. Thus, it suggests that additional mechanisms linked to astrocyte dysfunction might also contribute to the astrocytic neurotoxicity towards motoneurons (Castro et al., 2009; Staats and Van Den Bosch, 2009; Van Den Bosch and Robberecht, 2008).

Excess of glutamate appears to drive excitotoxicity through abnormal flow of calcium ions into neurons that

may activate multiple enzyme cascades (phospholipases, endonucleases, proteases...), damaging components of the cytoskeleton, cell membranes, mitochondria and DNA, which leads to neuron death. This glutamate-induced excitotoxicity represents one of the prominent pathophysiological hypothesis of ALS (Blasco et al., 2014; Heath and Shaw, 2002). Excitatory amino acid transporters in astrocytes (EAAT2) play a major role in keeping extracellular glutamate concentration below neurotoxic levels. Neuronal excitotoxicity in ALS has been associated with loss of EAAT2 protein and function (Rothstein et al., 1995). Consistent with these findings, the only therapeutic agent approved in ALS is riluzole, an anti-glutamatergic agent (Bensimon et al., 1994).

Metabolic alteration is a prominent feature of ALS pathogenesis (Dupuis et al., 2011) and we hypothesize that glutamate might play a decisive role by disturbing the metabolic coupling between astrocytes and motoneurons. Metabolomics (Dunn et al., 2011; Madji Hounoum et al., 2016a; Nicholson et al., 2002) may represent a pertinent approach to further understand the pathophysiological mechanisms that underlie motoneuron degeneration in ALS.

The aim of this work was to determine the metabolic changes that take place in astrocytes expressing a SOD1 mutation, co-cultured with motoneurons and after glutamate exposure. We used liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS) to determine intracellular metabolic profiles and nuclear magnetic resonance spectroscopy (NMR) to analyze the extracellular medium. We assessed metabolic profiles of astrocytes in the following three conditions: (1) Metabolomics profile analysis of SOD1^{G93A} astrocytes in mono- and co-culture with motoneurons to reveal the main metabolic changes induced by the presence of motoneurons; (2) Metabolomics profile analysis of astrocytes in co-culture to explore the impact of glutamate exposure on the metabolism of astrocytes expressing (or not) mutated SOD1; and (3) Metabolomics profile analysis of astrocytes in co-culture under both glutamate exposure and mutant SOD1 expression in astrocytes, and comparison with profiles of cells in co-culture under control condition (wildtype astrocyte-motoneuron co-cultures).

Materials and Methods

Animals

All animal experiments were approved by the National Ethics Committee on Animal Experimentation and were performed in compliance with national directives for the care and use of laboratory animals. Wildtype and transgenic SOD1^{G93A} mice were obtained from Jackson Laboratory. SOD1^{G93A} mice were maintained as hemizygotes by crossing transgenic males with non-transgenic females of the same C57BL/6 genetic background. Progeny were genotyped by

Abbreviations

4-Ace	4-acetamidobutanoic acid
AcCoA	acetyl-CoA
ALCAR	CoA, L-acetylcarnitine
ALS	amyotrophic lateral sclerosis
AMP	adenosine monophosphate
bHB	3-hydroxybutyric acid
cAMP	3'-5' cyclic adenosine monophosphate
CAR	carnitine
Creat	creatine
D2O	deuterium oxide
dCDP	2'-deoxycytidine diphosphate
DHA	docosahexaenoic acid
DMEM	Dulbecco's modified Eagle's medium
EAAT	excitatory amino-acid transporter
ESI	electrospray ionization
FALS	familial amyotrophic lateral sclerosis
FC	fold change
GLAST	glutamate/aspartate transporter
Gln	glutamine
GLT	glutamate transporter
Glu	glutamic acid
GLUT	glucose transporter
HMDB	human metabolome data base
KEGG	kyoto encyclopedia of genes and genomes
Lac	lactate
LC-HRMS	liquid chromatography coupled to high-resolution mass spectrometry
LDH	lactate dehydrogenase
MCTs	monocarboxylate transporters
NAD	nicotinamide adenine dinucleotide
NCBI	national center for biotechnology information
NMR	nuclear magnetic resonance
OPLS-DA	orthogonal partial least squares discriminant analysis
PBS	phosphate-buffered solution
PCA	principal component analysis
PCR	polymerase chain reaction
Pro	proline
QC	control quality
RSD	relative standard deviation
sALS	sporadic amyotrophic lateral sclerosis
SN1	glutamine transporter
SOD1	superoxide dismutase
TSP	trimethylsilylpropionic acid
UMP	uridine monophosphate
UV	unit variance
VIP	variable importance on projection

Hounoum Blandine et al.: Astrocyte Metabolism in ALS-Associated Conditions

polymerase chain reaction (PCR) as previously described (Raoul et al., 2002).

Primary Astrocyte Cell Cultures

Primary cultures of spinal astrocytes were prepared from transgenic SOD1^{G93A} newborn mice (2-3 days of age) and their non-transgenic littermates as previously described (Aebischer et al., 2011). Each spinal cord was treated individually and pups were screened for the SOD1 transgene using PCR (Aebischer et al., 2011). Briefly following trypsin treatment, cells were mechanically dissociated and after centrifugation, cells were seeded in DMEM containing 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C, 7.5% CO₂ (3-4 spinal cords of each genotype were seeded in T75 flask). Isolated astrocytes were then grown to confluence and separated from contaminating microglia via shaking. Cells were then split and plated onto 6-well Transwell® system with 0.4 μm pore polycarbonate membrane insert (Corning) at the density of 100,000 cells per well.

Primary Motoneuron Preparations and Separated Co-Cultures

Primary motoneuron cultures were established from spinal cords of C57BL/6 mice at embryonic day 12.5. Motoneuron enrichment was performed by mechanical dissociation after trypsin treatment, followed by an iodixanol (Sigma-Aldrich) density gradient as previously described (Madji Hounoum et al., 2016b; Raoul et al., 2002). The motoneuron-enriched fraction was seeded onto poly-ornithine/laminin-treated 6-well plates at the density of 15,000 cells per well in the presence of neurotrophic factors (0.1 ng/ml GDNF, 1 ng/ml BDNF, and 10 ng/ml CNTF) in a supplemented neurobasal medium (Invitrogen). The supplemented neurobasal medium contained 2% horse serum, 0.5% penicillin/streptomycin, 25 μM L-glutamate, 25 μM β-mercaptoethanol, 0.5 mM L-glutamine and 2% B-27 supplement (Life Technologies). Motoneurons were plated in triplicate for each culture condition. Cells were maintained at 37°C, 7.5% CO₂ for 8 days before performing co-culture by inserting Transwells containing astrocytes.

Astrocytes were either cultured in the presence (co-cultures) or absence (mono-cultures) of motoneurons, which were physically separated using a Transwell system (Fig. 1).

Glutamate Treatment and Cell Sampling

After 48 hours of co-culturing, glutamate (Sigma Aldrich) was added to the culture medium at the final concentration of 100 μM, the dose of glutamate that leads to excitotoxicity (Madji Hounoum et al., 2016b) while control cells were treated with the same volume of phosphate-buffered solution (PBS). Co-culture and mono-culture cells were exposed to glutamate for 16 hours. Transwells containing astrocytes were then removed to allow for the harvesting of astrocytes.

Before cell sampling for LC-HRMS analysis, 1 mL of culture media was removed and centrifuged (16,000g, 10 min). The supernatants were stored at -80°C until their preparation for ¹H-NMR analysis.

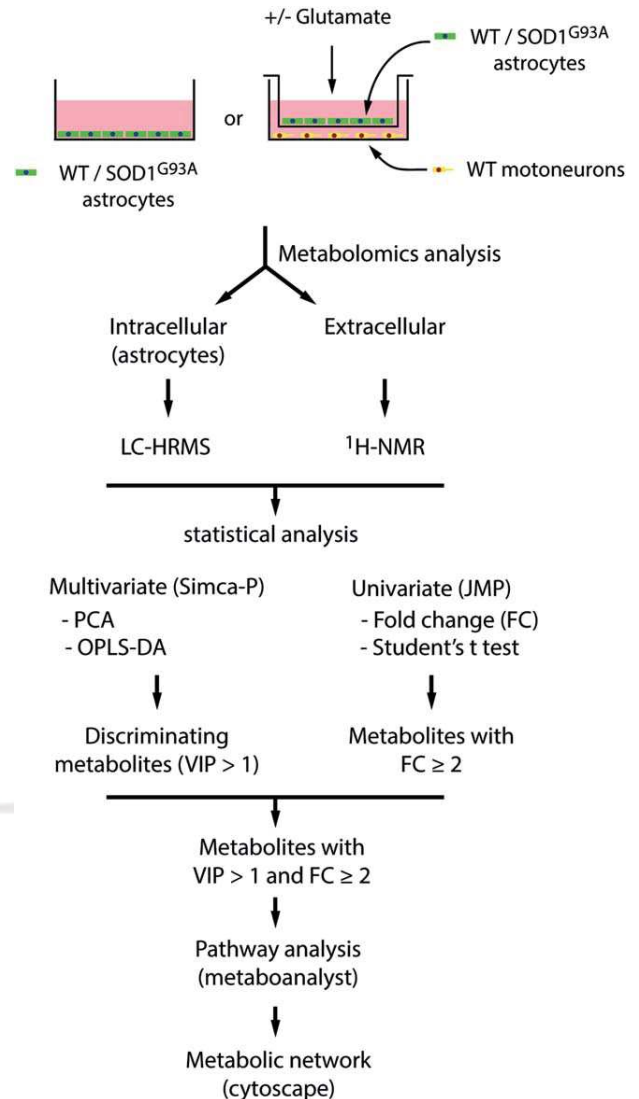


FIGURE 1: Workflow representing the different steps of the experimental design and data analysis.

¹H-NMR Analysis

500 μL of ice-cold methanol was added to 250 μL of culture media. The mixture was vigorously agitated for 20 sec, left at -20°C for 15 min, agitated again under the same conditions and centrifuged at 16,000g for 5 min. Then 700 μL of supernatant was evaporated using a SpeedVac Concentrator (Thermo, Fisher Scientific). The dried culture media were reconstituted with 208 μL of 0.2 M potassium phosphate buffered deuterium oxide (D₂O; pH = 7.4 ± 0.5) with 123 μM of 3-trimethylsilylpropionic acid (TSP) as an external reference. The solution was vortexed briefly, and centrifuged at 10,000g for 15 min at 4°C. The supernatant was transferred to 3 mm NMR tubes for ¹H-NMR analysis.

¹H-NMR spectra were acquired at 298 K on a Bruker Ascend 600 MHz spectrometer (Bruker, Sadis, Wissembourg, France), equipped with a TCI cryoprobe. Standard ¹H-NMR spectra were acquired using a “noesypr1d” pulse sequence with a relaxation delay

F1

COLOR IN PRINT

of 20 seconds and a mixing time of 10 milliseconds. Water suppression was achieved by presaturation during the relaxation delay and mixing time. ^1H -spectra were collected with 32 transients (and 8 dummy scans) into 64 k data points with a spectral width of 7,500 Hz.

LC-HRMS Analysis

Sample preparation and analysis were performed as described previously (Madji Hounoum et al., 2015). Briefly, metabolism was quenched with 1 mL of cold methanol at -40°C and 1 mL of cold water was added to the cell suspension for metabolite extraction. Then, the samples were centrifuged at $15,000g$ for 10 min at 4°C , the supernatant was evaporated, the dry residue was dissolved by 50 μL of methanol/water (50:50 v/v), and 10 μL were injected into the LC-HRMS system. LC-HRMS analysis was performed on a UPLC Ultimate WPS-3000 system (Dionex, Germany), coupled to a Q-Exactive mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) and operated in positive (ESI+) and negative (ESI-) electrospray ionization modes (one run for each mode).

Data Processing

NMR spectra were processed as previously described (Madji Hounoum et al., 2015). Briefly, spectra were processed using Topspin version 2.1 software (Bruker Daltonik, Karlsruhe, Germany). Each ^1H -NMR spectrum within a range of 0.8–9.4 ppm (excluding only the water region, $\delta = 4.67$ –5.0 ppm) was then reduced to 71 buckets of variable width (from 0.01 to 0.15 ppm) using AMIX software (Analysis of MIXture, version 3.9.2, Bruker, Karlsruhe, Germany). Spectral intensities were scaled to the internal reference region corresponding to TSP (-0.03 to 0.03ppm) trimethylsilyl propionate region. Spectra annotations were performed using Chenomx 7.1 software (Chenomx, Edmonton, Canada) and in-house database and human metabolome database (HMDB).

For the LC-HRMS analysis, a library of standard compounds (Mass Spectroscopy Metabolite Library of standards MSML®, IROA Technologies™) were analyzed with the same gradient of mobile phases and in the same conditions than those used to analyze metabolites extracted from cells. In order to validate the identity of each detected metabolite, several criteria were required: (1) the retention time of the metabolite detected must be within ± 20 sec of the standard reference, (2) the measured molecular exact mass of the metabolite must be within 10 ppm of the known mass of the reference compound, and (3) the isotope ratios of the metabolite must match the standard reference. The signal was calculated using Xcalibur® software (Thermo Fisher Scientific, San Jose, CA) by integrating selected ion chromatographic peak area. The data output provides only metabolites for which standard compounds have been analyzed. The metabolites identified after positive and negative electro spray ionization (ESI) mode analysis were combined to provide a non-redundant list of metabolites used for multivariate analysis. Metabolites with relative standard deviation (RSD) in control quality (QCs) higher than that in samples were excluded. Only metabolites with RSD in QCs below 30% and identified in all cell lysate samples were kept for further multivariate analysis. Metabolites greater than 30% variance in QCs were not considered, except if significant

variance was observed between groups, meaning that biological variability may exceed analytical variability (Want et al., 2013)

Statistical Analysis

The data obtained from LC-HRMS and ^1H -NMR analysis were analyzed by multivariate approach using Simca-P+-13.0 software (Umetrics, Umeå, Sweden). Data analysis was preceded by log transformation and unit variance (UV) scaling. The data analysis, including QC, was first conducted using principal component analysis (PCA). Discriminant metabolites were obtained by Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA), a supervised model of classes as previously described (Mavel et al., 2013; Westerhuis et al., 2010). A cross-validation analysis of variance was applied to further evaluate the significance of the contribution plot and to provide information about the variables that influence the cluster of samples. The most discriminating metabolites [variable importance on projection (VIP) representing a value >1] obtained from OPLS-DA analysis and determined by a fold change ≥ 2 (and this for each comparison) were used to analyze the potential impacted pathways.

For univariate analysis, statistical analysis was performed using the Mann–Withney test or student's test using JMP statistical software version 7.0.2 (SAS Institute, Cary, NC). A statistical correction for multiple tests was applied in order to adjust the p-value for significance by accounting for the number of metabolites evaluated (Bonferonni adjustment). Differences were deemed significant when $p < 0.05/n$ (n is the number of compounds analyzed by univariate analysis).

Pathway Analysis

The pathway analysis was conducted by using Metaboanalyst (www.Metaboanalyst.ca), a free web-based tool (Xia et al., 2015) to map pathways corresponding to the metabolites significantly highlighted in the models. The pathway plots were based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, and the National Center for Biotechnology Information (NCBI) database was searched to define gene functions. For ID conversions, the Chemical Translation Service (CTS: <http://cts.fiehnlab.ucdavis.edu/conversion/batch>) was used to convert the common chemical names into their KEGG, HMDB and InChiKeys.

Results

Metabolomics Analysis

The LC-HRMS analysis of intracellular astrocyte medium yielded 100 metabolites (mainly carnitines, amino acids, fatty acids and organic acids) and the ^1H -NMR analysis of culture medium provided 23 metabolites (see Supporting Information Table S1). The non-supervised multivariate analysis visualized by the principal component analysis (PCA) score plot revealed groups and differential responses of astrocytes to different culture conditions (SOD1^{G93A} mutation, presence of motoneurons and/or glutamate exposure; Fig. 2).

Hounoum Blandine et al.: Astrocyte Metabolism in ALS-Associated Conditions

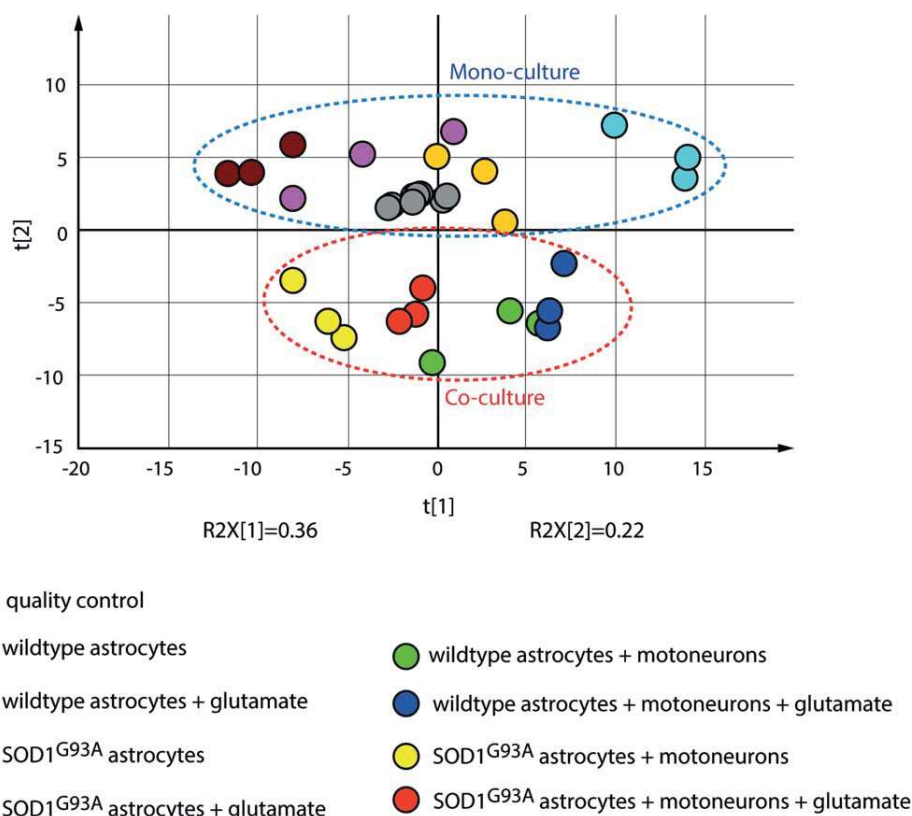


FIGURE 2: Principal component analysis (PCA) score plot of intracellular LC-HRMS analysis of quality controls and astrocyte extracts. Astrocytes expressing (or not) SOD1^{G93A} mutation, co-cultured (or not) to glutamate. Quality controls, wildtype astrocytes, wildtype astrocytes exposed to glutamate, wildtype astrocytes co-cultured with motoneurons, wildtype astrocytes co-cultured with motoneurons and exposed to glutamate, SOD1^{G93A} astrocytes, SOD1^{G93A} astrocytes exposed to glutamate, SOD1^{G93A} astrocytes co-cultured with motoneurons, SOD1^{G93A} astrocytes co-cultured with motoneurons and exposed to glutamate.

Impact of Genetic Mutation: SOD1^{G93A} Mutation Induces Metabolic Changes in Astrocytes

We first assessed metabolic changes in astrocyte metabolism induced by the expression of SOD1^{G93A}. The PCA score plot showed a distinct discrimination between wildtype and SOD1^{G93A} astrocytes, both co-cultured (or not) with motoneurons (Fig. 2). Comparing metabolic profiles between wildtype and SOD1^{G93A} astrocytes highlighted 14 and 15 discriminating metabolites when astrocytes were cultured alone and co-cultured with motoneurons, respectively (Fig. 3, Supporting Information Table S1), including four common discriminating metabolites (palmitate, urocanate, diethanolamine and glucose). Discriminating metabolites were involved in 19 metabolic pathways such as pyrimidine metabolism, purine metabolism, glutathione metabolism, and lysine metabolism pathways (Fig. 3), and thus showed that the expression of ALS-linked mutated SOD1 notably altered astrocyte metabolome.

OPLS-DA performed from ¹H-NMR data of extracellular media associated with wildtype and SOD1^{G93A} astrocytes revealed that nicotinate and lactate were the most

discriminating metabolites between these two groups of media (Table 1). Lactate was downregulated in the culture media from experiments comparing wildtype and SOD1^{G93A} astrocytes both cultured alone and co-cultured with motoneurons. All of these findings demonstrate that SOD1^{G93A} expression may affect the metabolism of astrocytes.

Impact of Co-Culture: Wildtype Motoneurons Induce Metabolic Changes in Wildtype and SOD1^{G93A} Astrocytes

To determine the impact of motoneurons on astrocytes metabolome, we compared the metabolic profile of astrocytes cultured alone to that of astrocytes co-cultured with motoneurons. PCA score plot showed that astrocytes cultured alone were highly distinguishable from those cultured with motoneurons (Fig. 2). OPLS-DA identified 34 metabolites discriminating SOD1^{G93A} astrocytes mono-cultured and SOD1^{G93A} astrocytes co-cultured with motoneurons, and 22 metabolites discriminating when wildtype astrocytes were compared to wildtype astrocytes co-cultured with motoneurons. Interestingly, SOD1^{G93A} and wildtype astrocytes

COLOR IN PRINT

F3

T1

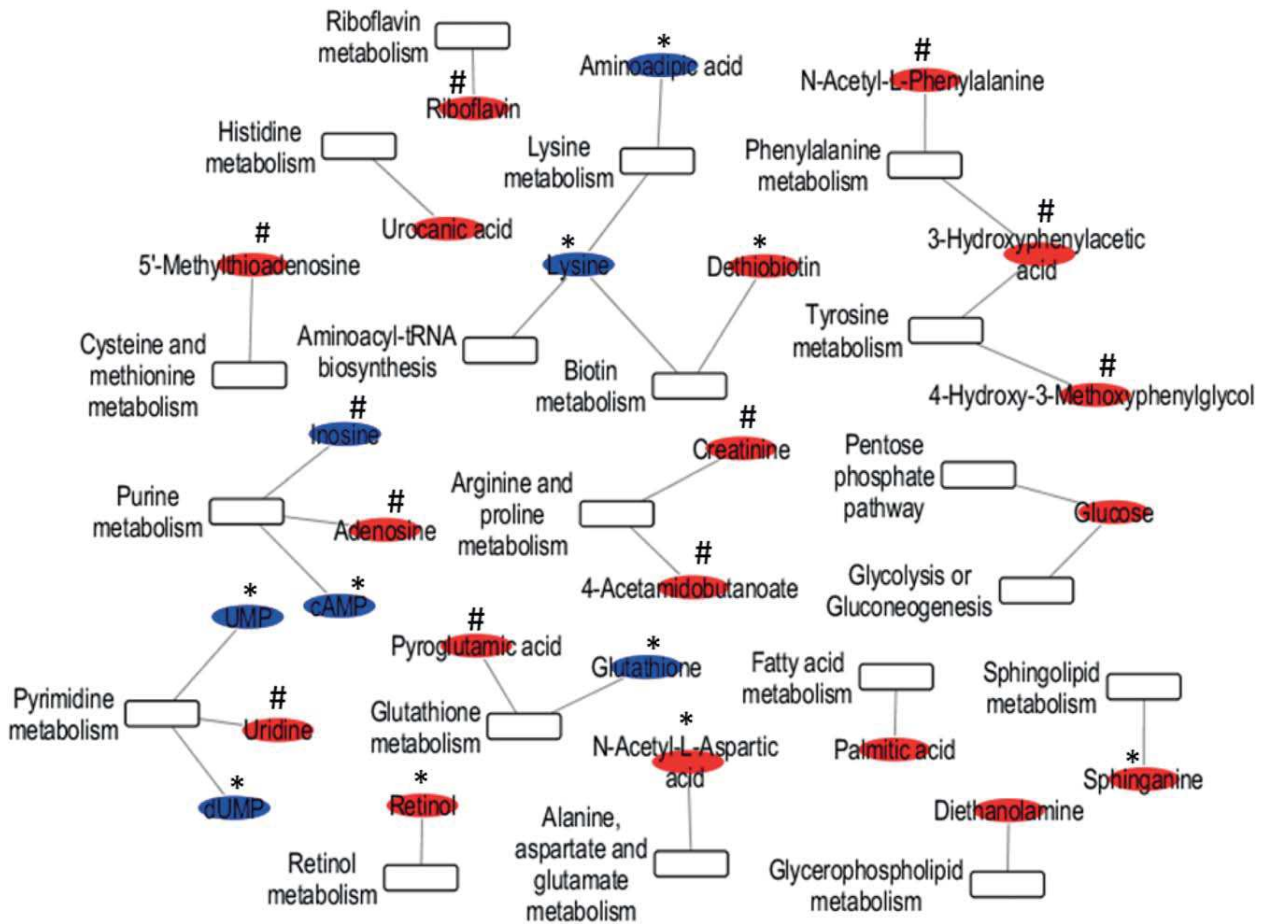


FIGURE 3: Network of metabolites altered by SOD1^{G93A} in mono- and co-cultured astrocytes with motoneurons. Discriminating and altered metabolites were determined by OPLS-DA (VIP >1 and univariate fold change ≥ 2). Rectangle nodes represent pathways, ellipse nodes represent metabolites: upregulated (red) and downregulated (blue) in wildtype astrocytes compared to those in SOD1^{G93A} astrocytes. "*" and "#" are used to underline metabolites altered by SOD1^{G93A} expression specifically in astrocytes cultured alone and co-cultured with motoneurons, respectively.

comparisons overlap greatly when co-cultured with motoneurons, sharing 19 discriminating metabolites. All these discriminating metabolites were upregulated (except palmitate)

in astrocytes cultured with motoneurons compared to those of astrocytes cultured alone (Fig. 4, Table 3). Metabolic pathway analysis revealed that these discriminating metabolites

F4

TABLE 1: Metabolic changes in extracellular media associated to SOD1^{G93A} expression in astrocytes

Extra-cellular metabolites	SOD1 impact on media from astrocytes cultures	
	Astrocytes	Astrocytes co-cultured with wildtype motoneurons
Nicotinic acid	↑ (0.51)	↓ (-0.41)
Lactic acid	↓ (-0.49)	↓ (-0.48)
Valine		↓ (-0.51)
Phosphocholine	↓ (-0.71)	
3-Hydroxybutyric acid	↓ (-0.77)	

Metabolic profiles of media from cultures containing wildtype astrocytes were compared to those containing SOD1^{G93A} mutant astrocytes. Metabolites discriminating culture media in each comparison were determined by OPLS-DA (VIP > 1). ↑ and ↓ denote higher and lower level, respectively in culture media of each investigated condition relative to those of control condition. In brackets the log₂ (fold change of SOD1/WT).

Hounoum Blandine et al.: Astrocyte Metabolism in ALS-Associated Conditions

COLOR IN PRINT

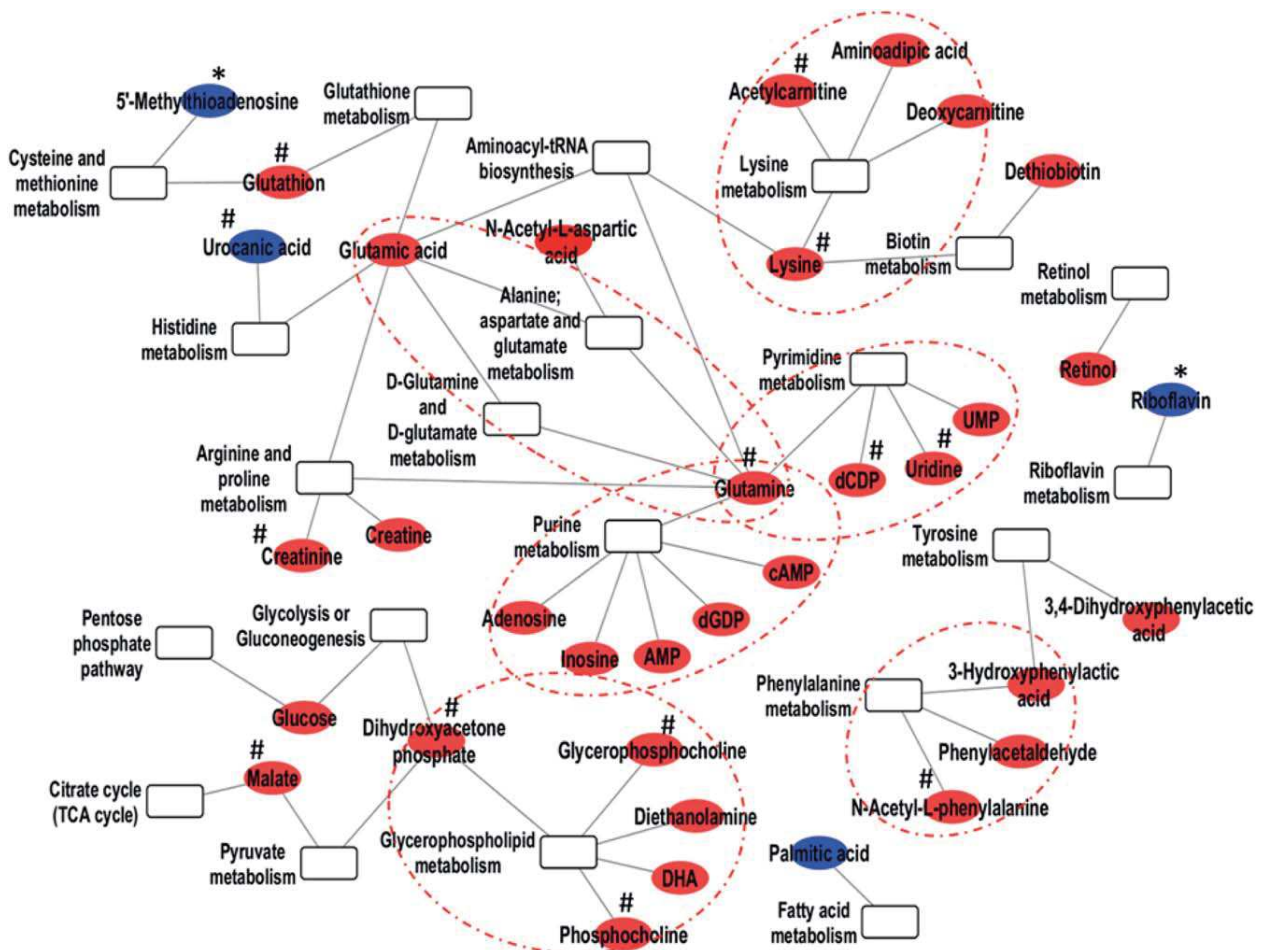


FIGURE 4: Network of metabolites altered by the presence of motoneurons in wildtype and $SOD1^{G93A}$ astrocytes. Discriminating and altered metabolites were determined by OPLS-DA (VIP >1 and univariate fold change ≥ 2). Rectangle nodes represent pathways, ellipse nodes represent metabolites: upregulated (red) and downregulated (blue) in astrocytes co-cultured with motoneurons compared to those in astrocytes cultured alone. "*" and "#" are used to underline metabolites altered by motoneurons presence specifically in wildtype and in $SOD1^{G93A}$ astrocytes, respectively.

were involved in 18 pathways. Pyrimidine metabolism, lysine metabolism, glutamine and glutamate metabolism, phenylalanine metabolism and glycerophospholipids metabolism pathways were the most prominent (Fig. 4). Therefore, the presence of motoneurons in the culture led to significant changes in the metabolic status of both $SOD1^{G93A}$ and wildtype astrocytes. Thus further experiments were focused on co-culture models, associating astrocytes and motoneurons.

Impact of Glutamate: Glutamate Leads to Metabolic Changes in $SOD1^{G93A}$ and Wildtype Astrocytes Cultured with Motoneurons

We first explored the metabolic changes that took place in wildtype astrocytes when co-cultured with motoneurons and exposed to a dose of glutamate that leads to excitotoxicity. The metabolic profile of wildtype astrocytes co-cultured with

motoneurons and treated with glutamate was compared to that of untreated wildtype astrocytes co-cultured with motoneurons (Supporting Information Fig. S1-A). Six discriminating metabolites were found and were involved in the five following metabolic pathways: pyrimidine, purine, biotin, and riboflavin metabolisms, as well as fatty acid metabolism (Table 2).

We then assessed glutamate effects on the metabolome of $SOD1^{G93A}$ astrocytes co-cultured with motoneurons. A PCA score plot showed a clear discrimination between metabolome of $SOD1^{G93A}$ astrocytes co-cultured with motoneurons exposed to glutamate and those non-exposed (Supporting Information Fig. S1-A). Metabolic profile analysis identified eight discriminating metabolites (uridine, phenylacetaldehyde, creatine, creatinine, L-acetylcarnitine, deoxycarnitine, lactate, and glutamate), which were all downregulated (except

T2

TABLE 2: Glutamate effects on wildtype and SOD1^{G93A} mutant astrocytes co-cultured with wildtype motoneurons

Pathways	a) Metabolites	Log2 (FC) ^a
<i>Glutamate effects on wildtype astrocytes</i>		
Biotin metabolism	Biotin	↑ (2.79)
Riboflavin metabolism	Riboflavin	↑ (1.66)
Purine metabolism	3'-5'cyclic AMP	↑ (1.03)
Fatty acid biosynthesis or metabolism	Palmitic acid	↑ (2.88)
Pyrimidine metabolism	Uridine	↓ (-1.40)
Phenylalanine, tyrosine and tryptophan biosynthesis	3-(4-Hydroxyphenyl)pyruvic acid	↓ (-1.01)
<i>Glutamate effects on SOD1^{G93A} mutant astrocytes</i>		
Glutamine and glutamate metabolism	Glutamic acid	↑ (1.33)
Alanine, aspartate and glutamate metabolism	Glutamic acid	↑ (1.33)
Glutathione metabolism	Glutamic acid	↑ (1.33)
Glycolysis or glycogenesis	Lactic acid	↓ (-1.98)
Pyruvate metabolism	Lactic acid	↓ (-1.98)
Arginine and proline metabolism	Glutamic acid	↑ (1.33)
	Creatine	↓ (-1.17)
	Creatinine	↓ (-1.07)
Phenylalanine metabolism	Phenylacetaldehyde	↓ (-1.97)
Lysine metabolism	Deoxycarnitine	↓ (-1.08)
	L-Acetylcarnitine	↓ (-1.16)
Pyrimidine metabolism	Uridine	↓ (-1.20)

Metabolites altered by glutamate exposure in wildtype astrocytes co-cultured, discriminating cells treated with glutamate from those untreated, and, pathways in which they were involved. Discriminating metabolites were determined by OPLS-DA (VIP > 1) and a fold change ≥ 2. ↑ and ↓ denote higher and lower level, respectively in cells treated with glutamate compared to those without treatment. In brackets the log2 (fold change treated/untreated).

glutamate) in SOD1^{G93A} astrocytes (Table 2). These discriminating metabolites were involved in nine metabolic pathways, of which, arginine, proline, and lysine metabolism were the most pronounced (Table 2).

We found one common discriminating metabolite (uridine) between wildtype and SOD1^{G93A} astrocytes comparisons. Astrocytes responded differently to glutamate exposure according to genetic context. SOD1^{G93A} astrocyte metabolism was more affected by glutamate exposure compared to wildtype astrocytes (Supporting Information Fig. S1-A). Glutamate, lactate, creatine, creatinine, L-acetylcarnitine, and deoxycarnitine were specifically modified in astrocytes when glutamate exposure was related to SOD1^{G93A} expression (Table 2).

The PCA score plot performed from ¹H-NMR data of extra-cellular metabolome showed a clear discrimination

between the astrocyte-motoneuron co-culture medium treated with glutamate and the one that was not treated with glutamate (Supporting Information Fig. S1-B) for both co-culture media containing wildtype and SOD1^{G93A} astrocytes. Data analysis revealed that valine, tryptophan, pyroglutamate, phosphocholine, and 3-hydroxybutyrate were the most discriminating metabolites and were all decreased in wildtype astrocyte-motoneuron co-culture medium treated with glutamate compared to untreated ones (Table 3). Nicotinate and 3-hydroxybutyrate were the most discriminating metabolites and were increased in SOD1^{G93A} astrocyte-motoneuron co-culture medium treated with glutamate compared to untreated co-cultures (Table 3). As for intracellular metabolic changes induced by glutamate in astrocytes, we found that glutamate impacted differently the extracellular metabolome depending on the astrocytes' genetic context.

T3

TABLE 3: Metabolic changes in extracellular media associated to glutamate effects on co-culture media containing wildtype and SOD1^{G93A} mutant astrocytes

Extracellular metabolites	Glutamate impact (co-culture)	
	Wildtype astrocytes	SOD1 ^{G93A} astrocytes
Nicotinic acid		↑ (0.38)
Valine	↓ (-0.49)	
Tryptophan	↓ (-0.53)	
Pyroglutamic acid	↓ (-0.44)	
Phosphocholine	↓ (-0.65)	
3-Hydroxybutyric acid	↓ (-0.70)	↑ (0.61)

Metabolic profiles of media from co-cultures exposed to glutamate were compared to those of untreated co-cultures. Metabolites discriminating culture media in each comparison were determined by OPLS-DA (VIP > 1).
 ↑ and ↓ denote higher and lower level, respectively in culture media of each investigated condition relative to those of control condition. In brackets the log₂ (fold change treated/untreated).

Pathogenic Impact: Astrocytic Metabolic Changes Associated with ALS Genetic and Exogenous Factors

To determine the global metabolic changes that occur in astrocytes placed in ALS-associated conditions, we compared the metabolic profile of SOD1^{G93A} astrocytes co-cultured with motoneurons and exposed to glutamate (referred to as “ALS-associated condition”) to the metabolic profile of untreated wildtype astrocytes co-cultured with motoneurons (referred to as “control condition”). The metabolic profile of astrocytes in ALS-associated condition was clearly different from that in control condition (Supporting Information Fig. S1-A). OPLS-DA identified 22 discriminating metabolites, 14 were downregulated and eight upregulated in astrocytes in ALS-associated condition compared to those in control condition (Table 4). The metabolic changes that take place in astrocytes in genetic and glutamate ALS-associated condition involved various metabolic pathways, including glutamate and glutamine metabolism, arginine and proline metabolism, purine metabolism, pyrimidine metabolism, lysine metabolism, glycerophospholipid metabolism, and glycolysis or gluconeogenesis pathways (Fig. 5, Table 4). Extra-cellular metabolic profiles displayed a clear discrimination between culture media of these two groups (Supporting Information Fig. S1-B). OPLS-DA and univariate analysis of ¹H-NMR media data showed that lactate and 3-hydroxybutyrate were discriminating metabolites and decreased in culture media of astrocytes in ALS-associated condition compared to those of astrocytes in control condition. Consistent with the decrease of extra-cellular levels of lactate, astrocytes showed a decreased in intracellular levels of lactate in ALS-associated condition (Fig. 5).

We then aimed to compare these findings on astrocytes and extra-cellular metabolic ALS signature with findings obtained from the cortex of mouse model of ALS [data not shown from a previous study done by our team (Patin et al., 2016)]. An OPLS-DA model and univariate analysis performed on cortex metabolites obtained from symptomatic transgenic SOD1^{G93A} mice revealed common discriminating metabolites between this *in vivo* model and our *in vitro* models (Table 4).

Discussion

Astrocytes play a fundamental role by providing metabolic support to neurons and increasing evidence indicates that they are implicated in the degeneration of motoneurons in ALS (Clement et al., 2003; Ferraiuolo et al., 2011; Papadeas et al., 2011; Van Den Bosch and Robberecht, 2008). We suggest that metabolism alteration largely described in ALS may play a key role in the pathogenesis of motoneuron degeneration, in particular through metabolism disturbances in astrocytes. In this study, we focused on the effects of the expression of a human SOD1 mutation in astrocytes as well as excitotoxicity induced by glutamate on intra- and extra-cellular metabolism of astrocytes co-cultured (or not) with motoneurons.

Wildtype Motoneurons Induce Pyrimidine, Purine, Lysine, Phenylalanine and Glycerophospholipid Metabolism Pathways Changes in Wildtype and SOD1^{G93A} Astrocytes

Several studies have shown that astrocytic energy metabolism plays a pivotal role in the regulation of neurotransmission and energy supply for neurons (Bouzier-Sore and Pellerin,

TABLE 4: Metabolic alterations by ALS-associated conditions in astrocytes, cortex of mice and the literature about human metabolomics studies in ALS

Pathways	Metabolites ^a	Astrocytes ^b	Cortex mice ^c	ALS Patients ^d
Acetyl CoA transfer	L-Acetylcarnitine	↓	↑	–
	Carnitine	↓	–	–
Aminoacyl-tRNA biosynthesis	Glutamic acid	↑	↓	[1]
	Glutamine	↑	↑	[1]
	Proline	↑	↑	[1]
Arginine and proline metabolism	4-Acetamidobutanoic acid	↑	–	–
	Creatine	↓	↓	[1]
	Glutamic acid	↑	↓	[1]
	Glutamine	↑	↑	[1]
	Proline	↑	↑	[1]
Fatty acid metabolism	Palmitic acid	↑	–	–
Glutamine and glutamate metabolism	Glutamic acid	↑	↓	[1]
	Glutamine	↑	↑	[1]
Glutathione metabolism	Glutamic acid	↑	↓	[1]
Glycerophospholipid metabolism	Diethanolamine	↑	–	–
	Docosahexaenoic acid (DHA)	↓	–	–
	Glycerophosphocholine	↓	↓	–
Glycolysis or Gluconeogenesis	Glucose	↑	–	[2]
	Lactic acid	↓	–	–
Lysine metabolism	Amino adipic acid	↓	↓	[2]
	Carnitine	↓	–	–
	Deoxycarnitine	↓	–	–
Nicotinate and nicotinamide metabolism	NAD+	↓	↓	–
Pantothenic and CoA biosynthesis	Pantothenic acid	↓	–	[1]
Pentose phosphate pathway	Glucose	↑	–	[2]
Phenylalanine metabolism	Phenylacetaldehyde	↓	–	[1]
Purine metabolism	Adenosine 3',5'-cyclic phosphate	↓	↓	–
	Glutamine	↑	↑	[1]
	Inosine	↓	–	[2]
Pyrimidine metabolism	2'-Deoxycytidine diphosphate	↓	–	–
	Glutamine	↑	↑	[1]
	Uridine	↑	↓	–

^aMetabolites were determined by OPLS-DA VIP > 1 and a univariate fold change ≥ 2, and pathways in which they were involved.

^bMetabolites altered by ALS associated condition (SOD1^{G93A} mutant astrocytes co-cultured with motoneurons and exposed to glutamate) and discriminating astrocytes in ALS associated condition from those in control condition (wildtype astrocytes co-cultured with wildtype motoneurons and untreated). ↑ and ↓ indicate higher and lower levels, respectively in astrocytes in ALS condition relative to those in control condition.

^cDiscriminating metabolites found in cortex of SOD1^{G93A} mice relative to those of wildtype mice [data not shown from a previous study (Patin et al. 2016)]. ↑ and ↓ indicate higher and lower levels, respectively in cortex of SOD1^{G93A} mice relative to those of wildtype mice.

^dDiscriminating metabolites reported in the literature about human metabolomics in ALS: [1], (Blasco et al. 2016); [2], (Wuolikainen et al. 2016).

2013; Brown and Ransom, 2007; Pellerin et al., 2007). In the present study, we observed that glutamate was upregulated in both wildtype and SOD1^{G93A} astrocytes co-cultured with motoneurons compared to those that were cultured alone

(Fig. 4, Supporting Information Table S3). Co-culturing astrocytes with neurons has been shown to induce the expression of glutamate transporter (GLT/EAAT2) and increase the levels of glutamate/aspartate transporter (GLAST/EAAT1)

Hounoum Blandine et al.: Astrocyte Metabolism in ALS-Associated Conditions

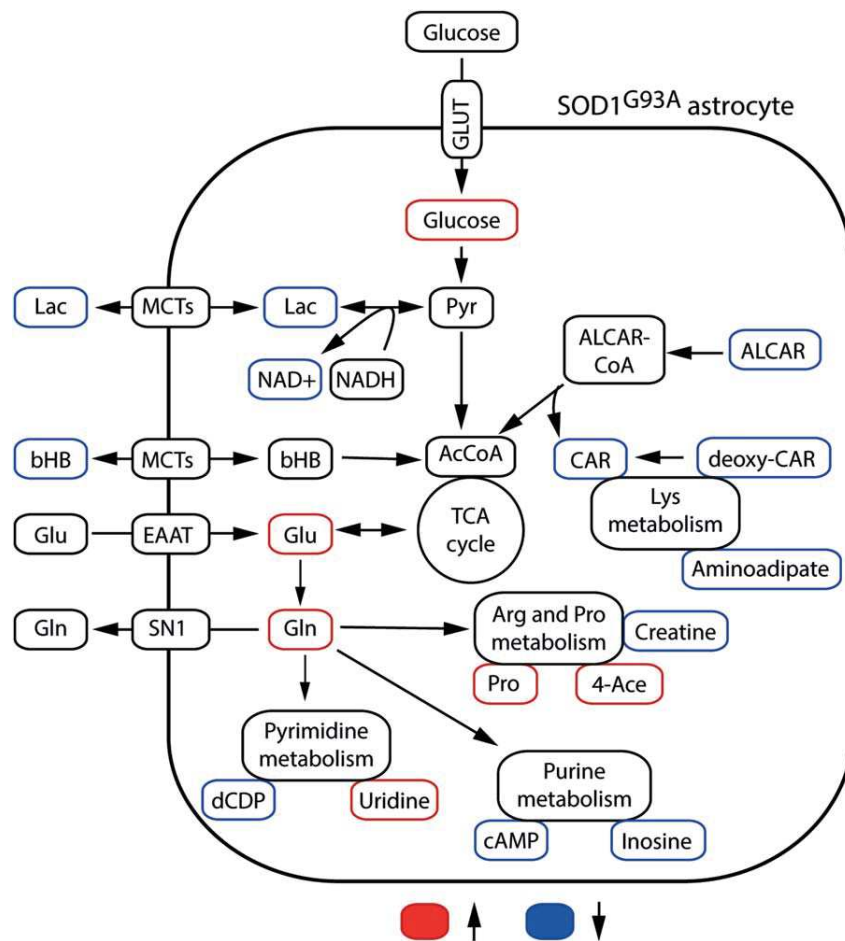


FIGURE 5: Schematic representation of astrocyte metabolic coupling mechanisms and related pathways in ALS conditions. Pathways (surrounded by a black circle) and metabolic profiles of astrocytes in ALS-associated condition ($SOD1^{G93A}$ astrocytes co-cultured with motoneurons and treated with glutamate) were compared to those in control conditions (wildtype astrocytes co-cultured with motoneurons and untreated), metabolites upregulated (red square) and downregulated (blue square) in astrocytes in ALS-associated condition compared to those in control condition.

(Gegelashvili and Schousboe, 1997; Swanson et al., 1997), which may increase glutamate levels observed in co-cultured astrocytes.

We also observed that most of the metabolites involved in purine and pyrimidine metabolic pathways were upregulated in wildtype and $SOD1^{G93A}$ astrocytes co-cultured with motoneurons. We found that adenosine 3',5'-cyclic phosphate (cAMP), adenosine monophosphate (AMP), adenosine, and inosine which derive from each other, as well as uridine and uridine monophosphate (UMP) were all upregulated (Fig. 4), suggesting the existence of an inflammatory circuit where the presence of motoneurons induced upregulation of purinergic machinery in wildtype and $SOD1^{G93A}$ astrocytes (Volonte et al., 2016). This result strongly suggests that the presence of motoneurons is necessary to study metabolism alteration in astrocytes in the context of ALS pathophysiology.

Glutamate Leads to Metabolic Changes in Astrocytes Cultured with Motoneurons

Astrocytes regulate extracellular glutamate concentration specifically through glutamate transporters $GLT-1$ and $GLAST$ (Lehre et al., 1995), which scavenge excess released glutamate from the synaptic cleft to prevent excitotoxicity neuronal death (Danbolt, 2001; Van Den Bosch and Robberecht, 2008). We show here that astrocytes respond differently to glutamate according to expression (or not) of a $SOD1$ mutation. $SOD1^{G93A}$ astrocytes co-cultured with motoneurons were most sensitive to glutamate (Supporting Information Fig. S1) with a prominent impact on arginine and proline metabolisms and on lysine metabolism pathways compared to wildtype astrocytes (Table 2). The accumulation of glutamate in $SOD1^{G93A}$ astrocytes exposed to glutamate suggests a deregulation of the glutamate-glutamine cycle (Massucci

et al., 2013; Pellerin and Magistretti, 1994; Tani et al., 2014). SOD1^{G93A} astrocytes treated with glutamate displayed decreased levels of lactate, creatine, creatinine, deoxycarnitine and L-acetylcarnitine (Table 2), which could be associated with mitochondrial impairment in ALS pathophysiology. This study highlighted the influence of a SOD1 mutation on the sensitivity of astrocytes to glutamate.

Metabolic Changes in Astrocytes with ALS-Associated Conditions

We have shown that astrocytes displayed increased levels of glutamine and glutamate in ALS-associated conditions (co-cultured with motoneurons and treated with glutamate) (Table 4). This is consistent with changes in glutamine and glutamate metabolism in ALS patients (Blasco et al., 2014; Blasco et al., 2016; D'Alessandro et al., 2011; Rothstein et al., 1990; Van Den Bosch et al., 2006). In ALS-associated conditions, carnitine and its acetic acid ester (L-acetyl-carnitine) were downregulated in astrocytes (Fig. 5, Table 4) and are consistent with the alteration in mitochondrial functions and oxidative metabolic disturbances associated with the disease. In addition to their energetic role, these metabolites are both antioxidants and neuroprotective agents (Silva-Adaya et al., 2008). Carnitine is synthesized in the brain from lysine and methionine and, interestingly, we found that metabolites involved in the lysine metabolism pathway, such as deoxycarnitine and aminoadipic acid, were downregulated (Fig. 5, Table 4). Astrocytes in ALS-associated conditions also displayed a decreased creatine level, which is known to be important to mitochondrial function.

Another interesting finding from this study is that ALS-associated conditions induced a decrease of lactate and an increase of glucose levels in astrocytes (Table 4). In accordance with intracellular lactate downregulation, we found that co-culture media of astrocytes in ALS-associated conditions also displayed lower lactate levels compared to control co-culture media. These findings suggest that the decreased lactate levels associated with ALS-like conditions might impair the lactate shuttling between astrocytes and motoneurons. Our results are consistent with an impairment of the astrocyte-motoneuron lactate shuttle hypothesis as previously suggested (Ferraiuolo et al., 2011; Martinez, 2012). In addition, we observed a downregulation of nicotinamide adenine dinucleotide (NAD⁺) in astrocytes in ALS associated conditions. Indeed, since both glycolysis (at the glyceraldehyde phosphate dehydrogenase-catalyzed step) and lactate dehydrogenase (LDH) required NAD⁺ (Kane, 2014), the decreased level of NAD⁺ observed in astrocytes in ALS-associated conditions could explain this lactate decrease and the glucose increase levels (induced by impaired glycolysis) in astrocytes in ALS. A recent study demonstrated that an increase of total

and mitochondrial NAD⁺ contents in ALS astrocytes increased oxidative stress resistance and reverted toxicity effects toward co-cultured motoneurons (Harlan et al., 2016). A decrease of NAD⁺ could make astrocytes vulnerable. Previously, an increase in cAMP concentration in cultured astrocytes has been shown to stimulate glycogen mobilization, glycolysis and lactate output (Pellerin et al., 1997, 1998). According to this observation, we found a decrease in astrocytes of cAMP levels but an increase of intra-cellular glucose levels and a decrease in extra-cellular lactate levels (Table 4 and Fig. 5). Lee and collaborators (Lee et al., 2012) found that lactate transporter expression was reduced in affected brain regions from patients with ALS and in the ventral horn of the spinal cord of SOD1^{G93A} mice. In addition to the downregulation of the lactate transporter, Ferraiuolo and collaborators (Ferraiuolo et al., 2011) showed decreased lactate levels in the spinal cord of SOD1^{G93A} mice. This disruption of lactate transporter could explain either reduced extracellular or intracellular lactate levels in astrocytes as observed in our study. The lactate shuttle impairment in ALS could also be associated with impaired aerobic glycolysis induced by NAD⁺ downregulation in astrocytes and consequently may lead to a lower production of lactate in astrocytes (Fig. 5; Magistretti and Allaman, 2015). These observations are consistent with our findings, that is, identification of a perturbation of lactate metabolism from experiments obtained from spinal cord of SOD1^{G93A} mice. We previously observed no change in lactate level in the cortex of SOD1^{G93A} mice [results from previous study done by our team (Patin et al., 2016)]. Importantly, as study design, disease evolution, brain regions and timing of sample collection were different, we consider that findings about the sense of metabolites levels changes are not comparable and cannot be interpreted. We can only point out the pathway highlighted by different independent experiments.

Nagai and collaborators (Nagai et al., 2007) showed that astrocytes expressing mutated SOD1 induced death of motoneurons *in vitro*, probably by soluble toxic factor(s) through a Bax-dependent mechanism. In 2011, it was shown that these soluble factors included an interferon gamma (Aebischer et al., 2011). However, additional astrocyte-derived soluble factor(s) are involved and could have important implications for ALS pathogenesis (Rossi, 2015). In our study, we analyzed culture media of astrocytes expressing mutated SOD1 and found decreased lactate levels in culture medium. This observation suggests that lactate decrease might be involved in non-cell-autonomous mechanisms described in ALS pathogenesis. We also observed the decrease in extra-cellular 3-hydroxybutyrate levels in ALS-associated condition. Astrocytes are known to consume ketone bodies and may provide them to neighboring neurons as oxidative substrate. Our results suggest that the astrocyte-neuron ketone body

Hounoum Blandine et al.: Astrocyte Metabolism in ALS-Associated Conditions

shuttle may also be impaired in ALS (Guzmán and Blázquez, 2001).

We have compared metabolites altered in this *in vitro* ALS model to those found in the ALS mouse model (Patin et al., 2016) and in ALS patients (Blasco et al., 2016; Wuolikainen et al., 2016) (Table 5). We observed that in ALS-associated condition, astrocytes displayed a low level of glycerophosphocholine as found in SOD1^{G93A} transgenic mice and additionally showed a decreased level of docosahexaenoic acid (DHA) as previously reported in SOD1^{G93A} mice (Arima et al., 2015). Phospholipids are used to synthesize sphingomyelins and are involved in the production of membranes (Moore et al., 1996). Low levels of phospholipids might limit the production of cell membranes and impair oxidative phosphorylation and cell growth (Tasseva et al., 2013). Tasseva and collaborators showed that depletion of mitochondrial phosphatidylethanolamine by only 20 to 30% reduced ATP production and activities of electron transport chain complexes (Tasseva et al., 2013). Our study strongly supports the crucial roles of astrocytes in neurotransmission and in the overall homeostasis of the brain and spinal cord. Astrocytes have a flexible and perhaps opportunistic metabolism that can use, synthesize and release many metabolites (McKenna, 2012). Moreover, the relevance of our findings was further validated by showing that these altered metabolites in astrocytes under ALS conditions were retrospectively found in human ALS data (Blasco et al., 2016; Wuolikainen et al., 2016; Table 5).

In summary, this work is the first metabolomics study performed on primary astrocyte-motoneuron co-cultures which allowed us to better understand the metabolic role of astrocytes in ALS conditions. We highlighted the main metabolic pathways linked with the pathophysiological ways of ALS, including excitotoxicity, mitochondrial dysfunction and oxidative stress, and more importantly we characterized the astrocytic metabolism in these dysfunctions. We have correlated for the first time the impairment of astrocytes lactate efflux in ALS-related SOD1 models to the downregulation of intracellular lactate levels in astrocytes. However, *in vitro* systems lack the complexity of animal models or the human body. This study needs to be completed with fluxomics and proteomics studies in order to highlight the enzyme and transporter regulations at the origin of these metabolic alterations. The metabolic deregulations linked with other genetic mutations found in ALS have to be investigated in order to study the specificity of metabolic changes. This work opens the perspective of therapeutic targets through focusing on the metabolic pathways highlighted.

Acknowledgment

Grant sponsor: La Région Centre [PhD graduate grant (2013-10)]; Grant sponsors: Institut National de la Santé et

de la Recherche Médicale (Inserm), the Association Française pour la Recherche sur la SLA (ARSLA) and ANR-14-RARE-0006 E-RARE 'FasSMALS'; Grant sponsor: Association Française contre les Myopathies (AFM; E.C.; Ph.D. fellowship).

We thank the staff of the PPF ASB platform of the University François-Rabelais of Tours, France, ~~“La Région Centre” for a PhD graduate grant (2013-10)~~ and Céline Salsac for technical assistance.

References

- Aebischer J, Cassina P, Otsmane B, Moumen A, Seilhean D, Meininger V, Barbeito L, Pettmann B, Raoul C. 2011. IFN γ triggers a LIGHT-dependent selective death of motoneurons contributing to the non-cell-autonomous effects of mutant SOD1. *Cell Death Differ* 18:754–768.
- Almad AA, Doreswamy A, Gross SK, Richard JP, Huo Y, Haughey N, Maragakis NJ. 2016. Connexin 43 in astrocytes contributes to motor neuron toxicity in amyotrophic lateral sclerosis. *Glia* 64:1154–1169.
- Arima H, Omura T, Hayasaka T, Masaki N, Hanada M, Xu D, Banno T, Kobayashi K, Takeuchi H, Kadomatsu K, et al. 2015. Reductions of docosahexaenoic acid-containing phosphatidylcholine levels in the anterior horn of an ALS mouse model. *Neuroscience* 297:127–136.
- Bensimon G, Lacomblez L, Meininger V, Group tARS. 1994. A Controlled Trial of Riluzole in Amyotrophic Lateral Sclerosis. *N Engl J Med* 330:585–591.
- Blasco H, Mavel S, Corcia P, Gordon PH. 2014. The glutamate hypothesis in ALS: Pathophysiology and drug development. *Curr Med Chem* 21:3551–3575.
- Blasco H, Patin F, Madji Hounoum B, Gordon PH, Vourc'h P, Andres CR, Corcia P. 2016. Metabolomics in amyotrophic lateral sclerosis: How far can it take us? *Eur J Neurol* 23:447–454.
- Bouzier-Sore AK, Pellerin L. 2013. Unraveling the complex metabolic nature of astrocytes. *Front Cell Neurosci* 7:179.
- Bowerman M, Salsac C, Coque E, Eiselt E, Deschaumes RG, Brodovitch A, Burkly LC, Scamps F, Raoul C. 2015. Tweak regulates astrogliosis, microgliosis and skeletal muscle atrophy in a mouse model of amyotrophic lateral sclerosis. *Hum Mol Genet* 24:3440–3456.
- Brown AM, Ransom BR. 2007. Astrocyte glycogen and brain energy metabolism. *Glia* 55:1263–1271.
- Castro MA, Beltrán FA, Brauchi S, Concha II. 2009. A metabolic switch in brain: Glucose and lactate metabolism modulation by ascorbic acid. *J Neurochem* 110:423–440.
- Clement AM, Nguyen MD, Roberts EA, Garcia ML, Boillée S, Rule M, McMahon AP, Doucette W, Siwek D, Ferrante RJ, et al. 2003. Wild-type non-neuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science* 302:113–117.
- D'Alessandro G, Calcagno E, Tartari S, Rizzardini M, Invernizzi RW, Cantoni L. 2011. Glutamate and glutathione interplay in a motor neuronal model of amyotrophic lateral sclerosis reveals altered energy metabolism. *Neurobiol Dis* 43:346–355.
- Danbolt NC. 2001. Glutamate uptake. *Prog Neurobiol* 65:1–105.
- Di Giorgio FP, Carrasco MA, Siao MC, Maniatis T, Eggan K. 2007. Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model. *Nat Neurosci* 10:608–614.
- Dunn WB, Broadhurst DI, Atherton HJ, Goodacre R, Griffin JL. 2011. Systems level studies of mammalian metabolomes: The roles of mass spectrometry and nuclear magnetic resonance spectroscopy. *Chem Soc Rev* 40:387–426.
- Dupuis L, Pradat P-F, Ludolph AC, Loeffler J-P. 2011. Energy metabolism in amyotrophic lateral sclerosis. *Lancet Neurol* 10:75–82.

- Ferraiuolo L, Higginbottom A, Heath PR, Barber S, Greenald D, Kirby J, Shaw PJ. 2011. Dysregulation of astrocyte-motoneuron cross-talk in mutant superoxide dismutase 1-related amyotrophic lateral sclerosis. *Brain* 134:2627–2641.
- Gegelashvili G, Schousboe A. 1997. High affinity glutamate transporters: Regulation of expression and activity. *Mol Pharmacol* 52:6–15.
- Geser F, Brandmeir NJ, Kwong LK, Martinez-Lage M, Elman L, McCluskey L, Xie SX, Lee VM, Trojanowski JQ. 2008. Evidence of multisystem disorder in whole-brain map of pathological TDP-43 in amyotrophic lateral sclerosis. *Arch Neurol* 65:636–641.
- Guzmán M, Blázquez C. 2001. Is there an astrocyte–neuron ketone body shuttle?. *Trends in Endocrinol Metabol* 12:169–173.
- Harlan BA, Pehar M, Sharma DR, Beeson G, Beeson CC, Vargas MR. 2016. Enhancing NAD⁺ salvage pathway reverts the toxicity of primary astrocytes expressing amyotrophic lateral sclerosis-linked mutant superoxide dismutase 1 (SOD1). *J Biol Chem* 291:10836–10846.
- Heath PR, Shaw PJ. 2002. Update on the glutamatergic neurotransmitter system and the role of excitotoxicity in amyotrophic lateral sclerosis. *Muscle Nerve* 26:438–458.
- Ilieva H, Polymenidou M, Cleveland DW. 2009. Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. *J Cell Biol* 187:761–772.
- Kane DA. 2014. Lactate oxidation at the mitochondria: A lactate-malate-aspartate shuttle at work. *Front Neurosci* 8:366.
- Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN, Liu Y, Tsingalia A, Jin L, Zhang P-W, *et al.* 2012. Oligodendroglia metabolically support axons and contribute to neurodegeneration. *Nature* 487:443–448.
- Lehre KP, Levy LM, Ottersen OP, Storm-Mathisen J, Danbolt NC. 1995. Differential expression of two glial glutamate transporters in the rat brain: Quantitative and immunocytochemical observations. *J Neurosci* 15:1835–1853.
- Madji Hounoum B, Blasco H, Emond P, Mavel S. 2016a. Liquid chromatography–high-resolution mass spectrometry-based cell metabolomics: Experimental design, recommendations, and applications. *TrAC Trends Anal Chem* 75:118–128.
- Madji Hounoum B, Blasco H, Nadal-Desbarats L, Dieme B, Montigny F, Andres CR, Emond P, Mavel S. 2015. Analytical methodology for metabolomics study of adherent mammalian cells using NMR, GC-MS and LC-HRMS. *Anal Bioanal Chem* 407:8861–8872.
- Madji Hounoum B, Vourc’h P, Romain F, Corcia P, Patin F, Gueguinou M, Potier-cartereau M, Vandier C, Raoul C, Andres C, *et al.* 2016b. NSC-34 motor neuron-like cells are unsuitable as experimental model for glutamate-mediated excitotoxicity. *Front Cellular Neurosci* 10:118.
- Magistretti PJ, Allaman I. 2015. A cellular perspective on brain energy metabolism and functional imaging. *Neuron* 86:883–901.
- Martinez BA. 2012. Lactate-starved neurons in ALS. *Dis Model Mech* 5:711–712.
- Massucci FA, DiNuzzo M, Giove F, Maraviglia B, Castillo IP, Marinari E, De Martino A. 2013. Energy metabolism and glutamate-glutamine cycle in the brain: A stoichiometric modeling perspective. *BMC Syst Biol* 7:103.
- Mavel S, Nadal-Desbarats L, Blasco H, Bonnet-Brilhault F, Barthélémy C, Montigny F, Sarda P, Laumonier F, Vourc’h P, Andres CR, *et al.* 2013. 1H–13C NMR-based urine metabolic profiling in autism spectrum disorders. *Talanta* 114:95–102.
- McKenna MC. 2012. Substrate competition studies demonstrate oxidative metabolism of glucose, glutamate, glutamine, lactate and 3-hydroxybutyrate in cortical astrocytes from rat brain. *Neurochem Res* 37:2613–2626.
- Moore C, Perry ACF, Love S, Hall L. 1996. Sequence analysis and immunolocalisation of phosphatidylethanolamine binding protein (PBP) in human brain tissue. *Mol Brain Res* 37:74–78.
- Nagai M, Re DB, Nagata T, Chalazonitis A, Jessell TM, Wichterle H, Przedborski S. 2007. Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. *Nat Neurosci* 10:615–622.
- Nicholson JK, Connelly J, Lindon JC, Holmes E. 2002. Metabonomics: A platform for studying drug toxicity and gene function. *Nat Rev Drug Discov* 1: 153–161.
- Papadeas ST, Kraig SE, O’Banion C, Lepore AC, Maragakis NJ. 2011. Astrocytes carrying the superoxide dismutase 1 (SOD1G93A) mutation induce wild-type motor neuron degeneration in vivo. *Proc Natl Acad Sci USA* 108: 17803–17808.
- Patin F, Baranek T, Vourc’h P, Nadal-Desbarats L, Goossens JF, Marouillat S, Dessein AF, Descat A, Hounoum BM, Bruno C, *et al.* 2016. Combined metabolomics and transcriptomics approaches to assess the IL-6 blockade as a therapeutic of ALS: Deleterious alteration of lipid metabolism. *Neurotherapeutics* 13:905–917.
- Pellerin L, Bouzier-Sore AK, Aubert A, Serres S, Merle M, Costalat R, Magistretti PJ. 2007. Activity-dependent regulation of energy metabolism by astrocytes: An update. *Glia* 55:1251–1262.
- Pellerin L, Magistretti PJ. 1994. Glutamate uptake into astrocytes stimulates aerobic glycolysis: A mechanism coupling neuronal activity to glucose utilization. *Proc Natl Acad Sci USA* 91:10625–9.
- Pellerin L, Pellegrini G, Bittar PG, Charnay Y, Bouras C, Martin JL, Stella N, Magistretti PJ. 1998. Evidence supporting the existence of an activity-dependent astrocyte–neuron lactate shuttle. *Dev Neurosci* 20: 291–299.
- Pellerin L, Stolz M, Sorg O, Martin J-L, Deschepper CF, Magistretti PJ. 1997. Regulation of energy metabolism by neurotransmitters in astrocytes in primary culture and in an immortalized cell line. *Glia* 21:74–83.
- Raoul C, Estevez AG, Nishimune H, Cleveland DW, deLapeyriere O, Henderson CE, Haase G, Pettmann B. 2002. Motoneuron death triggered by a specific pathway downstream of Fas. potentiation by ALS-linked SOD1 mutations. *Neuron* 35:1067–1083.
- Rojas F, Cortes N, Abarzua S, Dyrda A, van Zundert B. 2014. Astrocytes expressing mutant SOD1 and TDP43 trigger motoneuron death that is mediated via sodium channels and nitroxidative stress. *Front Cell Neurosci* 8:24.
- Rosen DR. 1993. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 362:59–62.
- Rossi D. 2015. Astrocyte physiopathology: At the crossroads of inter-cellular networking, inflammation and cell death. *Prog Neurobiol* 130: 86–120.
- Rothstein JD, Tsai G, Kuncl RW, Clawson L, Cornblath DR, Drachman DB, Pestronk A, Stauch BL, Coyle JT. 1990. Abnormal excitatory amino acid metabolism in amyotrophic lateral sclerosis. *Ann Neurol* 28:18–25.
- Rothstein JD, Van Kammen M, Levey AI, Martin LJ, Kuncl RW. 1995. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Ann Neurol* 38:73–84.
- Silva-Adaya D, Perez-De La Cruz V, Herrera-Mundo MN, Mendoza-Macedo K, Villeda-Hernandez J, Binienda Z, Ali SF, Santamaria A. 2008. Excitotoxic damage, disrupted energy metabolism, and oxidative stress in the rat brain: Antioxidant and neuroprotective effects of L-carnitine. *J Neurochem* 105:677–689.
- Staats KA, Van Den Bosch L. 2009. Astrocytes in amyotrophic lateral sclerosis: Direct effects on motor neuron survival. *J Biol Phys* 35:337–346.
- Swanson RA, Liu J, Miller JW, Rothstein JD, Farrell K, Stein BA, Longuemare MC. 1997. Neuronal regulation of glutamate transporter subtype expression in astrocytes. *J Neurosci* 17:932–940.
- Tani H, Dulla CG, Farzampour Z, Taylor-Weiner A, Huguenard JR, Reimer RJ. 2014. A local glutamate-glutamine cycle sustains synaptic excitatory transmitter release. *Neuron* 81:888–900.
- Tasseva G, Bai HD, Davidescu M, Haromy A, Michelakis E, Vance JE. 2013. Phosphatidylethanolamine deficiency in mammalian mitochondria impairs oxidative phosphorylation and alters mitochondrial morphology. *J Biol Chem* 288:4158–4173.
- Van Den Bosch L, Robberecht W. 2008. Crosstalk between astrocytes and motor neurons: What is the message? *Exp Neurol* 211:1–6.

Hounoum Blandine et al.: Astrocyte Metabolism in ALS-Associated Conditions

Van Den Bosch L, Van Damme P, Bogaert E, Robberecht W. 2006. The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis. *Biochim Biophys Acta* 1762:1068–1082.

Volonte C, Apolloni S, Parisi C, Amadio S. 2016. Purinergic contribution to amyotrophic lateral sclerosis. *Neuropharmacology* 104:180–193.

Want EJ, Masson P, Michopoulos F, Wilson ID, Theodoridis G, Plumb RS, Shockcor J, Loftus N, Holmes E, Nicholson JK. 2013. Global metabolic profiling of animal and human tissues via UPLC-MS. *Nat Protocols* 8:17–32.

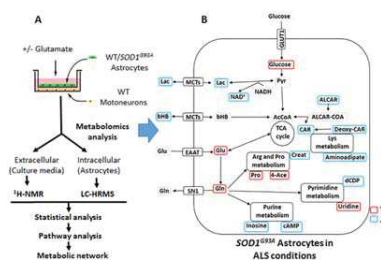
Westerhuis JA, van Velzen EJ, Hoefsloot HC, Smilde AK. 2010. Multivariate paired data analysis: Multilevel PLSDA versus OPLSDA. *Metabolomics* 6:119–128.

Wuolikainen A, Jonsson P, Ahnlund M, Antti H, Marklund SL, Moritz T, Forsgren L, Andersen PM, Trupp M. 2016. Multi-platform mass spectrometry analysis of the CSF and plasma metabolomes of rigorously matched amyotrophic lateral sclerosis, Parkinson's disease and control subjects. *Mol Biosyst* 12:1287–1298.

Xia J, Sinelnikov IV, Han B, Wishart DS. 2015. MetaboAnalyst 3.0—making metabolomics more meaningful. *Nucleic Acids Res* 43:W251–W257.

WILEY
Author Proof

SGML and CITI Use Only DO NOT PRINT



- First metabolomics study performed on primary cultures of astrocytes and motoneurons
- Motoneurons induced important changes in SOD1^{G93A} astrocyte metabolism
- Astrocytes in ALS conditions displayed a decrease of intra-and extra-cellular lactate

WILEY
Author Proof