NON-MOTOR SYMPTOMS IN PRIMARY MOTOR NEUROLOGICAL DISORDERS: FROM MOLECULAR PATHWAYS TO CLINICAL AND THERAPEUTIC IMPLICATIONS

EDITED BY: Francesca Trojsi, Kaylena Anastasia Ehgoetz Martens, Foteini Christidi, Rosa De Micco and Cristina Moglia

PUBLISHED IN: Frontiers in Neuroscience, Frontiers in Psychiatry and Frontiers in Neurology

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88963-389-0
DOI 10.3389/978-2-88963-389-0

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

NON-MOTOR SYMPTOMS IN PRIMARY MOTOR NEUROLOGICAL DISORDERS: FROM MOLECULAR PATHWAYS TO CLINICAL AND THERAPEUTIC IMPLICATIONS

Topic Editors:
Francesca Trojsi, University of Campania Luigi Vanvitelli, Italy
Kaylena Anastasia Ehgoetz Martens, University of Waterloo, Canada
Foteini Christidi, National and Kapodistrian University of Athens, Greece
Rosa De Micco, Second University of Naples, Italy
Cristina Moglia, University of Turin, Italy

Citation: Trojsi, F., Ehgoetz Martens, K. A., Christidi, F., De Micco, R., Moglia, C., eds. (2020). Non-Motor Symptoms in Primary Motor Neurological Disorders: from Molecular Pathways to Clinical and Therapeutic Implications. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88963-389-0

Table of Contents

frontiers in Neuroscience

EDITORIAL
published: 03 December 2019
doi: 10.3389/fnins.2019.01296

Editorial: Non-motor Symptoms in Primary Motor Neurological Disorders: From Molecular Pathways to Clinical and Therapeutic Implications

Foteini Christidi[1], *Rosa De Micco*[2], *Kaylena A. Ehgoetz Martens*[3], *Cristina Moglia*[4] *and Francesca Trojsi*[2]*

[1] First Department of Neurology, Aeginition Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece, [2] First Division of Neurology, Department of Advanced Medical and Surgical Sciences, University of Campania "Luigi Vanvitelli", Naples, Italy, [3] Department of Kinesiology, University of Waterloo, Waterloo, ON, Canada, [4] ALS Center, "Rita Levi Montalcini" Department of Neuroscience, University of Turin, Turin, Italy

Keywords: neurodegenerative diseases, movement disorders, motor neuron disease, non-motor symptoms, *C9orf72* hexanucleotide repeat expansion, neuropsychiatric disorders, sleep disorders, levodopa response

Editorial on the Research Topic

Non-motor Symptoms in Primary Motor Neurological Disorders: From Molecular Pathways to Clinical and Therapeutic Implications

OPEN ACCESS

Edited and reviewed by:
Einar M. Sigurdsson,
New York University, United States

***Correspondence:**
Francesca Trojsi
francesca.trojsi@unicampania.it

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: *27 October 2019*
Accepted: *18 November 2019*
Published: *03 December 2019*

Citation:
Christidi F, De Micco R, Ehgoetz Martens KA, Moglia C and Trojsi F (2019) Editorial: Non-motor Symptoms in Primary Motor Neurological Disorders: From Molecular Pathways to Clinical and Therapeutic Implications. Front. Neurosci. 13:1296. doi: 10.3389/fnins.2019.01296

There is increasing evidence that neurodegenerative diseases imply high emotional and economic burden (GBD 2016 Neurology Collaborators, 2019), substantially influenced by a variety of non-motor symptoms (NMS), such as gastrointestinal-, autonomic-, neuropsychiatric-, and sleep disorders, preceding classical motor signs or appearing during the disease course. A convincing explanation of these symptoms would be much better supported by the recently postulated "disease-spreading hypothesis" (Brundin et al., 2010), in that the pathology would not be limited to the initially affected cell populations, but disease would spread involving other non-motor regions, in the brain and beyond (Braak et al., 2003; Brettschneider et al., 2013). The 10 articles contributing to this Frontiers Research Topic will provide readers with an update on some of the most crucial aspects of NMS in neurodegenerative disorders, addressing both molecular and clinical issues.

Firstly, with regard to the use of animal models for investigating the pathogenesis of familial amyotrophic lateral sclerosis (fALS), Pharaoh et al. measured changes in metabolic pathways in spinal cords of the $SOD1^{G93A}$ mouse model of ALS using a targeted proteomic analysis. The protein content of metabolic proteins, including those involved in glycolysis, β-oxidation, and mitochondrial metabolism, was found altered in $SOD1^{G93A}$ mouse spinal cord before disease onset, recalling some hypotheses on the potential role of metabolism and nutrition in ALS pathogenesis (Gallo et al., 2013; Wills et al., 2014).

With regard to clinical studies, assuming the ALS as an emblematic neurodegenerative disease in which both motor and extra-motor symptoms may coexist from early stages, the neuropsychological profile described in ALS patients has been deeply reviewed by Benbrika et al. to provide readers with a clear picture of all the cognitive, emotional and psychological manifestations of ALS. Neuropsychological assessment needs the use of ALS-specific tools, such as the Edinburgh Cognitive and Behavioral ALS screen (ECAS) (Abrahams et al., 2014). Moreover, the increased understanding of the disease spectrum including ALS and frontotemporal dementia (FTD)

(Burrell et al., 2016) has led researchers to elaborate on a variety of symptoms not classically considered part of the ALS clinical picture, such as psychiatric symptoms, reviewed by Zucchi et al.. In particular, the link between ALS and schizophrenia was further supported by a substantial genetic correlation, only partially explained by pleiotropic gene variants, such as *C9orf72* (McLaughlin et al., 2017).

ALS patients carrying the *C9orf72* hexanucleotide repeat expansion (C9+ALS) had significantly more co-morbid behavioral variant FTD features than those without (Byrne et al., 2012) and increasing evidence has suggested that hippocampus and subcortical region degeneration also plays a role in the peculiar clinical picture of C9+ALS patients (Agosta et al., 2017). Furthermore, with regard to hippocampal involvement in sporadic ALS, Gómez-Pinedo et al. explored the expression of the intracellular pathway of Notch proteins, probably associated with amyloid precursor protein (APP) signaling pathways (Ables et al., 2011), by analyzing hippocampal samples from the autopsies of 12 patients with ALS or ALS-FTD. They reported lower levels of the Notch intracellular domain in patients with ALS than in controls, leading to increases in amyloid-β and to decrease in hippocampal neurogenesis.

Among the subcortical structures potentially involved in the cognitive status of ALS patients, Consonni et al. tested the relationship between cerebellar degeneration, cognitive syndromes, and *C9orf72* mutation in ALS patients, revealing that cerebellar involvement may reflect a signature of ALS-FTD (Bede et al., 2013) other than a signature of the *C9orf72* hexanucleotide repeat.

In light of the recent observation of a widespread extra-motor involvement in ALS, that has implied that, beyond motor neurons, peripheral axons and nerve terminals may also be affected as early events, Gentile et al. reviewed the most relevant literature regarding common molecular pathways (i.e., impairments of axonal transport, RNA metabolism, and proteostasis) shared by ALS and peripheral axonal neuropathies. They underlined that an extensive evaluation of the molecular events occurring in the peripheral nervous system could be fundamental to understand the pathogenic mechanisms of ALS as well as other neuropsychiatric disorders. In this regard, an interesting link between hereditary polyneuropathies and psychiatric disorders, such as schizophrenia, has been discussed by Endres et al., who presented the case of a patient with schizophrenia and comorbid hereditary neuropathy with liability to pressure palsy (HNPP), due to a deletion of the peripheral myelin protein 22 gene (*PMP22*). This potential association has been explained by the role of *PMP22*, mainly expressed in the peripheral nervous system, although its mRNA has also been detected in the brain (Chanson et al., 2013). In particular, *PMP22* seems to play an important role in regulating cell growth and myelination (Sanahuja et al., 2005), also impaired in schizophrenia.

A widespread appearance of pathologic proteins aggregates in both the central and peripheral nervous systems, including the enteric nervous system (ENS), has been recognized also in Parkinson's disease (PD) (Goedert, 2001). In particular, Fonseca Santos et al. focused on the hypothesis of a "gut-brain axis": gut toxins could induce the formation of α-synuclein aggregates in the ENS, which may then be transmitted in a prion-like manner to the central nervous system through the vagus nerve. From the therapeutic point of view, levodopa is actually considered the best current symptomatic treatment for PD, although characterizing levodopa response may be a challenge in early stages. In this regard, Serrano et al. presented the analysis of electroencephalography microstates (EEG-MS) default-mode network changes in response to dopaminergic stimulation as a potential tool to evaluate, in a non-invasive way, the levodopa response and to assess the suitability of the patients' medication dosage.

Finally, among other common NMS, Herzog-Krzywoszanska and Krzywoszanski addressed the role of sleep abnormalities in Huntington's disease (HD). Among the major criticisms raised, the authors underlined that many medications administered to HD patients to alleviate motor and psychiatric symptoms may change sleep architecture, thereby negatively impacting sleep quality (Arnulf et al., 2008). Furthermore, more detailed knowledge of these problems in HD can also provide more profound insight into the nature of the neurodegenerative process underlying the disease (Morton, 2013).

In conclusion, the substantial message of this Frontiers Research Topic is that the identification of extra-motor abnormalities may represent a core feature for supporting the diagnosis and predicting the prognosis of many primary motor neurodegenerative disorders and for shedding light on several dysfunctional pathways in order to prompt the development of combined therapies with synergistic neuroprotective effects on several neurodegenerative pathomechanisms.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

We are deeply grateful to all authors who contributed their valuable articles and expert reviewers for their significant contribution.

REFERENCES

Ables, J. L., Breunig, J. J., Eisch, A. J., and Rakic, P. (2011). Not(ch) just development: Notch signalling in the adult brain. *Nat. Rev. Neurosci.* 12, 269–283. doi: 10.1038/nrn3024

Abrahams, S., Newton, J., Niven, E., Foley, J., and Bak, T. (2014). Screening for cognition and behaviour changes in ALS. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 15, 9–14. doi: 10.3109/21678421.2013.805784

Agosta, F., Ferraro, P. M., Riva, N., Spinelli, E. G., Domi, T., Carrera, P., et al. (2017). Structural and functional brain signatures of

C9orf72 in motor neuron disease. *Neurobiol. Aging* 57, 206–219. doi: 10.1016/j.neurobiolaging.2017.05.024

Arnulf, I., Nielsen, J., Lohmann, E., Schieffer, J., Wild, E., Jennum, P., et al. (2008). Rapid eye movement sleep disturbances in Huntington disease. *Arch. Neurol.* 65, 482–488. doi: 10.1001/archneur.65.4.482

Bede, P., Bokde, A. L., Byrne, S., Elamin, M., McLaughlin, R. L., Kenna, K., et al. (2013). Multiparametric MRI study of ALS stratified for the *C9orf72* genotype. *Neurology* 81, 361–369. doi: 10.1212/WNL.0b013e31829c5eee

Braak, H., Del Tredici, K., Rüb, U., de Vos, R. A., Jansen Steur, E. N., and Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol. Aging* 24, 197–211. doi: 10.1016/s0197-4580(02)00065-9

Brettschneider, J., Del Tredici, K., Toledo, J. B., Robinson, J. L., Irwin, D. J., Grossman, M., et al. (2013). Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. *Ann. Neurol.* 74, 20–38. doi: 10.1002/ana.23937

Brundin, P., Melki, R., and Kopito, R. (2010). Prion-like transmission of protein aggregates in neurodegenerative diseases. *Nat. Rev. Mol. Cell Biol.* 11, 301–307. doi: 10.1038/nrm2873

Burrell, J. R., Halliday, G. M., Kril, J. J., Ittner, L. M., Götz, J., Kiernan, M. C., et al. (2016). The frontotemporal dementia-motor neuron disease continuum. *Lancet* 388, 919–931. doi: 10.1016/S0140-6736(16)00737-6

Byrne, S., Elamin, M., Bede, P., Shatunov, A., Walsh, C., Corr, B., et al. (2012). Cognitive and clinical characteristics of patients with amyotrophic lateral sclerosis carrying a *C9orf72* repeat expansion: a population-based cohort study. *Lancet Neurol.* 11, 232–240. doi: 10.1016/S1474-4422(12)70014-5

Chanson, J. B., Echaniz-Laguna, A., Blanc, F., Lacour, A., Ballonzoli, L., Kremer, S., et al. (2013). Central nervous system abnormalities in patients with *PMP22* gene mutations: a prospective study. *J. Neurol. Neurosurg. Psychiatry*. 84, 392–397. doi: 10.1136/jnnp-2012-303725

Gallo, V., Wark, P. A., Jenab, M., Pearce, N., Brayne, C., Vermeulen, R., et al. (2013). Prediagnostic body fat and risk of death from amyotrophic lateral sclerosis: the EPIC cohort. *Neurology* 80, 829–838. doi: 10.1212/WNL.0b013e3182840689

GBD 2016 Neurology Collaborators (2019). Global, regional, and national burden of neurological disorders, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 18, 459-480. doi: 10.1016/S1474-4422(18)30499-X

Goedert, M. (2001). Alpha-synuclein and neurodegenerative diseases. *Nat. Rev. Neurosci.* 2, 492–501. doi: 10.1038/35081564

McLaughlin, R. L., Schijven, D., van Rheenen, W., van Eijk, K. R., O'Brien, M., Kahn, R. S., et al. (2017). Genetic correlation between amyotrophic lateral sclerosis and schizophrenia. *Nat. Commun* 8:14774. doi: 10.1038/ ncomms14774

Morton, A. J. (2013). Circadian and sleep disorder in Huntington's disease. *Exp. Neurol.* 243, 34–44. doi: 10.1016/j.expneurol.2012.10.014

Sanahuja, J., Franco, E., Rojas-García, R., Gallardo, E., Combarros, O., Begué, R., et al. (2005). Central nervous system involvement in hereditary neuropathy with liability to pressure palsies: description of a large family with this association. *Arch. Neurol.* 62, 1911–1914. doi: 10.1001/archneur.62.12.1911

Wills, A.-M., Hubbard, J., Macklin, E. A., Glass, J., Tandan, R., Simpson, E. P., et al. (2014). Hypercaloric enteral nutrition in patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet* 383, 2065–2072. doi: 10.1016/S0140-6736(14)60222-1

Conflict of Interest: 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.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 15 October 2018
doi: 10.3389/fnins.2018.00714

EEG Microstates Change in Response to Increase in Dopaminergic Stimulation in Typical Parkinson's Disease Patients

*J. Ignacio Serrano[1], María Dolores del Castillo[1], Verónica Cortés[2], Nuno Mendes[3], Aida Arroyo[2], Jorge Andreo[2], Eduardo Rocon[1], María del Valle[4], Jaime Herreros[5] and Juan Pablo Romero[1,6]**

[1] Neural and Cognitive Engineering Group, Centre for Automation and Robotics, Spanish National Research Council – Technical University of Madrid, Madrid, Spain, [2] Faculty of Experimental Sciences, Francisco de Vitoria University, Madrid, Spain, [3] Faculty of Sciences, University of Lisbon, Lisbon, Portugal, [4] Department of Neurology, Fuenlabrada University Hospital, Madrid, Spain, [5] Department of Neurology, Infanta Leonor University Hospital, Madrid, Spain, [6] Brain Damage Unit, Hospital Beata Maria Ana, Madrid, Spain

Objectives: Characterizing pharmacological response in Parkinson's Disease (PD) patients may be a challenge in early stages but gives valuable clues for diagnosis. Neurotropic drugs may modulate Electroencephalography (EEG) microstates (MS). We investigated EEG-MS default-mode network changes in response to dopaminergic stimulation in PD.

Methods: Fourteen PD subjects in HY stage III or less were included, and twenty-one healthy controls. All patients were receiving dopaminergic stimulation with levodopa or dopaminergic agonists. Resting EEG activity was recorded before the first daily PD medication dose and 1 h after drug intake resting EEG activity was again recorded. Time and frequency variables for each MS were calculated.

Results: Parkinson's disease subjects MS A duration decreases after levodopa intake, MS B appears more often than before levodopa intake. MS E was not present, but MS G was. There were no significant differences between control subjects and patients after medication intake.

Conclusion: Clinical response to dopaminergic drugs in PD is characterized by clear changes in MS profile.

Significance: This work demonstrates that there are clear EEG MS markers of PD dopaminergic stimulation state. The characterization of the disease and its response to dopaminergic medication may be of help for early therapeutic diagnosis.

Keywords: Parkinson's disease, electroencephalography, microstates, levodopa, diagnosis

OPEN ACCESS

Edited by:
Foteini Christidi, National and Kapodistrian University of Athens, Greece

Reviewed by:
Gabriella Santangelo, Università degli Studi della Campania Luigi Vanvitelli, Italy
Ales Holobar, University of Maribor, Slovenia

***Correspondence:**
Juan Pablo Romero
p.romero.prof@ufv.es

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: *16 June 2018*
Accepted: *19 September 2018*
Published: *15 October 2018*

Citation:
Serrano JI, del Castillo MD, Cortés V, Mendes N, Arroyo A, Andreo J, Rocon E, del Valle M, Herreros J and Romero JP (2018) EEG Microstates Change in Response to Increase in Dopaminergic Stimulation in Typical Parkinson's Disease Patients. Front. Neurosci. 12:714. doi: 10.3389/fnins.2018.00714

INTRODUCTION

Parkinson's Disease (PD) is a neurodegenerative disease affecting up to 3% of the population $\geq$ 65 years of age (Poewe et al., 2017). PD has been associated with several risk factors common to other age related diseases and some chemicals exposure (Beitz, 2014) but its ultimate cause is still unknown.

In typical PD, progressive degeneration of dopaminergic neurons in the substantia nigra is correlated with the wide known motor symptoms of bradykinesia, rigidity, and tremor (Kalia and Lang, 2015). However, the phenotypical profile of each patient gives rise to the identification of several subtypes such as tremor-dominant subtype and on the other hand bradykinesia/rigidity dominant (Marras and Lang, 2013). Dopaminergic treatment usually provides substantial alleviation in motor symptoms. Some other symptoms such as gait disturbance and postural instability do not usually have a substantial improvement (Hely et al., 2005). There are several syndromes that share some features similar to Parkinson's disease, but the progression and onset of symptoms are different. One of the main characteristics of this atypical Parkinsonism is the lack of or incomplete response to levodopa (Goetz et al., 2008; Stamelou and Hoeglinger, 2013).

Levodopa is actually considered the best current symptomatic treatment for Parkinson's Disease. One of the main obstacles for the treatment of the disease is its pharmacodynamics, meaning there is a low penetrance of the drug into the central nervous system (Khor and Hsu, 2007; Britz et al., 2010; Musso et al., 2010; Van de Ville et al., 2010; Michel and Koenig, 2017). The action of this drug determines important changes not only in motor but also in non-motor symptoms. The state where patients show a marked improvement is called "ON state" and the one with no effects is called "OFF state." Dopamine (DA) receptor agonists are also used to treat the symptoms of the disease since such drugs mimic the action of dopamine, their action is achieved by stimulation of pre-synaptic (auto receptors) and post-synaptic DA receptors (Radad et al., 2005). Levodopa equivalent dose (LED) can be calculated from dopamine receptor agonist doses so the total daily levodopa administration can be estimated (Tomlinson et al., 2010).

Dopaminergic stimulation is surely alleviating typical PD symptoms in most patients, but the degree of its effects shows major inter-individual differences. These differences in levodopa motor response are evident even between same disease-severity-stage patients (Goetz et al., 2000). Every patient has their own needs of medication to reach their "ON state" and this varies according to the disease progression (Nyholm et al., 2012) and degree of denervation (Kostrzewa et al., 2005).

The clinical diagnosis of PD is currently based on clinical symptoms and other support criteria such as response to medication. This clinical diagnosis is very difficult, especially in early stages of the disease when there are no remarkable motor features.

Identification of neurophysiological variables with diagnostic value in early-stage PD would raise the chances of improving diagnostic certainty (Valls-Solé and Valldeoriola, 2002).

Electroencephalography (EEG) is a well-known technique used to record the electrical field produced by the electrical activity in the brain. This technique is characterized by a high temporal resolution and high test–retest reliability (Lopes da Silva, 1991). It has been published that quantifying EEG rhythms and their variations could be the source of biomarker for several neuropsychiatric disorders, such as schizophrenia, major depressive disorder, or even neurodegenerative diseases as Alzheimer's disease (Gandal et al., 2012; Han et al., 2013).

Electroencephalography data can be analyzed according to momentary states of the topographical brain activation, called microstates (MS). "Microstate analysis is a method in which states are defined by topographies of electrical potentials on a set of multichannel electrodes that remain stable for 80–120 ms before rapidly moving to a different microstate" (Khanna et al., 2015). Unlike other EEG processing techniques, in microstates, the simultaneous analysis of the signals from all the electrodes is used to create a global representation of a functional state. In fact, many studies have shown that time series of EEG microstates vary through behavioral states (Stevens and Kircher, 1998; Lehmann et al., 2010), personality types (Schlegel et al., 2012) and neuropsychiatric disorders (Dierks et al., 1997; Lehmann et al., 2005; Kikuchi et al., 2011; Khanna et al., 2015). Consequently, changes in the duration or frequency of appearance of specific microstates can be considered as biomarkers for different neurological and neuropsychiatric conditions.

Interestingly, several studies that have examined resting-state EEG report the same four archetypal microstates that explain most of the global topographic variance (Koenig et al., 2002; Khanna et al., 2015). The four canonical EEG microstates (A, B, C, D) seem to represent the neurophysiological correlates of four known Resting State Networks identified by fMRI, suggesting that Resting State Networks of fMRI may be the same ones that give rise to microstates (Michel and Koenig, 2017). The dynamics of these networks may imply various brain functions, and their alteration can be associated with the pathophysiology of several neurological and neuropsychiatric diseases (Khanna et al., 2015).

When the microstate time series is convolved with the resting-state fMRI BOLD signal, each microstate map correlates with the activity of particular RSNs (Britz et al., 2010; Musso et al., 2010; Yuan et al., 2016). Britz et al. (2010) showed that microstate A is associated with the phonological processing network, B with the visual network, C with the salience network, and D related to the attentional network.

There is evidence that neurotropic drugs may modulate EEG microstates (Kikuchi et al., 2007, 2011; Yoshimura et al., 2007), but there are no studies showing EEG microstate changes in response to the administration of levodopa or dopaminergic agonist drugs in typical PD patients.

The aim of the study presented in this paper is to identify EEG microstate changes that characterize levodopa response. The data obtained from this study can be used to support typical PD diagnosis in difficult clinical scenarios where a therapeutic trial of levodopa is not feasible or not well tolerated by the patient because of the gastric effects of its administration.

MATERIALS AND METHODS

Participants

The protocol was approved by the CEIC Fuenlabrada Hospital, Madrid, Spain. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Fourteen patients were included in the study after signing informed consent forms (4 female: mean age 66.25 $\pm$ 12.9, range 52–80 and 10 male: mean age 66.9 $\pm$ 7.41, range 50–76).

All the patients had been diagnosed with Parkinson's Disease according to London Brain Bank criteria (mean time from onset 7.29 ± 2.33 years, range 4–13), with Hoehn and Yahr scale 2 ± 0.8 (range 1–3), and were taking levodopa or dopaminergic agonists (mean daily amount 324.28 ± 232.77 levodopa milligrams equivalent dose (LED), range 100–733) in stable dosing regimen for at least 90 days with a clear ON effect (good clinical effect). There was not head tremor in any of the patients. **Table 1** shows a description of included patients. In addition, twenty-one healthy subjects were recruited as control participants (6 female: mean age 67.4 ± 10.21, range 50–77 and 15 male: mean age 69.6 ± 10.14, range 50–93).

Intervention

Participants were asked to come to the hospital early in the morning without their corresponding daily levodopa or agonist intake (at least 8 h after the last levodopa or dopamine agonist dose). Resting EEG activity was recorded over 2 min by 64 electrodes placed according to the 10–20 system as depicted in **Figure 1**. They were comfortably seated with their hands on their laps, relaxed jaw and eyes open, looking at a white wall. Immediately afterward, the EEG electrodes were removed, and they took their daily Levodopa or agonist dose with a glass of water, 30 min before they had a light breakfast and were given free time. The resting EEG activity was analogously recorded 1 h after the levodopa intake, once the patient had asserted they were in their usual ON state.

Materials

An actiCHamp amplifier (Brain Vision LLC, NC, United States) was used to amplify and digitize the EEG data at a sampling frequency of 512 Hz. The EEG data were stored in a PC running Windows 7 (Microsoft Corporation, Washington, DC, United States). EEG activity was recorded from 64 positions with active Ag/AgCl scalp electrodes (actiCAP electrodes, Brain Vision LLC, NC, United States). The ground and reference electrodes were placed on AFz and on FCz, respectively (see **Figure 1**).

Electroencephalography acquisition was carried out by NeuroRT Studio software (Mensia Technologies SA, Paris, France). The EEG signal processing procedure was performed using MATLAB functions (MathWorks Inc., Natick MA, United States), specifically the EEGLab toolbox (Delorme and Makeig, 2004). EEG microstates were extracted and characterized by LORETA-KEY v20170220 software (the Key Institute for Brain-Mind Research, Zurich, Switzerland). Statistical analyses were performed by SPSS for Windows, version 23.0 (IBM Inc., Chicago, IL, United States).

EEG Processing and Outcome Measurements

The continuous EEG signal for each channel was artifact-corrected by the Artifact Subspace Reconstruction algorithm (Mullen et al., 2013), disabling all parameters except the high-pass filter band width (0.25–0.75) and burst repairing (*kurtosis* > 5). The signal was then band-pass filtered between 2 and 31 Hz with a Finite Impulse Response (FIR) filter (order 846). Finally, a common average reference (CAR) spatial filter was applied.

The processed EEG was the input to an EEG microstate detection and characterization algorithm (Pascual-Marqui et al., 2014). The algorithm requires an initial interval for the number of microstates searched. This interval was set to 4–10. The algorithm was run independently for PD PRE, PD POST and CONTROL conditions. Mean microstate topographies in each condition were manually assigned to canonical microstates reported in previous studies. The assignment was individually performed by three judges (among the authors) by visual analysis.

TABLE 1 | Description of the patients included in the study.

	Gender	Age	Initial side	Years from onset	Hoehn Yahr	UPDRS*	Levodopa equivalent dose (mg) (LED)	Levodopa dose (mg)	Total morning dose (mg)
1	Male	73	Right	8	1	14	100[2,3]	50	150
2	Male	67	Left	6	1	8	100[2]	0	100
3	Female	59	Left	6	1	10	420[2,4]	50	470
4	Female	74	Left	9	3	12	400[2,5]	250	650
5	Male	71	Left	8	2.5	15	0	100	100
6	Female	80	Right	9	3	14	520[2,4,7]	200	720
7	Male	76	Left	13	3	30	220[3,5,6]	0	220
8	Male	50	Left	5	2	24	100[2]	0	100
9	Male	68	Left	6	2	11	310[1,2]	0	310
10	Male	69	Left	6	1	22	105[1]	250	355
11	Male	69	Left	8	2	16	205[1,2,3]	100	305
12	Female	52	Right	4	2	12	100[2]	67	167
13	Male	59	Right	9	3	11	550[1,3,5,6]	283	833
14	Male	67	Right	5	1.5	11	260[2,4]	0	260
Avg (std)	28.57% Female	66.71 (8.77)	35.71% Right	7.29 (2.33)	2.00 (0.81)	14.46 (6.09)	504.00 (366.68)	96.43 (105.39)	338.57 (242.17)

**UPDRS, Unified Parkinson's Disease Rating Scale. [1]Pramipexole, [2]Rasagiline, [3]Benserazide, [4]Ropirinole, [5]Rotigotine, [6]Safinamide, [7]Amantadine.*

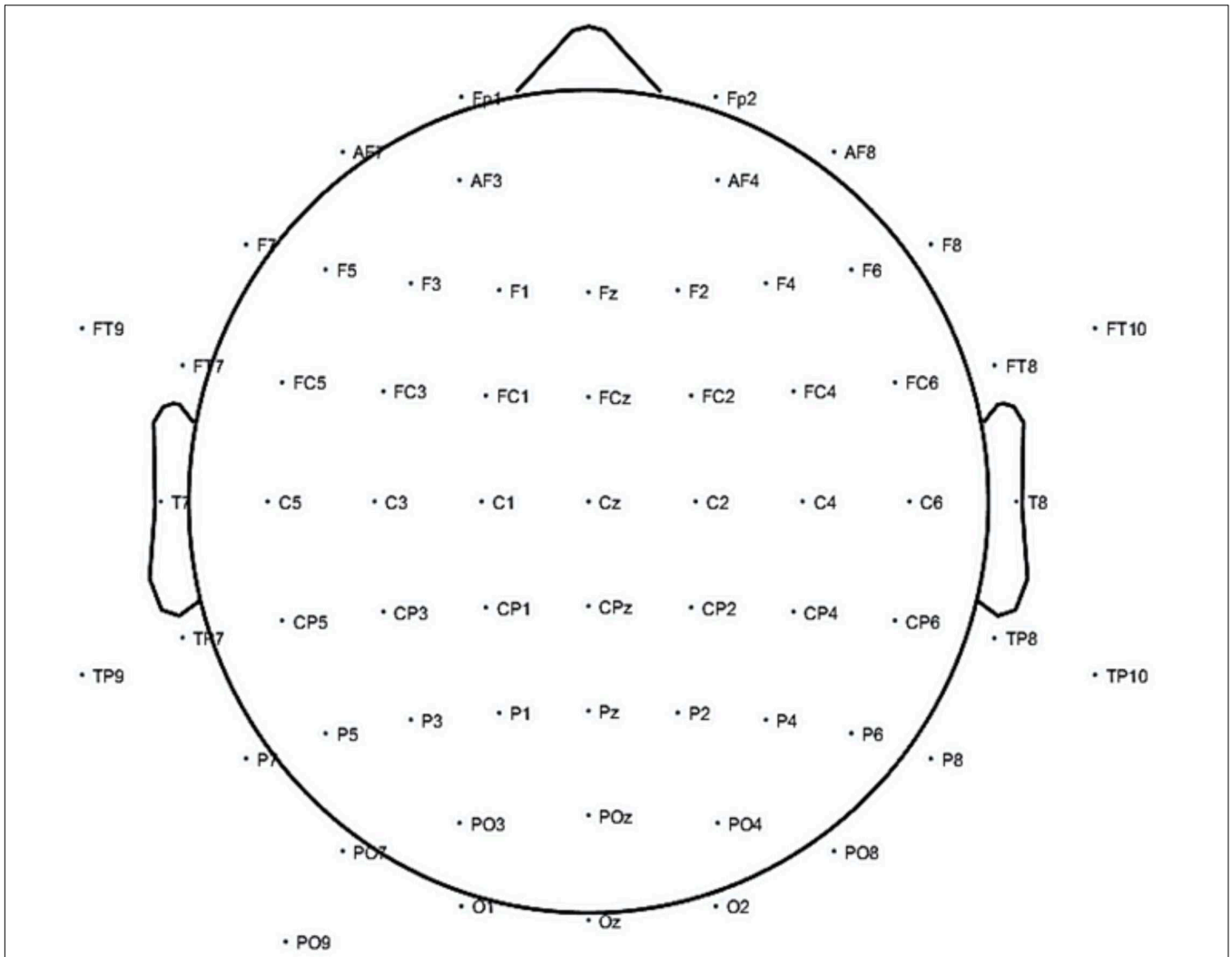

FIGURE 1 | Electroencephalography (EEG) electrode placement for the study.

This procedure avoids the likely mislabeling introduced by the common topography correlation analysis in the presence of irregular topographies and more than four microstates (Custo et al., 2017). Labels agreed by two or more judges were assigned (all mean microstate topographies labels were agreed by two judges at least). From this algorithm, the microstates accounting for most of the variance were selected. For each microstate, the percentage of the total time in the microstate (coverage), the percentage of times entered in the microstate (frequency), the number of times entered in the microstate (occurrence) and the average duration of the microstate are calculated. In addition, the frequency and probability of change from each microstate to each other one are also calculated, giving a total of 6 features for each microstate.

Statistical Analysis

The microstate features mentioned above are compared between the pre- and post-levodopa intake conditions. The difference of averages between pre- and post-conditions for each feature was checked by a *t*-test for repeated measurements with bootstrapping ($n = 1000$). Differences with a significance $p < 0.05$ and confidence intervals (lower and upper) with the same sign were considered as statistically significant.

RESULTS

Figure 2 shows the microstates topographies found in pre- (first column) and post- (second column) levodopa intake conditions. Control group (third column) is examined with no medication.

In the pre-condition (OFF state), canonical A, B and C microstates were found with a percentage of explained variance of 19.55, 20.34 and 18.55%, respectively. Canonical microstate D was not identified. Microstates B and C presented altered patterns with respect to the findings in the literature (Michel and Koenig, 2017). In the absence of levodopa intake, a microstate E was also

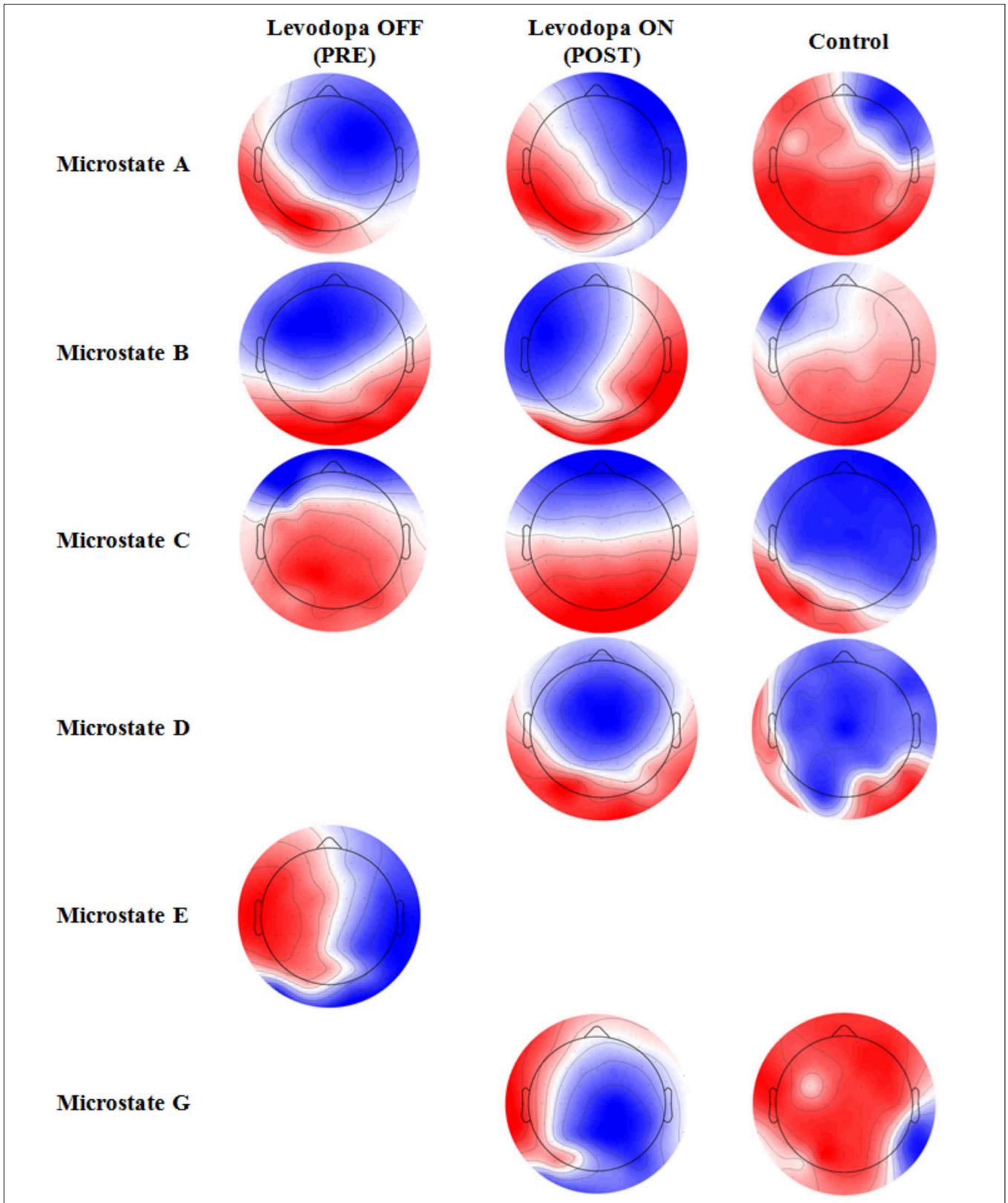

FIGURE 2 | Average topographic distributions of found microstates in pre- and post-levodopa intake conditions and control condition.

TABLE 2 | Statistically significant differences in features of Electroencephalography (EEG) microstates between pre- and post-levodopa intake in Parkinson's patients.

N = 14	*Average difference (POST-PRE)*	*Bootstrapping simulation*			
		SE	*p*	*95% CI*	
				Lower	*Upper*
Average duration A	−0.00006864	0.00002078	0.009	−0.00010	−0.000031
Occurrence B	549.28571	47.85855	< 0.0001	419.67657	642.10915
Frequency B to A	−680.17248	214.30544	0.033	−1026.77105	−271.71723

SE, Standard error; p, significance value; CI, Confidence interval.

TABLE 3 | Statistically significant differences in features of EEG microstates between post-levodopa intake of Parkinson's patients and control participants.

N = 21	*Average difference (Control-PD POST)*	*Bootstrapping simulation (n = 1000)*			
		SE	*p*	*95% CI*	
				Lower	*Upper*
% EV D	−5.198024	1.425808	0.004	−8.223715	−2.748249
Coverage D	−12.136262	2.076990	0.001	−16.321933	−8.071208
Average duration D	−0.00009076	0.00003172	0.024	−0.00015279	−0.00002818
Occurrence A	−86.500	33.004	0.015	−150.628	−21.874
Occurrence C	−89.167	31.995	0.011	−154.717	−28.991
Occurrence D	−213.238	26.329	0.001	−262.567	−161.391
Occurrence G	−106.167	34.890	0.005	−171.316	−37.608
Probability A to D	−0.15608055	0.025070321	0.001	−0.207682815	−0.10978173
Probability B to D	−0.134840881	0.021954275	0.001	−0.175374815	−0.0919498
Probability C to D	−0.174758333	0.026128700	0.001	−0.229032260	−0.1263252
Probability G to D	−0.111732048	0.027221222	0.001	−0.163486738	−0.0606472

EV, Explained variance; SE, Standard error; p, significance value; CI, Confidence interval.

found, in congruence with the definition of Custo et al. (2017), with an explained variance of 19.58%.

After levodopa intake (ON state), the four canonical microstates A, B, C, and D were found with normal patterns (Michel and Koenig, 2017), and explained variances of 20.12, 18.39, 20.00, and 16.67%, respectively. Microstate E was no longer present after levodopa intake. However, the microstate G, according to Custo et al. (2017), was found with an explained variance of 15.39%. Control participants presented the same latter microstates (**Figure 2**, third column), with A, B, C, D, and G percentages of global explained variance of 20.85, 22.51, 17.49, 7.06, and 12.42%, respectively.

Given that the two patient conditions only share three microstates (A, B, and C), **Table 2** presents the statistically significant differences in the features of those three microstates between the OFF and ON states. The remaining features not present in **Table 2** did not show significant differences.

According to the results in **Table 2**, the microstate A shows a decreased duration after levodopa intake. Moreover, microstate B appears more often than before levodopa intake. Finally, the frequency of transition from microstate B to A got decreased with levodopa.

Table 3 presents the statistically significant differences in microstate features between control participants and patients after levodopa intake. No significant differences in microstate features were found between control participants and patients before levodopa intake. All microstate types except B occurred more often in post-levodopa patients than in control participants. This difference is especially high for microstate D, which also presented a higher explained variance and coverage. The probability of shifting from all microstates to D was also higher in post-levodopa patients, as is justified by the mentioned increased occurrence of the latter.

DISCUSSION

The fact that control subjects and PD patients after taking levodopa show the same microstate types is supported by the fact that levodopa is known to restore altered motor and non-motor functions in PD patients.

A higher duration of microstate A has been related to clinical variables such as disability and cognitive fatigue in patients with multiple sclerosis (Gschwind et al., 2016), and could be related to the cognitive fatigue presented by patients with PD. Such fatigue decreases with dopaminergic stimulation, and therefore it can lead to a decrease in the duration of microstate A, as observed in the results obtained in our study. As we said earlier that Britz et al. (2010) showed that microstate B is associated with the visual network, then the observed increase in the number of times microstate B is present after levodopa or dopaminergic agonist intake might be explained by a lower

fatigability in visual monitoring and a better functioning of PD patients thanks to the medication (Lou, 2009, 2015), reflecting the tendency of visual network generators to be active in the absence of cognitive fatigue (Milz et al., 2017). Cognitive fatigability is most likely associated with neurotransmitter (dopaminergic, cholinergic, and noradrenergic) abnormalities in PD. Levodopa may be effective in treating fatigue and fatigability (Lou, 2015). The decrease of frequency of transition from microstate B to A with dopaminergic stimulation does not seem to be related to any known clinical or behavioral condition. Therefore, more research is needed on the importance and functional correlation of the transition of microstates.

There are no differences in characteristics for the microstates shared by patients before medication intake and the controls. However, after levodopa intake there are differences, mainly in the microstate D. There are studies that demonstrate reduced duration (Kikuchi et al., 2007; Nishida et al., 2013) and also a lower frequency of appearance (Lehmann et al., 2010) of microstate D in patients with schizophrenia. Schizophrenia is believed to have a dopaminergic deficit up to a certain point that could explain this common finding in PD (Van Den Brink et al., 2018). Consequently, it is to be expected that patients diagnosed with PD have a lower frequency of appearance of microstate D before taking the medication. Patients presented a greater frequency and duration of microstate D after increasing dopaminergic stimulation, as a consequence of taking the medication. This increase is even greater than in healthy controls, which, assuming that microstate D reflects dopaminergic activity, could be a result of an acute increase of this activity in the brain.

Regarding topographic considerations, microstate D is mainly due to the activation of the right inferior parietal (BA40), the right middle and superior frontal gyri and the right insula (BA13) (Custo et al., 2017). The right inferior parietal area is related to executive control and vision-guided movements (Lasaponara et al., 2018) and the insula has a direct relationship with motor planning (Beurze et al., 2007). The right middle and superior frontal areas seem to explain the changes related to the improvement in attention (Angelidis et al., 2018). The appearance of microstate D, after taking medication, is congruent and consistent with the disappearance of certain motor and non-motor symptoms after levodopa.

Apart from the four canonical microstates (A–D) (Custo et al., 2017), two additional microstates (E and G) were identified. The microstate E corresponds to the activation of the middle frontal gyrus, the dorsal part of the anterior cingulate, the cuneus and the thalamus. Dopamine has an inhibitory effect (D2 receptors). Consequently, it is plausible to attribute a relative hyperactivity in its absence to the thalamus, and that this relative hyperactivity disappears after the intake of levodopa. Besides, according to Yoo et al. (2015), the anterior cingulate and frontal areas correspond to the presence of non-motor symptoms. Therefore, this justifies the presence of microstate E in patients before dopaminergic stimulation and its disappearance after they took levodopa (Lou, 2009, 2015). Finally, the cuneus is also related with oculomotor control (Darby et al., 1996), which is well-known to also be a function specifically regulated by the basal ganglia, whose function is altered in PD (Pretegiani and Optican, 2017).

The microstate G corresponds to the activation of the right inferior parietal lobe, extending to the superior temporal gyrus and also the cerebellum (Khanna et al., 2015). Both areas are closely related to motor behavior (Pirondini et al., 2017). Therefore, the appearance of microstate G after levodopa intake is strongly consistent with the improvement of motor symptoms. Since the visible and clinically evaluable motor symptoms disappear with the medication intake, this results in the observation of the microstate G.

The present study is not without limitations. First, the sample size is relatively small. A larger population might have yielded more significant results. Second, the sample population is heterogeneous in medication terms, with different types and doses of drugs, although they are all in their optimal ON state. Heterogeneity in medication is usual in PD patient cohorts because such variations correspond to the different treatment strategies that can be initiated even in the same disease stage. Dopaminergic agonists play a key role in actual treatment of the disease and their diversity makes them comparable only by their conversion to dopaminergic equivalents as we did in our study. Besides, cognitive fatigue, related to microstates A and B, was not assessed in this study. Cognitive fatigability and cognitive fatigue are usually evaluated through self-reporting scales. Given that our measures were performed in sequential OFF and ON state (in less than 2 h), we considered that this evaluation would have had a very important bias of motor and emotional symptoms as product of dopaminergic deprivation. In addition, dopaminergic therapy optimization is one of the main management recommendations for treating fatigue in PD (Kostić, 2016). Finally, no cognitive evaluation was performed, specially attention changes that are highly related to microstate D. Nevertheless, cognitive symptoms improvement, included attention span, has been widely reported in PD patients in response to dopaminergic treatment and can be assumed as a well stablished effect of medication. These limitations should be taken into consideration when interpreting the conclusions of the study.

CONCLUSION

Electroencephalography microstate analysis can be performed by means of an economical and minimally invasive technique with high temporal resolution. Since the EEG microstate correlation with RSNs has been evidenced, the results obtained from microstate analysis have been interpreted based on the known findings about these networks. Our work has demonstrated that there is an alteration of EEG microstate features and occurrence in typical PD in response to levodopa administration. These changes correlate with known clinical effects of the substance on such patients and are coherent with related changes in RSNs.

In spite of the differences between controls and PD patients, the microstates found in patients after levodopa intake are closer to controls' microstates than before taking the medication. Thus, the analysis performed in this study can be considered as a means to assess the suitability of the patients' medication dosage.

Further, not every Parkinsonian patient has a good response to levodopa or dopamine agonist treatment, and patients who were non-respondent to levodopa are excluded from typical PD diagnosis. Absence of complete clinical response to Levodopa is common on atypical parkinsonian patients. This lack of effect of dopaminergic stimulation is considered a red flag and implies the exclusion for typical Parkinson's Disease diagnosis (Postuma et al., 2015). Considering our results, we would not expect a microstate "normalization" in atypical PD patients in response to dopamine administration. Consequently, the microstate analysis can be considered of great utility to characterize the levodopa response prior to making a diagnosis of typical vs. atypical Parkinsonism in a non-invasive way suitable for outpatients. Nevertheless, further studies are required to characterize EEG microstate changes due to levodopa administration on atypical PD patients.

AUTHOR CONTRIBUTIONS

VC, JS, MDC, and JR contributed conception and design of the study. AA, JA, NM, JH, MDV, and JR organized the database. JS, MDC, and JR performed the statistical analysis. VC and JR wrote the first draft of the manuscript. VC, JS, MDC, ER, and JR wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

FUNDING

This research was supported mainly by FEDER funds. JR was supported by the Ministry of Economy and Competitiveness (Grant DPI2015-68664-C4-3-R (MINECO/FEDER), NeuroMOD Therapy development and evaluation of motor and cognitive impact for Parkinson's disease rehabilitation). ER, MDC, and JS were supported by the Ministry of Economy and Competitiveness (Grant DPI2015-68664-C4-1-R (MINECO/FEDER), NeuroMOD and by MD project (PIE-201650E055).

ACKNOWLEDGMENTS

We would like to thank Lucía Esteban and Paz Fernández for their assistance with the project.

REFERENCES

Angelidis, A., Hagenaars, M., van Son, D., van der Does, W., and Putman, P. (2018). Do not look away! spontaneous frontal eeg theta/beta ratio as a marker for cognitive control over attention to mild and high threat. *Biol. Psychol.* 135, 8–17. doi: 10.1016/j.biopsycho.2018.03.002

Beitz, J. M. (2014). Parkinson's disease: a review. *Front. Biosci.* 6, 65–74. doi: 10.2741/S415

Beurze, S. M., de Lange, F. P., Toni, I., and Medendorp, W. P. (2007). Integration of target and effector information in the human brain during reach planning. *J. Neurophysiol.* 97, 188–199. doi: 10.1152/jn.00456.2006

Britz, J., Van De Ville, D., and Michel, C. M. (2010). Bold correlates of EEG topography reveal rapid resting-state network dynamics. *Neuroimage* 52, 1162–1170. doi: 10.1016/j.neuroimage.2010.02.052

Custo, A., Van De Ville, D., Wells, W. M., Tomescu, M. I., Brunet, D., and Michel, C. M. (2017). Electroencephalographic resting-state networks: source localization of microstates. *Brain Connect.* 7, 671–682. doi: 10.1089/brain.2016.0476

Darby, D. G., Nobre, A. C., Thangaraj, V., Edelman, R., Mesulam, M. M., and Warach, S. (1996). Cortical activation in the human brain during lateral saccades using epistar functional magnetic resonance imaging. *Neuroimage* 3, 53–62. doi: 10.1006/nimg.1996.0006

Delorme, A., and Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J. Neurosci. Methods* 134, 9–21. doi: 10.1016/j.jneumeth.2003.10.009

Dierks, T., Jelic, V., Julin, P., Maurer, K., Wahlund, L. O., Almkvist, O., et al. (1997). EEG-microstates in mild memory impairment and Alzheimer's disease: possible association with disturbed information processing. *J. Neural Transm.* 104, 483–495. doi: 10.1007/BF01277666

Gandal, M. J., Edgar, J. C., Klook, K., and Siegel, S. J. (2012). Gamma synchrony: towards a translational biomarker for the treatment-resistant symptoms of Schizophrenia. *Neuropharmacology* 62, 1504–1518. doi: 10.1016/j.neuropharm.2011.02.007

Goetz, C. G., Stebbins, G. T., and Blasucci, L. M. (2000). Differential progression of motor impairment in levodopa-treated parkinson's disease. *Mov. Disord.* 15, 479–484. doi: 10.1002/1531-8257(200005)15:3<479::AID-MDS1009>3.0.CO;2-P

Goetz, C. G., Tilley, B. C., Shaftman, S. R., Stebbins, G. T., Fahn, S., Martinez-Martin, P., et al. (2008). Movement disorder society-sponsored revision of the unified parkinson's disease rating scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Mov. Disord.* 23, 2129–2170. doi: 10.1002/mds.22340

Gschwind, M., Hardmeier, M., Van De Ville, D., Tomescu, M. I., Penner, I. K., Naegelin, Y., et al. (2016). Fluctuations of spontaneous EEG topographies predict disease state in relapsing-remitting multiple sclerosis. *Neuroimage Clin.* 12, 466–477. doi: 10.1016/j.nicl.2016.08.008

Han, C.-X., Wang, J., Yi, G.-S., and Che, Y.-Q. (2013). Investigation of EEG abnormalities in the early stage of Parkinson's disease. *Cogn. Neurodyn.* 7, 351–359. doi: 10.1007/s11571-013-9247-z

Hely, M. A., Morris, J. G., Reid, W. G., and Trafficante, R. (2005). Sydney multicenter study of Parkinson's Disease: non-l-dopa–responsive problems dominate at 15 years. *Mov. Disord.* 20, 190–199. doi: 10.1002/mds.20324

Kalia, L. V., and Lang, A. E. (2015). Parkinson's Disease. *Lancet* 386, 896–912. doi: 10.1016/S0140-6736(14)61393-3

Khanna, A., Pascual-Leone, A., Michel, C. M., and Farzan, F. (2015). Microstates in resting-state EEG: current status and future directions. *Neurosci. Biobehav. Rev.* 49, 105–113. doi: 10.1016/j.neubiorev.2014.12.010

Khor, S. P., and Hsu, A. (2007). The pharmacokinetics and pharmacodynamics of levodopa in the treatment of Parkinson's Disease. *Curr. Clin. Pharmacol.* 2, 234–243. doi: 10.2174/157488407781668802

Kikuchi, M., Koenig, T., Munesue, T., Hanaoka, A., Strik, W., Dierks, T., et al. (2011). EEG microstate analysis in drug-naive patients with panic disorder. *PLoS One* 6:e22912. doi: 10.1371/journal.pone.0022912

Kikuchi, M., Thomas, K., Yuji, W., Higashima, M., Koshino, Y., Strik, W., et al. (2007). Native EEG and treatment effects in neuroleptic-naïve schizophrenic patients: time and frequency domain approaches. *Schizophr. Res.* 97, 163–172. doi: 10.1016/j.schres.2007.07.012

Koenig, T., Prichep, L., Lehmann, D., Sosa, P. V., Braeker, E., Kleinlogel, H., et al. (2002). Millisecond by millisecond, year by year: normative EEG microstates and developmental stages. *Neuroimage* 16, 41–48. doi: 10.1006/nimg.2002.1070

Kostić, V. S., Tomić, A., and Ječmenica-Lukić, M. (2016). The pathophysiology of fatigue in Parkinson's Disease and its pragmatic management. *Mov. Disord. Clin. Pract.* 3, 323–330. doi: 10.1002/mdc3.12343

Kostrzewa, R. M., Nowak, P., Kostrzewa, J. P., Kostrzewa, R. A., and Brus, R. (2005). Peculiarities of L-DOPA treatment of Parkinson's disease. *Amino Acids* 28, 157–164. doi: 10.1007/s00726-005-0162-4

Lasaponara, S., D'Onofrio, M., Pinto, P., Dragone, A., Menicagli, D., Bueti, D., et al. (2018). EEG correlates of preparatory orienting, contextual updating

and inhibition of sensory processing in left spatial neglect. *J. Neurosci.* 38, 3792–3808. doi: 10.1523/JNEUROSCI.2817-17.2018

Lehmann, D., Faber, P. L., Galderisi, S., Herrmann, W. M., Kinoshita, T., Koukkou, M., et al. (2005). EEG microstate duration and syntax in acute, medication-naive, first-episode schizophrenia: a multi-center study. *Psychiatry Res.* 138, 141–156. doi: 10.1016/j.pscychresns.2004.05.007

Lehmann, D., Pascual-Marqui, R. D., Strik, W. K., and Koenig, T. (2010). Core networks for visual-concrete and abstract thought content: a brain electric microstate analysis. *Neuroimage* 49, 1073–1079. doi: 10.1016/j.neuroimage.2009.07.054

Lopes da Silva, F. (1991). Neural mechanisms underlying brain waves: from neural membranes to networks. *Electroencephalogr. Clin. Neurophysiol.* 79, 81–93. doi: 10.1016/0013-4694(91)90044-5

Lou, J.-S. (2009). Physical and mental fatigue in Parkinson's Disease: epidemiology, Pathophysiology and Treatment. *Drugs Aging* 26, 195–208. doi: 10.2165/00002512-200926030-00002

Lou, J. S. (2015) Fatigue in Parkinson's Disease and potential interventions. *Neurorehabilitation* 37, 25–34.

Marras, C., and Lang, A. (2013). Parkinson's Disease subtypes: lost in translation? *J. Neurol. Neurosurg Psychiatry* 84, 409–415. doi: 10.1136/jnnp-2012-303455

Michel, C. M., and Koenig, T. (2017). EEG microstates as a tool for studying the temporal dynamics of whole-brain neuronal networks: a review. *Neuroimage* 180(Pt B), 577–593. doi: 10.1016/j.neuroimage.2017.11.062

Milz, P., Pascual-Marqui, R. D., Achermann, P., Kochi, K., and Faber, P. L. (2017). The EEG microstate topography is predominantly determined by intracortical sources in the alpha band. *Neuroimage* 162, 353–361. doi: 10.1016/j.neuroimage.2017.08.058

Mullen, T., Makeig, S., Kothe, C., Chi, Y. M., Ojeda, A., and Kerth, T. (2013). "Real-time modeling and 3d visualization of source dynamics and connectivity using wearable EEG," in *Proceedings of the 2013 35th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*, Victoria, VIC. 2184–2187. doi: 10.1109/EMBC.2013.6609968

Musso, F., Brinkmeyer, J., Mobascher, A., Warbrick, T., and Winterer, G. (2010). Spontaneous brain activity and EEG microstates. a novel EEG/fMRI analysis approach to explore resting-state networks. *Neuroimage* 52, 1149–1161. doi: 10.1016/j.neuroimage.2010.01.093

Nishida, K., Morishima, Y., Yoshimura, M., Isotani, T., Irisawa, S., Jann, K., et al. (2013). EEG microstates associated with salience and frontoparietal networks in frontotemporal dementia, schizophrenia and Alzheimer's Disease. *Clin. Neurophysiol.* 124, 1106–1114. doi: 10.1016/j.clinph.2013.01.005

Nyholm, D., Johansson, A., Aquilonius, S.-M., Hellquist, E., Lennernäs, H., and Askmark, H. (2012). Complexity of motor response to different doses of duodenal levodopa infusion in parkinson disease. *Clin. Neuropharmacol.* 35, 6–14. doi: 10.1097/WNF.0b013e31823b1ffd

Pascual-Marqui, R. D., Lehmann, D., Faber, P., Milz, P., Kochi, K., Yoshimura, M., et al. (2014). The resting microstate networks (RMN): cortical distributions, dynamics, and frequency specific information flow. *arXiv* [Preprint]. arXiv:1411.1949

Pirondini, E., Coscia, M., Minguillon, J., Millán, J. d. R., Van De Ville, D., and Micera, S. (2017). EEG topographies provide subject-specific correlates of motor control. *Sci. Rep.* 7:13229. doi: 10.1038/s41598-017-13482-1

Poewe, W., Seppi, K., Tanner, C. M., Halliday, G. M., Brundin, P., Volkmann, J., et al. (2017). Parkinson Disease. *Nat. Rev. Dis. Primers* 3:17013. doi: 10.1038/nrdp.2017.13

Postuma, R. B., Berg, D., Stern, M., Poewe, W., Olanow, C. W., Oertel, W., et al. (2015). MDS clinical diagnostic criteria for Parkinson's Disease. *Mov. Disord.* 30, 1591–1601. doi: 10.1002/mds.26424

Pretegiani, E., and Optican, L. M. (2017). Eye movements in Parkinson's Disease and inherited parkinsonian syndromes. *Front. Neurol.* 8:592. doi: 10.3389/fneur.2017.00592

Radad, K., Gille, G., and Rausch, W. D. (2005). Short review on dopamine agonists: insight into clinical and research studies relevant to Parkinson's Disease. *Pharmacol. Rep.* 57, 701–712.

Schlegel, F., Lehmann, D., Faber, P. L., Milz, P., and Gianotti, L. R. (2012). EEG microstates during resting represent personality differences. *Brain Topogr.* 25, 20–26. doi: 10.1007/s10548-011-0189-7

Stamelou, M., and Hoeglinger, G. U. (2013). Atypical parkinsonism: an update. *Curr. Opin. Neurol.* 26, 401–405. doi: 10.1097/WCO.0b013e3283632da6

Stevens, A., and Kircher, T. (1998). Cognitive decline unlike normal aging is associated with alterations of EEG temporo-spatial characteristics. *Eur. Arch. Psychiatry Clin. Neurosci.* 248, 259–266. doi: 10.1007/s004060050047

Tomlinson, C. L., Stowe, R., Patel, S., Rick, C., Gray, R., Clarke, C. E., et al. (2010). Systematic review of levodopa dose equivalency reporting in Parkinson's Disease. *Mov. Disord.* 25, 2649–2653. doi: 10.1002/mds.23429

Valls-Solé, J., and Valldeoriola, F. (2002). Neurophysiological correlate of clinical signs in Parkinson's Disease. *Clin. Neurophysiol.* 113, 792–805. doi: 10.1016/S1388-2457(02)00080-9

Van de Ville, D., Britz, J., and Michel, C. M. (2010). EEG microstate sequences in healthy humans at rest reveal scale-free dynamics. *Proc. Natl. Acad. Sci. U.S.A.* 107, 18179–18184. doi: 10.1073/pnas.1007841107

Van Den Brink, W. J., Palic, S., Köhler, I., and de Lange, E. C. M. (2018). Access to the CNS: biomarker strategies for dopaminergic treatments. *Pharm. Res.* 35:64. doi: 10.1007/s11095-017-2333-x

Yoo, K., Chung, S. J., Kim, H. S., Choung, O. H., Lee, Y. B., Kim, M. J., et al. (2015). Neural substrates of motor and non-motor symptoms in Parkinson's Disease: a resting FMRI study. *PLoS One* 10:e0125455. doi: 10.1371/journal.pone.0125455

Yoshimura, M., Koenig, T., Irisawa, S., Isotani, T., Yamada, K., Kikuchi, M., et al. (2007). A pharmaco-EEG study on antipsychotic drugs in healthy volunteers. *Psychopharmacology* 191, 995–1004. doi: 10.1007/s00213-007-0737-8

Yuan, H., Ding, L., Zhu, M., Zotev, V., Phillips, R., Bodurka, J., et al. (2016). Reconstructing large-scale brain resting-state networks from high-resolution EEG: spatial and temporal comparisons with fMRI. *Brain Connect.* 6, 122–135. doi: 10.1089/brain.2014.0336

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.

frontiers in Neuroscience

REVIEW
published: 11 March 2019
doi: 10.3389/fnins.2019.00175

Psychiatric Symptoms in Amyotrophic Lateral Sclerosis: Beyond a Motor Neuron Disorder

*Elisabetta Zucchi[1], Nicola Ticozzi[2,3] and Jessica Mandrioli[4]**

[1] Department of Neuroscience, Azienda Ospedaliero Universitaria di Modena, University of Modena and Reggio Emilia, Modena, Italy, [2] Department of Neurology and Laboratory of Neuroscience, Istituto Auxologico Italiano, Istituto di Ricovero e Cura a Carattere Scientifico, Milan, Italy, [3] Department of Pathophysiology and Transplantation, 'Dino Ferrari' Center, Università degli Studi di Milano, Milan, Italy, [4] Department of Neuroscience, Azienda Ospedaliera Universitaria Modena, St. Agostino- Estense Hospital, Modena, Italy

The historical view that Amyotrophic Lateral Sclerosis (ALS) as a pure motor disorder has been increasingly challenged by the discovery of cognitive and behavioral changes in the spectrum of Frontotemporal Dementia (FTD). Less recognized and still significant comorbidities that ALS patients may present are prior or concomitant psychiatric illness, such as psychosis and schizophrenia, or mood disorders. These non-motor symptoms disturbances have a close time relationship with disease onset, may constitute part of a larger framework of network disruption in motor neuron disorders, and may impact ALS patients and families, with regards to ethical choices and end-of-life decisions. This review aims at identifying the most common psychiatric alterations related to ALS and its prognosis, looking at a common genetic background and shared structural brain pathology.

Keywords: amyotrophic lateral sclerosis, frontotemporal dementia, psychosis, depression, c9orf72, psychiatric symptoms and disorders

OPEN ACCESS

Edited by:
Foteini Christidi, National and Kapodistrian University of Athens Medical School, Greece

Reviewed by:
Giorgia Querin, INSERM U1146 Laboratoire d'Imagerie Biomédicale, France
Panagiotis Ferentinos, National and Kapodistrian University of Athens, Greece

****Correspondence:***
Jessica Mandrioli
mandrioli.jessica@aou.mo.it

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: *14 December 2018*
Accepted: *14 February 2019*
Published: *11 March 2019*

Citation:
Zucchi E, Ticozzi N and Mandrioli J (2019) Psychiatric Symptoms in Amyotrophic Lateral Sclerosis: Beyond a Motor Neuron Disorder. Front. Neurosci. 13:175. doi: 10.3389/fnins.2019.00175

INTRODUCTION: AMYOTROPHIC LATERAL SCLEROSIS AND ITS PHENOTYPES

Amyotrophic Lateral Sclerosis (ALS) has traditionally been defined since the first reports as a disorder characterized by progressive degeneration of upper and lower motor neurons (UMN and LMN, respectively), leading invariably to paralysis of voluntary muscles, with a variable proportion of spasticity and atrophy. Despite a uniformly fatal outcome, extreme variability exists within ALS, with heterogeneity of initial presentation, spreading of disease, progression, and survival (Brown and Al-Chalabi, 2017; Hardiman et al., 2017). The observation of distinct patterns within ALS clinical variability has led to the recognition of homogeneous phenotypic subgroups. A first classification system is based on the differential involvement of upper and lower motor neuron, with primary lateral sclerosis (PLS) and progressing muscular atrophy (PMA) representing the extremes of the spectrum. The type of involvement of different body regions at onset is another common identifier, with bulbar patients constituting 25% of the total ALS population (Chiò et al., 2011). This phenotype is more consistently associated with cognitive alterations and displays decreased survival compared to the classic spinal-onset, "Charcot-type" phenotype (Chiò et al., 2011; Talman et al., 2016). A prevalent LMN involvement define flail arm and flail leg variants (Wijesekera et al., 2009), whereas a prevalence of UMN signs with spasticity, increased and pathological reflexes and pseudobulbar affect, identify the UMN-predominant phenotype (UMN-p); these phenotypes

display a relatively long survival (Sabatelli et al., 2008; Chiò et al., 2011). Lastly, the respiratory phenotype, characterized by an early and prominent involvement of the respiratory muscles, is characterized by the worst prognosis (Shoesmith et al., 2007). Moreover, while the majority of patients report a pattern of spreading of the disease from one limb to the contralateral, as by means of contiguity in cortical representations, there is a substantial fraction of ALS population describing a close sequential involvement of two distal sites (Walhout et al., 2017).

The clinical heterogeneity of ALS is reflected at molecular level in many ways. First of all, up to 20% of ALS patients show familiality for the disease, most typically transmitted by an autosomal dominant pattern (Gibson et al., 2014; Ryan et al., 2018). Among familial ALS (fALS), two thirds of the cases can be explained by pathogenic mutations in the C9orf72, SOD1, TARDBP, and FUS genes (Zou et al., 2017; Chiò et al., 2014), which also occur in 10–15% of sporadic ALS (sALS) (Renton et al., 2014). The relative contribution of each gene mutation to the epidemiology of the disease differs according to the population origin, with C9orf72 repeat expansion representing the most frequent alteration in ALS patients of European descent, while SOD1 mutations dominate the genetic landscape of ALS in China, Korea, and Japan (Zou et al., 2017). Overall, variable penetrance, genetic pleiotropy and the finding of double pathogenic mutations in the same patient point to an oligogenic mode of inheritance for many cases (Van Blitterswijk et al., 2012). At the histopathological level, diverse pathological signatures correspond to this fragmented genetic scenario. In the majority of sALS, as well as nearly half of frontotemporal dementia (FTD) cases, ubiquitinated and phosphorylated cytosolic TDP-43 aggregates are found in the frontal cortex (Neumann et al., 2006; Braak et al., 2010), whereas motor neurons of ALS patients harboring mutations in SOD1 or FUS display either neurofilamentous hyaline conglomerate inclusions and aggregates of misfolded SOD1 or cytoplasmic inclusions immunoreactive for FUS (Shibata et al., 1996; Kwiatkowski et al., 2009). C9orf72 associated diseases are characterized by TDP-43 pathology, but also by the presence of repeat-containing RNA (Al-Sarraj et al., 2011; Ash et al., 2013). These expanded C9orf72 RNAs form nuclear foci and can sequester various RNA-binding proteins, indirectly impairing their function on nucleic acid life cycle (Gendron et al., 2013; Zu et al., 2013). In addition, C9orf72 repeat expansions produce, via non-canonical Repeat-Associated Non-ATG (RAN) translation, several dipeptide repeat proteins (DPRs) that are highly aggregation-prone, thus compromising proteostasis (Mori et al., 2013; Kumar et al., 2017). Moreover, the human C9orf72 protein has been recently shown to play a role in endosomal degradation and lysosomal homeostasis and to target stress granules (SGs) to autophagy for clearance, acting in concert with SQSTM1 (Chitiprolu et al., 2018; Corrionero and Horvitz, 2018).

The majority of ALS-associated mutations displays an extreme variability in clinical manifestations, which may present asALS-plus phenotypes in the same individuals (e.g., ALS and parkinsonism) and/or different clinical pictures in the carriers belonging to the same family (e.g., ALS, FTD, or both in C9orf72 families).

All these recent achievements in the understanding of the disease pathogenesis led to the general consensus that ALS is a multisystem disorder in which the clinical, pathological and genetic features largely overcome the boundaries of a pure motor neuron involvement.

ALS AS AN EXTRA-MOTOR DISORDER WITH COGNITIVE INVOLVEMENT

The common notion of ALS as a disease affecting exclusively motor neurons has been initially cast into doubt by the early clinical observations of an association with FTD. In particular, clinicians observed some degrees of motor neuron diseases (MND) in FTD patients, and conversely, signs of cognitive and behavioral changes in ALS patients (Talbot et al., 1995; Neary et al., 2000).

FTD is the second most common form of early onset dementia, characteristically presenting in the fifth–sixth decade. The term FTD is used as an umbrella which encompasses a variety of clinical subtypes defined by clinical and pathological consensus criteria (Neary et al., 2000; Gorno-Tempini et al., 2011; Rascovsky et al., 2011). FTD can present as two main types, primarily affecting behavior (behavioral variant FTD, bvFTD) or language (primary progressive aphasia, PPA), the latter of which can be further divided in semantic variant (SD), non-fluent agrammatic variant (PNFA), and logopenic variant (lvPPA) (Chare et al., 2014; Finger, 2016). Although these subtypes can have very distinct neuroanatomical substrates, with time patients with bvFTD develop impairment in language functions and vice versa. Notably, ALS is most typically associated with behavioral FTD, whereas PPA variants with MND are rare (Saxon et al., 2017). In general, nearly 15% ALS patients satisfy diagnostic criteria for FTD (Raaphorst et al., 2012a,b; Phukan et al., 2012), constituting the syndrome of ALS-FTD, while larger fractions of ALS patients exhibit mild to moderate behavioral (ALSbi) and/or cognitive deficits (ALS-eci if executive impairment is present; ALS-neci if other intellectual functions are affected). An impairment of executive functions and verbal fluency has been found in 34–55% of ALS patients (Murphy et al., 2007a,b, 2016; Lillo and Hodges, 2009; Phukan et al., 2012; van Es et al., 2017), while behavioral disturbances have been observed in 14–40% of cases (Witgert et al., 2010; Phukan et al., 2012; Abrahams et al., 2014). Even more subtle cognitive and behavioral changes can be detected by recently validated batteries especially designed for screening ALS patients (Strong et al., 2017).

On the other side, almost 15% of bvFTD patients develop ALS during the course of disease, while signs of motor neuron impairment are observed in 40% of cases (Burrell et al., 2011; Bang et al., 2015). In conclusion, ALS and FTD can be regarded as the extremes of a disease continuum sharing some common histopathological and genetic background, which reflects a much extensive involvement of the sole motor neuron pathology.

The increased understanding of this diseases spectrum, has led researchers to study a variety of symptoms not classically considered part of the ALS clinical picture, the main ones being sensory and coordination impairment, pain and autonomic

involvement, sleep alteration and sphincter abnormalities. More recently, psychiatric symptoms have gained attention from several points of view: their increased presence preceding or following ALS onset, their relationship with FTD, familiality, prognosis, and treatment options.

In this review we aim to examine and elaborate on the reported aspects of psychiatric features in relation to the ALS spectrum.

PSYCHIATRIC DISTURBANCES IN ALS AND FTD

Early clinical observations reported several cases in which psychiatric illnesses such as schizophrenia co-occurred in ALS patients, raising the hypothesis of a common genetic background (Howland, 1990). More recently, register-based nationwide studies have proven an higher occurrence of psychiatric illnesses both before and after ALS diagnosis. In particular, the presence of depression, neurotic disorders and history of drug abuse or dependence, was associated to an increased odds ratio (OR) for ALS; in-depth analysis revealed that a diagnosis of schizophrenia may also represent a risk factor for ALS (OR 5.0) (Turner et al., 2016). Moreover, the risk of presenting depression, a neurotic or stress-related disorder following the diagnosis appeared to be increased as well (Longinetti et al., 2017).

Along with these findings, family members of ALS patients, especially children, showed increased risk for manifesting psychiatric disturbances both before and after their relative's diagnosis (Longinetti et al., 2017). Further strengthening this link, aggregation studies suggest neuropsychiatric illnesses and ALS cluster in families. In a population-based cohort study the relative risk of developing a neuropsychiatric condition such as schizophrenia or psychosis was significantly higher in first or second degree relatives of ALS patients (Byrne et al., 2013; O'Brien et al., 2017). Whether this can be explained by genetic pleiotropy of few variants into several kindreds or by a shared polygenic risk between psychiatric diseases and ALS spectrum remains to be determined (O'Brien et al., 2017).

Moving to FTD, psychosis is a recognized symptom, affecting 32% of patients in the largest autoptically confirmed case series, though psychiatric disturbances are not included in the diagnostic criteria (Landqvist Waldö et al., 2015). Prevalence of hallucinations in FTD cohorts varies considerably, with auditory being the most common, and delusions affect one quarter of the patients (Hall and Finger, 2015). When retrospectively evaluating clinical features in a FTD cohort that later evolved to motor neuron disorder, the presence of delusions was the best predictor of such progression, with a hazard ratio of 4.4 (Lillo et al., 2010).

Psychosis and Schizophrenia

Even before the discovery of a genetic overlap between schizophrenia and ALS, a relation between the two diseases was already suggested by early historical studies (Meltzer and Crayton, 1974; Howland, 1990; Larner, 2008). Moreover, disturbances in motor neuron function both at central (Goode and Manning, 1988) and peripheral level (Crayton et al., 1977; Crayton and Meltzer, 1979) exist in schizophrenia. Population-based studies have long corroborated the relation between single psychotic events, as well as schizophrenia, and ALS. In particular, an increased risk of hospitalization for schizophrenia could be observed in the 5 years preceding ALS, with higher statistical significance especially 1 year before onset of motor symptoms (Turner et al., 2016; Longinetti et al., 2017). This close relationship between psychotic features and motor symptoms in ALS may underlie the prodromal nature of these extra-motor symptoms in the framework of ALS pathogenesis (Turner et al., 2016). The link between ALS and schizophrenia was further supported by a large genome-wide association study which found a substantial genetic correlation, only partially explained by pleiotropic gene variants such as c9orf72 (McLaughlin et al., 2017). As previously highlighted, increased risk for schizophrenia and single psychotic episodes is observed among kindreds of c9orf72 carriers (Devenney et al., 2018). Moreover, psychosis was the presenting symptoms in 38% of c9orf72 carriers in a FTD-motor neuron disorder cohort, with florid psychotic symptoms such as delusional psychosis, somatoform psychosis or paranoid schizophrenia, and frontotemporal atrophy or hypoperfusion were noted on neuroimaging (Snowden et al., 2012). Delusions and hallucinations in this cohort of patients were reported to be mainly negative in nature, not related to their personal life experience (Snowden et al., 2012). Single psychotic episodes were also observed in elderly patients carrying c9orf72 expansion (Devenney et al., 2018). Overall, late-onset psychosis should always raise concern for familiality with motor neuron disease and thus warrant genetic testing for c9orf72 repeat expansion (Sommerlad et al., 2014). Furthermore, Snowden and colleagues noted that, though similar in appearance, in the case of c9orf72 expansion carriers bizarre behaviors and complex motor stereotypes had a distinct trait compared to those of other FTD and FTD-MND patients. According to the authors, it might therefore be hypothesized that this background activity of delusional thinking guides and reinforces the behavioral aberrancies typical of symptomatic FTD (Snowden et al., 2012).

Some of the cognitive changes associated to ALS, such as sensory behavioral disturbances, which were found in more than half of a large ALS cohort (Gibbons et al., 2008), have also been implicated in schizophrenia network dysfunction, with hyperactivation of secondary somatosensory cortex (Rains et al., 2012) and failed integration of sensory signaling (Carter et al., 2017). Regarding the pathophysiology of schizophrenia, a plethora of putative mechanisms have been implicated so far, mostly involving cerebral metabolic abnormalities in the pre-frontal cortex, anterior cingulate, caudate nucleus, basal ganglia, thalamus, and the cerebellum (Gross-Isseroff et al., 2003). Moreover, disruption of cortical inhibitory circuits by a reduction of overall GABAergic transmission has been advocated in schizophrenia by neurophysiological studies, mainly using transcranial magnetic stimulation (Fitzgerald et al., 2002), which demonstrated a reduction of long-interval cortical inhibition (LICI) especially in prefrontal cortex (Radhu et al., 2015). This not only relates to, similarly, enhanced cortical excitability in motor neuron disease (Geevasinga et al., 2016), but also to likewise affected tracts, with prefrontal cortex

involvement in ALS patients showing verbal fluency, attention and executive function impairment (Lomen-Hoerth et al., 2003; Meier et al., 2010).

Depression and Anxiety

Exogenous depression in ALS can be partly justified by the dismal prognosis of such a diagnosis, with patients experiencing everyday continuous motor decay. Nevertheless, literature show contrasting results in terms of the prevalence of depressive disorders in ALS, partly explained by the different testing scales employed, partly by the cross-sectional or prospective nature of these studies, and partly by the emotional adjustment or concomitant cognitive symptoms affecting ALS patients. The prevalence of depression in different studies thus ranges from impressively high values as 48–75% (McElhiney et al., 2009; Körner et al., 2015; Wei et al., 2016) to as low as 0.9–12% (Ferentinos et al., 2011; Lulé et al., 2012; Rabkin et al., 2015). In a large observational study, 17% of ALS patients were diagnosed with a major depressive disorder, and more than half of them were on antidepressant medications (Thakore and Pioro, 2016). There is evidence of an increased risk of depression prior to motor symptoms in ALS patients, particularly evident 1 year before disease onset, suggesting that the mood disorder is part of the prodromal cascade (Roos et al., 2016; Turner et al., 2016; Longinetti et al., 2017). Importantly, patients receiving a diagnosis of depression have a 3.6 OR of developing ALS compared to controls within 1 year (Roos et al., 2016). Likewise, a diagnosis of depression is more probable after ALS onset, in particular within 1 year from the appearance of motor symptoms (Roos et al., 2016; Turner et al., 2016). The same increased prevalence before and after ALS onset is true also for anxiety symptoms and neuroticism (Longinetti et al., 2017). Familial history of suicide was extremely overrepresented in ALS kindreds (Byrne et al., 2013; O'Brien et al., 2017).

On the other hand, many of the behavioral alterations demonstrated in ALS patients may be confused with depressive symptoms, such as apathy, which is found in 31–88% of patients (Witgert et al., 2010; Lillo et al., 2011), self-centredness, blunting of primary emotions, and lack of concern for personal hygiene (Gibbons et al., 2008). Interestingly, some studies failed to relate these symptoms to measures of depression (Grossman et al., 2007), arguing that this may reflect a behavioral disturbance due to the intrinsic ALS-FTD pathological continuum, rather than being secondary to the mood disorder. Another potential confounder of depressive symptoms in ALS is pseudobulbar affect (PBA), a neurobehavioral phenomenon manifesting with pathological overwhelming laughter or crying which are either incongruent with or excessive for the context. However, it has been demonstrated that crying-predominant PBA is associated with depression, while laughter-predominant is not (Thakore and Pioro, 2016). This finding may be due to mutual contributions from these conditions, with depression presenting as crying in the setting of PBA, and crying from PBA reinforcing the feeling of sadness and underlying depression. Further studies will be needed to determine whether these emotional phenomena in ALS are related to a shared pathological mechanism with depression.

Autism, Obsessiveness and Other Psychiatric Disorders Associated With ALS

Among other psychiatric disorders associated with ALS, Turner et al. found increased rate of bipolar disorder, with a relative risk of 3.2 to develop ALS within 1 year from hospitalization (Turner et al., 2016). This was later confirmed by a large registry-based study in which bipolar, neurotic and stress-related disorders, as well as a history of drug abuse/dependence, represented risk factors for subsequently developing ALS (Longinetti et al., 2017). Clustering of autism spectrum disorders has been observed within ALS and c9orf72 positive ALS-FTD kindreds (O'Brien et al., 2017; Devenney et al., 2018). Lack of empathy is commonly reported among the cognitive deficits of ALS patients (Cerami et al., 2014). Other abnormalities typically associated to autism such as stereotypical behaviors, social cognition impairment, obsessive-compulsive traits and mental rigidity have been reported in patients and corresponds to the clinical ALS-FTD continuum (Gibbons et al., 2008; Lillo et al., 2010; Mioshi et al., 2014). Theory of Mind (ToM) refers to the ability to infer mental states of oneself and others such as beliefs, emotions, intentions, and desires, thus allowing for an understanding of other people's behavior; these capacities are typically compromised in autistic patients (Hoogenhout and Malcolm-Smith, 2017). ToM processes have been further subdivided in cognitive and affective components. In ALS, 36% of patients displayed impairment in cognitive abilities, whereas 27% were dysfunctional in the affective ToM (van der Hulst et al., 2015).

Medial and orbitolateral prefrontal cortices have been involved in ToM capacity (Gallagher and Frith, 2003; Mitchell et al., 2006; Völlm et al., 2006), and several studies have largely demonstrated dysfunctional networks in these areas (Meier et al., 2010; Trojsi et al., 2017). Overall, a selective neurochemical or neuroanatomical network disruption may lie beneath ALS and autism mediated by unknown pathological mechanisms that warrant further research.

HISTOPATHOLOGICAL SIGNATURE OF THE ALS-FTD SPECTRUM AND HINTS FOR CORRELATIONS TO PSYCHIATRIC SYMPTOMS

The most common histopathological feature in ALS is represented by TDP-43 inclusions in motor neurons, either large and round (Lewy bodies-like) or skein-like (Braak et al., 2010). TDP-43 can be mislocalized within neuronal cytoplasmatic inclusion (NCIs) or dystrophic neurites (DN), and is enriched in post-translational modifications such as ubiquitination and phosphorylation (Tan et al., 2013).

Larger works proved TDP-43 pathology to be present in about half of all FTD cases (Davidson et al., 2007; Mann and Snowden, 2017), whereas the remnant 45% is represented by protein tau, and less than 5% by FUS or other aggregate-prone proteins (Mann and Snowden, 2017).

Interestingly, based on morphology and distribution of the inclusions, TDP-43 pathology in ALS and FTD can be subclassified into four types (A, B, C, D), the first two of them displaying round intracytoplasmatic aggregates (Tan et al., 2013). Type B is the most typically observed inclusion pattern in MND, while in FTD it is observed only in patients showing concomitant motor neuron involvement (Burrell et al., 2016).

Propagation of TDP-43 pathology from its core anatomical substrate, i.e., the motor cortex, was shown in ALS from a large cross-sectional autopsy study in which four stages of progression were identified. In stage 1, TDP-43 proteinopathy can be observed in the granular motor neocortex, alpha-motoneurons of the ventral horn, and bulbar motor neurons of cranial nerves. Stage 2 is characterized by involvement of reticular formation and precerebellar nuclei. In stage 3 TDP-43 inclusions are present in the prefrontal neocortex (firstly, gyrus rectus and orbital gyri, and, secondly, sensory areas and temporal neocortical area) and basal ganglia (striatum and inferior colliculus). In the final stage (4), anteromedial areas of the temporal lobe and the hippocampal formation display signs of pathology (Brettschneider et al., 2013).

A similar mechanism of spreading has been observed for bvFTD, where involvement of the orbital gyri, gyrus rectus, and amygdala characterizes the cases at the very initial phase (stage I). At an increasing burden of disease, the middle frontal and anterior cingulate gyrus as well as anteromedial temporal lobe areas, superior and medial temporal gyri, striatum, red nucleus, thalamus, and precerebellar nuclei are involved (stage II). More advanced phases of disease are characterized by motor cortex, bulbar somatomotor neurons, and spinal cord anterior horn propagation (stage III), and ultimately, visual cortex is affected (stage IV) (Brettschneider et al., 2014).

With regard to psychosis and schizophrenia, though they are considered more as diseases of connectivity and abnormal neurochemical transmission, some histopathological studies have found small but significant areas of atrophy in hippocampus, prefrontal and superior temporal cortex, and thalamus. This is accompanied by hemispheric asymmetry, decreased cortical thickness and gyrification, and abnormalities in hippocampal shape. Moreover, an early neurodevelopmental anomaly in schizophrenia may be postulated since the discovery of abnormally placed and clustered neurons in lamina II of entorhinal cortex or in the neocortex (Harrison and Weinberger, 2005). The finding of a decreased number of dendrites and arborization at hippocampal and neocortical level further support the view of reduced or aberrant wiring in schizophrenic patients, while the major neurochemical findings at cortical levels are represented by reduced number of serotoninergic (5-HT2A) and muscarinic (M1) receptors in patients with schizophrenia (Dean et al., 2016).

A clear neuropathological hallmark similar to the ALS/FTD spectrum does not exist for schizophrenia; nevertheless, in an autopsy case series on schizophrenic patients, tau-positive glial tangles were found in the dorsal aspect of temporal horn, arcuate fibers in gyri of frontal cortex, and within parahippocampal gyrus, while neurofibrillary tangles were observed in transentorhinal cortex, entorhinal region, subiculum and anterior hippocampus, in almost one third of cases, with increasing prevalence in elderly patients (Casanova et al., 2002). Altogether, these data may point to a restricted limbic tauopathy in presenile or senile psychotic patients, with no evidence of progression unlike Alzheimer disease. Though it may not be excluded that aberrancies in tau metabolism are due to neuroleptic drugs (Wisniewski et al., 1994), it is reasonable to hypothesize neurodegenerative processes occur in schizophrenia as well, given the accelerated aging and overall atrophy resulting in severe cognitive decline and motor abnormalities from duration of untreated psychosis (Anderson et al., 2014).

A further neuropathological link between psychosis and ALS may be found in microglia activation.

In fact, ALS arises in part by non-cell-autonomous mechanisms, from a combination of damage within MNs and their glial partners (Boillée et al., 2006). During the disease course, microglia switches from a neuroprotective M2 phenotype to an activated M1 phenotype which secretes proinflammatory interleukines, cytokines and neurotoxic factors, leading to the progression of neuronal injury (Henkel et al., 2013). Similarly, in schizophrenic patients' autopsies, increased markers of microglia activation were observed in the prefrontal cortex, anterior cingulate and temporal cortex (Radewicz et al., 2000).

NEUROIMAGING ACROSS PSYCHIATRIC AND BEHAVIORAL SYMPTOMS IN ALS

Neuroimaging studies have been crucial to better investigate functional and structural alterations in ALS patients showing psychiatric symptoms. By studying brain volume in a cohort of bvFTD and FTD-MND patients, a precise network of cortical and subcortical areas could be identified in patients with psychotic symptoms, which display bilateral medial prefrontal and occipital cortices, right thalamus and left cerebellum atrophy (Devenney et al., 2016). Sub-analysis within c9orf72 expansion carriers with psychosis prior to FTD or FTD-MND revealed that, besides presenting higher psychotic index, more extensive network disruption occurred, with volume reduction of bilateral medial frontal cortex, anterior cingulate and orbitofrontal cortex, bilateral insula, caudate, putamen and thalamic nuclei, middle, inferior and superior temporal gyrus, temporal fusiform gyrus, lateral occipital cortex and right cerebellum (Devenney et al., 2016). These areas roughly corresponds to those with the highest degree of atrophy in schizophrenic and schizoaffective patients (Amann et al., 2016). Moreover, anterior cingulate cortex and insula are strongly connected in the salience network, whose main function is to detect, analyze and integrate emotionally salient stimuli with respect to the internal environment, and which is involved in symptom generation in both FTD and schizophrenia (Seeley et al., 2007; Zhou and Seeley, 2014). In addition to schizophrenia, late-onset obsessive compulsive disorder in the setting of an upper motor neuron disease with concomitant FTD presents with bilateral hippocampal atrophy with sclerosis of right hippocampus on MRI and moderate right temporal cortex thinning at PET imaging (Bersano et al., 2018).

As already mentioned, abnormal behaviors are found in ALS patients along the FTD spectrum. Among these, apathy is one of the most commonly reported, and is correlated with

cortical thickness reduction in bilateral orbitofrontal lobe and left precentral gyrus. On the other hand, a hostile, disinhibited pattern of personality as identified by PCA analysis is more associated to thinning of temporal and cingular regions of the right hemisphere (Consonni et al., 2018).

A COMMON GENETIC BACKGROUND

Genome wide association studies (GWAS) allowed for exploring the genetic relationship between schizophrenia and ALS through SNPs-based heritability estimates, obtaining a genetic correlation of 14% (McLaughlin et al., 2017) due to polygenic overlap. Intriguingly, conditional false discovery rate was used to investigate novel ALS-associated genomic loci, confirming some of the known pleiotropic risk loci discussed below. A further study found clustering of schizophrenia and psychosis, suicide, autism, rigid personality disorders, and alcoholism in ALS kindreds suggesting that shared pleiotropic oligogenic variants may be responsible for co-segregation of psychiatric illnesses and ALS (O'Brien et al., 2017).

c9orf72

In 2011 a worldwide effort identified a hexanucleotidic expansion in the c9orf72 gene as the major genetic determinant of both ALS and FTD (DeJesus-Hernandez et al., 2011; Renton et al., 2011), thus revolutionizing our knowledge of genetic pleiotropy of ALS. C9orf72 pathological expansion accounts for almost 40% of fALS, 8% of sALS, and almost 30% of familial FTD in Caucasian population (Ng et al., 2015; Ng and Tan, 2017). This prevalence is increased in ALS-FTD, where it is found in 50–70% of familial and 15–20% of apparently sporadic cases (van der Zee et al., 2013). Noteworthy, c9orf72 repeat expansion display a high phenotypic variability, spanning from parkinsonism (Floris et al., 2012), to corticobasal degeneration (Lindquist et al., 2013), psychosis (Watson et al., 2016), and suicidal behavior (Synofzik et al., 2012). Penetrance is incomplete and age-dependent (Murphy et al., 2017), with anticipation phenomena similar to other repeat expansions diseases (Van Mossevelde et al., 2017). When analyzing the clinical feature best discriminating c9orf72 carriers from non-carriers in a cohort of bvFTD patients, psychosis and familiality for ALS appeared the most reliable clues (Devenney et al., 2014). A recent study investigating the risk of psychiatric disorders in c9orf72 positive kindreds, extrapolated from FTD and ALS cohorts, revealed an association with increased risk of autism spectrum disorders (HR: 2.7), schizophrenia (HR for a family member: 4.9) or a single psychotic episode (HR: 17.9), and mood disorder (HR: 1.9) (Devenney et al., 2018). Overall, this study confirms previous reports from an aggregation study in which stratification of ALS probands in carriers and non-carriers of c9orf72 repeat expansion was associated with major risk of presenting psychiatric disturbances in family members (Byrne et al., 2013). Importantly, among the referred psychiatric disorders associated to c9orf72 expansion, obsessive-compulsive disorder seems to be excluded (Arthur et al., 2017), though rigid stereotyped behavior with obsessiveness is frequently observed in carriers (Snowden et al., 2012).

C9orf72 is an alternatively spliced gene encoding for three protein transcripts, whose functions have not been fully elucidated. Molecular studies showed that the protein localizes in the nucleus and is structurally similar to DENN (differentially expressed in normal and neoplasia) proteins, which contain a guanine nucleotide exchange factor allowing them to interact with RAB GTPase proteins and regulate membrane trafficking from the nucleus (Levine et al., 2013; Aoki et al., 2017). A striking characteristic of c9orf72 alterations is that differential repeat length is observed across different tissues (Van Blitterswijk et al., 2013), suggesting instability and possibly the occurrence of epigenetic phenomena such as hypermethylation as a potential source of this variability (Xi et al., 2014).

Healthy individuals carry up to 25 repeats of GGGGCC in c9orf72, with the majority having a couple of repeats, while in ALS and FTD cases the number of repeats ranges from 100s to 1000s (DeJesus-Hernandez et al., 2011; Renton et al., 2011; Beck et al., 2013). Uncertainty surrounds the role of intermediate length (22–30) repeats, though they seem to be associated with a higher frequency of psychiatric symptoms in FTD, FTD-ALS, and atypical parkinsonism cohorts (Ng and Tan, 2017).

Recently some studies focused on biomarkers that may help predict the so-called "phenoconversion," since genetic therapy may become an option for in C9orf72 carriers (Floeter and Gendron, 2018). Biological markers might guide pharmacological response to potential therapies, as in the case of Poly(GP) proteins (Gendron et al., 2017a), or predict the prognosis (Gendron et al., 2017b) and anticipate the onset of symptoms by a year (Benatar et al., 2018), as in the case of neurofilament heavy and light chain, respectively. Furthermore, imaging studies showed that atrophy of several cortical and subcortical structures have been observed in asymptomatic carriers, including the thalamus (Papma et al., 2017; Bertrand et al., 2018; Floeter and Gendron, 2018), the left caudate and putamen, besides diffuse cortical thinning in defined temporal, parietal, and occipital regions (Walhout et al., 2015). Similarly, white matter tracts are not spared either before symptoms onset: functional studies have shown salience and medial pulvinar networks, who are known connectivity networks prominently affected in bvFTD, to be severely disrupted in carriers already in their 40s (Lee et al., 2016). Increased radial diffusivity has been reported as well in the right anterior thalamic radiation and the right forceps, even in younger c9orf72 expansion carriers (Bertrand et al., 2018). Notwithstanding, asymptomatic carriers did not show significant atrophy before symptoms onset in a longitudinal voxel-based morphometry study (Floeter et al., 2016). When testing the hypothesis that psychiatric disturbances might be prodromal of the structural and functional brain abnormalities observed in c9orf72 presymptomatic carriers, Lee et al. (2016) found that carriers and non-carrier family members had comparable lifetime histories of psychiatric symptoms, non-carriers family members doubled the amount of psychiatric medications compared to carriers, and underwent similar rates of hospitalization for psychiatric disturbances. Overall, we cannot exclude that familiality for psychiatric diseases, which are known polygenic conditions, runs independently of the expansion among c9orf72-families, however, further studies are warranted to better explore

prodromal disturbances of thought in expansion carriers because of the high variability between personality and behavioral tests, which might not be suited to detect subtle changes in non-demented cohorts.

Other Genes Associated With Psychiatric Disturbances

Notwithstanding the major role of c9orf72, a non-trivial residual association between ALS and psychiatric disorders persists even after excluding repeat expansion carriers from genetic analyses (Byrne et al., 2013). Isolated cases of concomitant psychiatric disorders such as schizophrenia have been found in kindreds with specific mutations in FUS (Yan et al., 2010) and TARDBP (Quadri et al., 2011). Variable rates of psychiatric illnesses, generally less common than in c9orf72 repeat carriers, were also observed in non-c9orf72 ALS-FTD cases, carrying PRGN (Hall and Finger, 2015), TBK1 (Van Mossevelde et al., 2016), and VCP (Weihl, 2011) mutation. ATXN2, has also been associated to both ALS and schizophrenia risk (Zhang et al., 2014).

It is reasonable to speculate that the numerous genetic loci known to be involved in the ALS-FTD disease spectrum, such as TBK1, PGRN, CHCHD10, TUB4A, VCP, may predispose to psychiatric illnesses by analogous mechanisms to c9orf72. The rarity of these cases, together with the relatively small populations studied, and the difficulty in discerning psychiatric disturbances from other aspects of behavioral FTD, make proving this assumption a daunting task.

PROGNOSTIC ROLE OF PSYCHIATRIC DISTURBANCES IN ALS

Concomitant psychiatric diseases in ALS patients, whether prior or after this fatal diagnosis, may add strain on caregivers and pose important ethical challenges for support and end-of-life decisions along the course of this disease. Until now, only few register-based studies have taken into account the prognostic significance of simultaneous psychiatric illness in ALS, showing a mild negative influence of anxiety symptoms and other psychiatric disturbances in univariate analysis, whose effect was later unconfirmed in multivariate analysis (Körner et al., 2013; Mandrioli et al., 2018). Other studies examining the impact of neuropsychiatric symptoms in ALS, expressed mainly as behavioral alterations, failed to demonstrate any impact on survival (Mioshi et al., 2014; Burke et al., 2017). However, in a prospective study evaluating depression in ALS by validated outcome measures, a concurrent diagnosis of major depressive disorder corresponded to decreased survival, and any increasing score matched increased death hazard ratio (Thakore and Pioro, 2016). This discordance in findings may be related to an underestimation of depression in the ALS population. In addition, the use of more subtle evaluating psychometric measures may aid the clinician to formulate such a diagnosis and address these disorders, which are increasingly reported as a major burden for carers (Creemers et al., 2016).

CONCLUSION

In conclusion, psychiatric diseases often anticipate the onset of motor symptoms in ALS, and their timely relation with motor neuron pathology may be due to underlying common pathogenic mechanisms affecting non-motor structures within the central nervous system. Similar changes in structural framework between ALS, ALS-FTD and schizophrenia exist, and some degree of genetic overlap between these diseases has been found, strengthening a common pathological signature. Overall, psychiatric illness do not appear to influence significantly the prognosis and survival of ALS patients, but may constitute an increased burden for caregivers and challenge ethical choices with regards to end-of-life decisions. Thus, clinicians should be aware of the tight relationship between ALS and psychiatric disorders and timely address specialist interventions to better assist ALS families.

AUTHOR CONTRIBUTIONS

JM, EZ, and NT contributed to conceptualization, data curation, formal analysis, and methodology. EZ wrote the first draft. and JM and NT reviewed and edited it.

REFERENCES

Abrahams, S., Newton, J., Niven, E., Foley, J., and Bak, T. H. (2014). Screening for cognition and behaviour changes in ALS. *Amyotroph. Lateral Scler. Frontotemporal. Degener.* 15, 9–14. doi: 10.3109/21678421.2013.805784

Al-Sarraj, S., King, A., Troakes, C., Smith, B., Maekawa, S., Bodi, I., et al. (2011). p62 positive, TDP-43 negative, neuronal cytoplasmic and intranuclear inclusions in the cerebellum and hippocampus define the pathology of C9orf72-linked FTLD and MND/ALS. *Acta Neuropathol.* 122, 691–702. doi: 10.1007/s00401-011-0911-2

Amann, B. L., Canales-Rodríguez, E. J., Madre, M., Radua, J., Monte, G., Alonso-Lana, S., et al. (2016). Brain structural changes in schizoaffective disorder compared to schizophrenia and bipolar disorder. *Acta Psychiatr. Scand.* 133, 23–33. doi: 10.1111/acps.12440

Anderson, K. K., Voineskos, A., Mulsant, B. H., George, T. P., and Mckenzie, K. J. (2014). The role of untreated psychosis in neurodegeneration: a review of hypothesized mechanisms of neurotoxicity in first-episode psychosis. *Can. J. Psychiatry* 59, 513–517. doi: 10.1177/070674371405901003

Aoki, Y., Manzano, R., Lee, Y., Dafinca, R., Aoki, M., Douglas, A. G. L., et al. (2017). C9orf72 and RAB7L1 regulate vesicle trafficking in amyotrophic lateral sclerosis and frontotemporal dementia. *Brain* 140, 887–897. doi: 10.1093/brain/awx024

Arthur, K. C., Rivera, A. M., Samuels, J., Wang, Y., Grados, M., Goes, F. S., et al. (2017). C9orf72 hexanucleotide repeat expansions are not a common cause of obsessive-compulsive disorder. *J. Neurol. Sci.* 375, 71–72. doi: 10.1016/j.jns.2017.01.040

Ash, P. E. A., Bieniek, K. F., Gendron, T. F., Caulfield, T., Lin, W. L., Dejesus-Hernandez, M., et al. (2013). Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. *Neuron* 77, 639–646. doi: 10.1016/j.neuron.2013.02.004

Bang, J., Spina, S., and Miller, B. L. (2015). Frontotemporal dementia. *Lancet* 386, 1672–1682. doi: 10.1016/S0140-6736(15)00461-4

Beck, J., Poulter, M., Hensman, D., Rohrer, J. D., Mahoney, C. J., Adamson, G., et al. (2013). Large C9orf72 hexanucleotide repeat expansions are seen in multiple

neurodegenerative syndromes and are more frequent than expected in the UK population. *Am. J. Hum. Genet.* 92, 345–353. doi: 10.1016/j.ajhg.2013.01.011

Benatar, M., Wuu, J., Andersen, P. M., Lombardi, V., and Malaspina, A. (2018). Neurofilament light: a candidate biomarker of presymptomatic amyotrophic lateral sclerosis and phenoconversion. *Ann. Neurol.* 84, 130–139. doi: 10.1002/ana.25276

Bersano, E., Sarnelli, M. F., Solara, V., De Marchi, F., Sacchetti, G. M., Stecco, A., et al. (2018). A case of late-onset OCD developing PLS and FTD. *Amyotroph. Lateral Scler. Frontotemporal. Degener.* 19, 463–465. doi: 10.1080/21678421.2018.1440405

Bertrand, A., Wen, J., Rinaldi, D., Houot, M., Sayah, S., Camuzat, A., et al. (2018). Early cognitive, structural, and microstructural changes in presymptomatic C9orf72 carriers younger than 40 Years. *JAMA Neurol.* 75, 236–245. doi: 10.1001/jamaneurol.2017.4266

Boillée, S., Vande Velde, C., and Cleveland, D. W. (2006). ALS: a disease of motor neurons and their nonneuronal neighbors. *Neuron* 52, 39–59. doi: 10.1016/j.neuron.2006.09.018

Braak, H., Ludolph, A., Thal, D. R., and Del Tredici, K. (2010). Amyotrophic lateral sclerosis: dash-like accumulation of phosphorylated TDP-43 in somatodendritic and axonal compartments of somatomotor neurons of the lower brainstem and spinal cord. *Acta Neuropathol.* 120, 67–74. doi: 10.1007/s00401-010-0683-0

Brettschneider, J., Del Tredici, K., Irwin, D. J., Grossman, M., Robinson, J. L., Toledo, J. B., et al. (2014). Sequential distribution of pTDP-43 pathology in behavioral variant frontotemporal dementia(bvFTD). *Acta Neuropathol.* 127, 423–439. doi: 10.1007/s00401-013-1238-y

Brettschneider, J., Del Tredici, K., Toledo, J. B., Robinson, J. L., Irwin, D. J., Grossman, M., et al. (2013). Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. *Ann. Neurol.* 74, 20–38. doi: 10.1002/ana.23937

Brown, R. H., and Al-Chalabi, A. (2017). Amyotrophic lateral sclerosis. *N. Engl. J. Med.* 377, 162–172. doi: 10.1056/NEJMra1603471

Burke, T., Pinto-Grau, M., Lonergan, K., Bede, P., O'Sullivan, M., Heverin, M., et al. (2017). A Cross-sectional population-based investigation into behavioral change in amyotrophic lateral sclerosis: subphenotypes, staging, cognitive predictors, and survival. *Ann. Clin. Transl. Neurol.* 4, 305–317. doi: 10.1002/acn3.407

Burrell, J. R., Halliday, G. M., Kril, J. J., Ittner, L. M., Götz, J., Kiernan, M. C., et al. (2016). The frontotemporal dementia-motor neuron disease continuum. *Lancet* 388, 919–931. doi: 10.1016/S0140-6736(16)00737-6

Burrell, J. R., Kiernan, M. C., Vucic, S., and Hodges, J. R. (2011). Motor neuron dysfunction in frontotemporal dementia. *Brain* 134, 2582–2594. doi: 10.1093/brain/awr195

Byrne, S., Heverin, M., Elamin, M., Bede, P., Lynch, C., Kenna, K., et al. (2013). Aggregation of neurologic and neuropsychiatric disease in amyotrophic lateral sclerosiskindreds: a population-based case control cohort study of familial and sporadic amyotrophic lateral sclerosis. *Ann. Neurol.* 74, 699–708. doi: 10.1002/ana.23969

Carter, O., Bennett, D., Nash, T., Arnold, S., Brown, L., Cai, R. Y., et al. (2017). Sensory integration deficits support a dimensional view of psychosis and are not limited to schizophrenia. *Transl. Psychiatry.* 7:e1118. doi: 10.1038/tp.2017.69

Casanova, M., Stevens, J. R., Brown, R., Royston, C., and Bruton, C. (2002). Disentangling the pathology of schizophrenia and paraphrenia. *Acta Neuropathol.* 103, 313–320. doi: 10.1007/s00401-001-0468-6

Cerami, C., Dodich, A., Canessa, N., Crespi, C., Iannaccone, S., Corbo, M., et al. (2014). Emotional empathy in amyotrophic lateral sclerosis: a behavioural and voxel-based morphometry study. *Amyotroph. Lateral Scler. Frontotemporal. Degener.* 15, 21–29. doi: 10.3109/21678421.2013.785568

Chare, L., Hodges, J. R., Leyton, C. E., McGinley, C., Tan, R. H., Kril, J. J., et al. (2014). New criteria for frontotemporal dementia syndromes: clinical and pathological diagnostic implications. *J. Neurol. Neurosurg. Psychiatry* 85, 865–870. doi: 10.1136/jnnp-2013-306948

Chiò, A., Battistini, S., Calvo, A., Caponnetto, C., Conforti, F. L., Corbo, M., et al. (2014). Genetic counselling in ALS: facts, uncertainties and clinical suggestions. *J. Neurol. Neurosurg. Psychiatry* 85, 478–485. doi: 10.1136/jnnp-2013-305546

Chiò, A., Calvo, A., Moglia, C., Mazzini, L., and Mora, G. (2011). Phenotypic heterogeneity of amyotrophic lateral sclerosis: a population based study. *J. Neurol. Neurosurg. Psychiatry* 82, 740–746. doi: 10.1136/jnnp.2010.235952

Chitiprolu, M., Jagow, C., Tremblay, V., Bondy-Chorney, E., Paris, G., and Savard, A. (2018). A complex of C9ORF72 and p62 uses arginine methylation to eliminate stress granules by autophagy. *Nat. Commun.* 9:2794. doi: 10.1038/s41467-018-05273-7

Consonni, M., Cappa, S. F., Dalla Bella, E., Contarino, V. E., and Lauria, G. (2018). Cortical correlates of behavioural change in amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry*. doi: 10.1136/jnnp-2018-318619 [Epub ahead of print].

Corrionero, A., and Horvitz, H. R. (2018). A C9orf72 ALS/FTD ortholog acts in endolysosomal degradation and lysosomal homeostasis. *Curr. Biol.* 28, 1522–1535.e5. doi: 10.1016/j.cub.2018.03.063

Crayton, J. W., and Meltzer, H. Y. (1979). Degeneration and regeneration of motor neurons in psychotic patients. *Biol. Psychiatry* 14, 803–819.

Crayton, J. W., Meltzer, H. Y., and Goode, D. J. (1977). Motoneuron excitability in psychiatric patients. *Biol. Psychiatry* 12, 545–561.

Creemers, H., de Morée, S., Veldink, J. H., Nollet, F., van den Berg, L. H., and Beelen, A. (2016). Factors related to caregiver strain in ALS: a longitudinal study. *J. Neurol. Neurosurg. Psychiatry* 87, 775–781. doi: 10.1136/jnnp-2015-311651

Davidson, Y., Kelley, T., Mackenzie, I. R., Pickering-Brown, S., Du Plessis, D., and Neary, D. (2007). Ubiquitinated pathological lesions in frontotemporal lobar degeneration contain the TAR DNA-binding protein, TDP-43. *Acta Neuropathol.* 113, 521–533. doi: 10.1007/s00401-006-0189-y

Dean, B., Copolov, D., and Scarr, E. (2016). Understanding the pathophysiology of schizophrenia: contributions from the Melbourne Psychiatric Brain Bank. *Schizophr. Res.* 177, 108–114. doi: 10.1016/j.schres.2016.04.037

DeJesus-Hernandez, M., Mackenzie, I. R., Boeve, B. F., Boxer, A. L., Baker, M., Rutherford, N. J., et al. (2011). Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 72, 245–256. doi: 10.1016/j.neuron.2011.09.011

Devenney, E., Hornberger, M., Irish, M., Mioshi, E., Burrell, J., Tan, R., et al. (2014). Frontotemporal dementia associated with the C9ORF72 mutation: a unique clinical profile. *JAMA Neurol.* 71, 331–339. doi: 10.1001/jamaneurol.2013.6002

Devenney, E. M., Ahmed, R. M., Halliday, G., Piguet, O., Kiernan, M. C., and Hodges, J. R. (2018). Psychiatric disorders in C9orf72 kindreds: study of 1,414 family members. *Neurology* 91, e1498–e1507. doi: 10.1212/WNL.0000000000006344

Devenney, E. M., Landin-Romero, R., Irish, M., Hornberger, M., Mioshi, E., Halliday, G. M., et al. (2016). The neural correlates and clinical characteristics of psychosis in the frontotemporal dementia continuum and the C9orf72 expansion. *Neuroimage* 13, 439–445. doi: 10.1016/j.nicl.2016.11.028

Ferentinos, P., Paparrigopoulos, T., Rentzos, M., Zouvelou, V., Alexakis, T., and Evdokimidis, I. (2011). Prevalence of major depression in ALS: comparison of a semi-structured interview and four self-report measures. *Amyotroph. Lateral Scler.* 12, 297–302. doi: 10.3109/17482968.2011.556744

Finger, E. (2016). Frontotemporal Dementias. *Continuum* 22, 464–489. doi: 10.1212/CON.0000000000000300

Fitzgerald, P. B., Brown, T. L., Daskalakis, Z. J., and Kulkarni, J. (2002). A transcranial magnetic stimulation study of inhibitory deficits in the motor cortex in patients with schizophrenia. *Psychiatry Res.* 114, 11–22. doi: 10.1016/S0925-4927(02)00002-1

Floeter, M. K., Bageac, D., Danielian, L. E., Braun, L. E., Traynor, B. J., and Kwan, J. Y. (2016). Longitudinal imaging in C9orf72 mutation carriers: relationship to phenotype. *Neuroimage Clin.* 12, 1035–1043. doi: 10.1016/j.nicl.2016.10.014

Floeter, M. K., and Gendron, T. F. (2018). Biomarkers for amyotrophic lateral sclerosis and frontotemporal dementia associated with hexanucleotide expansion mutations in C9orf72. *Front. Neurol.* 9:1063. doi: 10.3389/fneur.2018.01063

Floris, G., Borghero, G., Cannas, A., Di Stefano, F., Costantino, E., Murru, M. R., et al. (2012). Frontotemporal dementia with psychosis, parkinsonism, visuospatial dysfunction, upper motor neuron involvement associated to expansion of C9ORF72: a peculiar phenotype? *J. Neurol.* 259, 1749–1751. doi: 10.1007/s00415-012-6444-3

Gallagher, H. L., and Frith, C. D. (2003). Functional imaging of 'theory of mind'. *Trends Cogn. Sci.* 7, 77–83. doi: 10.1016/S1364-6613(02)00025-6

Geevasinga, N., Menon, P., Özdinler, P. H., Kiernan, M. C., and Vucic, S. (2016). Pathophysiological and diagnostic implications of cortical dysfunction in ALS. *Nat. Rev. Neurol.* 12, 651–661. doi: 10.1038/nrneurol.2016.140

Gendron, T. F., Bieniek, K. F., Zhang, Y.-J., Jansen-West, K., Ash, P. E., Caulfield, T., et al. (2013). Antisense transcripts of the expanded C9ORF72 hexanucleotide repeat form nuclear RNA foci and undergo repeat-associated non-ATG translation in c9FTD/ALS. *Acta Neuropathol.* 126, 829–844. doi: 10.1007/s00401-013-1192-8

Gendron, T. F., Chew, J., Stankowski, J. N., Hayes, L. R., Zhang, Y. J., Prudencio, M., et al. (2017a). Poly(GP) proteins are a useful pharmacodynamic marker for C9ORF72-associated amyotrophic lateral sclerosis. *Sci. Transl. Med.* 9:eaai7866. doi: 10.1126/scitranslmed.aai7866

Gendron, T. F., Daughrity, L. M., Heckman, M. G., Diehl, N. N., Wuu, J., Miller, T. M., et al. (2017b). Phosphorylated neurofilament heavy chain: a biomarker of survival for C9ORF72-associated amyotrophic lateral sclerosis. *Ann. Neurol.* 82, 139–146. doi: 10.1002/ana.24980

Gibbons, Z. C., Richardson, A., Neary, D., and Snowden, J. S. (2008). Behaviour in Amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* 9, 67–74. doi: 10.1080/17482960701642437

Gibson, S. B., Figueroa, K. P., Bromberg, M. B., Pulst, S. M., and Cannon-Albright, L. (2014). Familial clustering of ALS in a population-based resource. *Neurology* 82, 17–22. doi: 10.1212/01.wnl.0000438219.39061.da

Goode, D. J., and Manning, A. A. (1988). Specific imbalance of right and left sided motor neuron excitability in schizophrenia. *J. Neurol. Neurosurg. Psychiatry* 51, 626–629. doi: 10.1136/jnnp.51.5.626

Gorno-Tempini, M. L., Hillis, A. E., Weintraub, S., Kertesz, A., Mendez, M., and Cappa, S. F. (2011). Classification of primary progressive aphasia and its variants. *Neurology* 76, 1006–1014. doi: 10.1212/WNL.0b013e31821103e6

Gross-Isseroff, R., Hermesh, H., Zohar, J., and Weizman, A. (2003). Neuroimaging communality between schizophrenia and obsessive compulsive disorder: a putative basis for schizo-obsessive disorder? *World J. Biol. Psychiatry* 4, 129–134. doi: 10.1080/15622970310029907

Grossman, A. B., Woolley-Levine, S., Bradley, W. G., and Miller, R. G. (2007). Detecting neurobehavioral changes in amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* 8, 56–61. doi: 10.1080/17482960601044106

Hall, D., and Finger, E. C. (2015). Psychotic symptoms in frontotemporal dementia. *Curr. Neurol. Neurosci. Rep.* 15:46. doi: 10.1007/s11910-015-0567-8

Hardiman, O., Al-Chalabi, A., Chio, A., Corr, E. M., Logroscino, G., Robberecht, W., et al. (2017). Amyotrophic lateral sclerosis. *Nat. Rev. Dis. Primers.* 3:17071. doi: 10.1038/nrdp.2017.71

Harrison, P. J., and Weinberger, D. R. (2005). Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. *Mol. Psychiatry* 10, 40–68. doi: 10.1038/sj.mp.4001558

Henkel, J. S., Beers, D. R., Wen, S., Rivera, A. L., Toennis, K. M., Appel, J. E., et al. (2013). Regulatory T-lymphocytes mediate amyotrophic lateral sclerosis progression and survival. *EMBO Mol. Med.* 5, 64–79. doi: 10.1002/emmm.201201544

Hoogenhout, M., and Malcolm-Smith, S. (2017). Theory of mind predicts severity level in autism. *Autism* 21, 242–252. doi: 10.1177/1362361316636758

Howland, R. H. (1990). Schizophrenia and amyotrophic lateral sclerosis. *Compr. Psychiatry* 31, 327–336. doi: 10.1016/0010-440X(90)90039-U

Körner, S., Kollewe, K., Abdulla, S., Zapf, A., Dengler, R., and Petri, S. (2015). Interaction of physical function, quality of life and depression in Amyotrophic lateral sclerosis: characterization of a large patient cohort. *BMC Neurol.* 15:84. doi: 10.1186/s12883-015-0340-2

Körner, S., Kollewe, K., Ilsemann, J., Müller-Heine, A., Dengler, R., Krampfl, K., et al. (2013). Prevalence and prognostic impact of comorbidities in amyotrophic lateral sclerosis. *Eur. J. Neurol.* 20, 647–654. doi: 10.1111/ene.12015

Kumar, V., Hasan, G. M., and Hassan, M. I. (2017). Unraveling the Role of RNA Mediated Toxicity of C9orf72 Repeats in C9-FTD/ALS. *Front. Neurosci.* 11:711. doi: 10.3389/fnins.2017.00711

Kwiatkowski, T. J. Jr., Bosco, D. A., Leclerc, A. L., Tamrazian, E., Vanderburg, C. R., Russ, C., et al. (2009). Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* 323, 1205–1208. doi: 10.1126/science.1166066

Landqvist Waldö, M., Gustafson, L., Passant, U., and Englund, E. (2015). Psychotic symptoms in frontotemporal dementia: a diagnostic dilemma? *Int. Psychogeriatr.* 27, 531–539. doi: 10.1017/S1041610214002580

Larner, A. J. (2008). Delusion of pregnancy in frontotemporal lobar degeneration with motor neurone disease (FTLD/MND). *Behav. Neurol.* 19, 199–200. doi: 10.1155/2008/149086

Lee, S. E., Sias, A. C., Mandelli, M. L., Brown, J. A., Brown, A. B., Khazenzon, A. M., et al. (2016). Network degeneration and dysfunction in presymptomatic C9ORF72 expansion carriers. *Neuroimage Clin.* 14, 286–297. doi: 10.1016/j.nicl.2016.12.006

Levine, T. P., Daniels, R. D., Gatta, A. T., Wong, L. H., and Hayes, M. J. (2013). The product of C9orf72, a gene strongly implicated in neurodegeneration, is structurally related to DENN Rab-GEFs. *Bioinformatics* 29, 499–503. doi: 10.1093/bioinformatics/bts725

Lillo, P., Garcin, B., Hornberger, M., Bak, T. H., and Hodges, J. R. (2010). Neurobehavioral features in frontotemporal dementia with amyotrophic lateral sclerosis. *Arch. Neurol.* 67, 826–830. doi: 10.1001/archneurol.2010.146

Lillo, P., and Hodges, J. R. (2009). Frontotemporal dementia and motor neurone disease: overlapping clinic-pathological disorders. *J. Clin. Neurosci.* 16, 1131–1135. doi: 10.1016/j.jocn.2009.03.005

Lillo, P., Mioshi, E., Zoing, M. C., Kiernan, M. C., and Hodges, J. R. (2011). How common are behavioural changes in amyotrophic lateral sclerosis? *Amyotroph. Lateral Scler.* 12, 45–51. doi: 10.3109/17482968.2010.520718

Lindquist, S., Duno, M., Batbayli, M., Puschmann, A., Braendgaard, H., Mardosiene, S., et al. (2013). Corticobasal and ataxia syndromes widen the spectrum of C9ORF72 hexanucleotide expansion disease. *Clin. Genet.* 83, 279–283. doi: 10.1111/j.1399-0004.2012.01903.x

Lomen-Hoerth, C. M. D., Murphy, J., Langmore, S., Kramer, J. H., Olney, R. K. M., and Miller, B. L. (2003). Are amyotrophic lateral sclerosis patients cognitively normal? *Neurology* 60, 1094–1097.

Longinetti, E., Mariosa, D., Larsson, H., Ye, W., Ingre, C., Almqvist, C., et al. (2017). Neurodegenerative and psychiatric diseases among families with amyotrophic lateral sclerosis. *Neurology* 89, 578–585. doi: 10.1212/WNL.0000000000004179

Lulé, D., Pauli, S., Altintas, E., Singer, U., Merk, T., Uttner, I., et al. (2012). Emotional adjustment in amyotrophic lateral sclerosis (ALS). *J. Neurol.* 259, 334–341. doi: 10.1007/s00415-011-6191-x

Mandrioli, J., Ferri, L., Fasano, A., Zucchi, E., Fini, N., Moglia, C., et al. (2018). Cardiovascular diseases may play a negative role in the prognosis of amyotrophic lateral sclerosis. *Eur. J. Neurol.* 25, 861–868. doi: 10.1111/ene.13620

Mann, D., and Snowden, J. (2017). Frontotemporal lobar degeneration: pathogenesis, pathology and pathways to phenotype. *Brain Pathol.* 27, 723–736. doi: 10.1111/bpa.12486

McElhiney, M. C., Rabkin, J. G., Gordon, P. H., Goetz, R., and Mitsumoto, H. (2009). Prevalence of fatigue and depression in ALS patients and change over time. *J. Neurol. Neurosurg. Psychiatry* 80, 1146–1149. doi: 10.1136/jnnp.2008.163246

McLaughlin, R. L., Schijven, D., van Rheenen, W., van Eijk, K. R., O'Brien, M., Kahn, R. S., et al. (2017). Genetic correlation between amyotrophic lateral sclerosis and schizophrenia. *Nat. Commun*:8, 14774. doi: 10.1038/ncomms14774

Meier, S. L., Charleston, A. J., and Tippett, L. J. (2010). Cognitive and behavioural deficits associated with the orbitomedial prefrontal cortex in amyotrophic lateral sclerosis. *Brain* 133, 3444–3457. doi: 10.1093/brain/awq254

Meltzer, H. Y., and Crayton, J. W. (1974). Subterminal motor nerve abnormalities in psychotic patients. *Nature* 249, 373–375. doi: 10.1038/249373a0

Mioshi, E., Caga, J., Lillo, P., Hsieh, S., Ramsey, E., Devenney, E., et al. (2014). Neuropsychiatric changes precede classic motor symptoms in ALS and do not affect survival. *Neurology* 82, 149–155. doi: 10.1212/WNL.0000000000000023

Mitchell, J. P., Macrae, C. N., and Banaji, M. R. (2006). Dissociable medial prefrontal contributions to judgments of similar and dissimilar others. *Neuron* 50, 655–663. doi: 10.1016/j.neuron.2006.03.040

Mori, K., Arzberger, T., Grässer, F. A., Gijselinck, I., May, S., Rentzsch, K., et al. (2013). Bidirectional transcripts of the expanded C9orf72 hexanucleotide repeat are translated into aggregating dipeptide repeat proteins. *Acta Neuropathol.* 126, 881–893. doi: 10.1007/s00401-013-1189-3

Murphy, J. M., Factor-Litvak, P., Goetz, R., Lomen-Hoerth, C., Nagy, P. L., Hupf, J., et al. (2016). Cognitive-behavioral screening reveals prevalent impairment in

a large multicenter ALS cohort. *Neurology* 86, 813–820. doi: 10.1212/WNL.0000000000002305

Murphy, J. M., Henry, R. G., Langmore, S., Kramer, J. H., Miller, B. L., and Lomen-Hoerth, C. (2007a). Continuum of frontal lobe impairment in amyotrophic lateral sclerosis. *Arch. Neurol.* 64, 530–534. doi: 10.1001/archneur.64.4.530

Murphy, J. M., Henry, R., and Lomen-Hoerth, C. (2007b). Establishing subtypes of the continuum of frontal lobe impairment in amyotrophic lateral sclerosis. *Arch. Neurol.* 64, 330–334.

Murphy, N. A., Arthur, K. C., Tienari, P. J., Houlden, H., Chiò, A., and Traynor, B. J. (2017). Age-related penetrance of the C9orf72 repeat expansion. *Sci. Rep.* 7:2116. doi: 10.1038/s41598-017-02364-1

Neary, D., Snowden, J. S., and Mann, D. M. (2000). Cognitive change in motor neurone disease/amyotrophic lateral sclerosis (MND/ALS). *J. Neurol. Sci.* 180, 15–20. doi: 10.1016/S0022-510X(00)00425-1

Neumann, M., Sampathu, D. M., Kwong, L. K., Truax, A. C., Micsenyi, M. C., Chou, T. T., et al. (2006). Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 314, 130–133. doi: 10.1126/science.1134108

Ng, A. S. L., and Tan, E. K. (2017). Intermediate C9orf72 alleles in neurological disorders: does size really matter? *J. Med. Genet.* 54, 591–597. doi: 10.1136/jmedgenet-2017-104752

Ng, A. S., Rademakers, R., and Miller, B. L. (2015). Frontotemporal dementia: a bridge between dementia and neuromuscular disease. *Ann. N. Y. Acad. Sci.* 1338, 71–93. doi: 10.1111/nyas.12638

O'Brien, M., Burke, T., Heverin, M., Vajda, A., McLaughlin, R., Gibbons, J., et al. (2017). Clustering of neuropsychiatric disease in first-degree and second-degree relatives of patientswith amyotrophic lateral sclerosis. *JAMA Neurol.* 74, 1425–1430. doi: 10.1001/jamaneurol.2017.2699

Papma, J. M., Jiskoot, L. C., Panman, J. L., Dopper, E. G., den Heijer, T., Donker Kaat, L., et al. (2017). Cognition and gray and white matter characteristics of presymptomatic C9orf72 repeat expansion. *Neurology* 89, 1256–1264. doi: 10.1212/WNL.0000000000004393

Phukan, J., Elamin, M., Bede, P., Jordan, N., Gallagher, L., Byrne, S., et al. (2012). The syndrome of cognitive impairment in amyotrophic lateral sclerosis: a population-based study. *J. Neurol. Neurosurg. Psychiatry* 83, 102–108. doi: 10.1136/jnnp-2011-300188

Quadri, M., Cossu, G., Saddi, V., Simons, E. J., Murgia, D., Melis, M., et al. (2011). Broadening the phenotype of TARDBP mutations: the TARDBP Ala382Thr mutation and Parkinson's disease in Sardinia. *Neurogenetics* 12, 203–209. doi: 10.1007/s10048-011-0288-3

Raaphorst, J., Beeldman, E., De Visser, M., De Haan, R. J., and Schmand, B. (2012a). A systematic review of behavioural changes in motor neuron disease. *Amyotroph. Lateral Scler.* 13, 493–501. doi: 10.3109/17482968.2012.656652

Raaphorst, J., Beeldman, E., Schmand, B., Berkhout, J., Linssen, W. H., van den Berg, L. H., et al. (2012b). The ALSFTD-Q: a new screening tool for behavioral disturbances in ALS. *Neurology* 79, 1377–1383.

Rabkin, J. G., Goetz, R., Factor-Litvak, P., Hupf, J., McElhiney, M., Singleton, J., et al. (2015). Depression and wish to die in a multicenter cohort of ALS patients. *Amyotroph. Lateral Scler. Frontotemporal. Degener.* 16, 265–273. doi: 10.3109/21678421.2014.980428

Radewicz, K., Garey, L. J., Gentleman, S. M., and Reynolds, R. (2000). Increase in HLA-DR immunoreactive microglia in frontal and temporal cortex of chronic schizophrenics. *J. Neuropathol. Exp. Neurol.* 59, 137–150. doi: 10.1093/jnen/59.2.137

Radhu, N., Garcia Dominguez, L., Farzan, F., Richter, M. A., Semeralul, M. O., Chen, R., et al. (2015). Evidence for inhibitory deficits in the prefrontal cortex in schizophrenia. *Brain* 138, 483–497. doi: 10.1093/brain/awu360

Rains, L. S., Fallica, G., O'Daly, O., Gilleen, J., Giampetro, V., Morley, L., et al. (2012). Exploring psychotic symptoms: a comparison of motor related neuronal activation during and after acute psychosis. *BMC Psychiatry* 12:102. doi: 10.1186/1471-244X-12-102

Rascovsky, K., Hodges, J. R., Knopman, D., Mendez, M. F., Kramer, J. H., Neuhaus, J., et al. (2011). Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain* 134, 2456–2477. doi: 10.1093/brain/awr179

Renton, A. E., Chiò, A., and Traynor, B. J. (2014). State of play in amyotrophic lateral sclerosis genetics. *Nat. Neurosci.* 17, 17–23. doi: 10.1038/nn.3584

Renton, A. E., Majounie, E., Waite, A., Simón-Sánchez, J., Rollinson, S., Gibbs, J. R., et al. (2011). A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 72, 257–268. doi: 10.1016/j.neuron.2011.09.010

Roos, E., Mariosa, D., Ingre, C., Lundholm, C., Wirdefeldt, K., Roos, P. M., et al. (2016). Depression in amyotrophic lateral sclerosis. *Neurology* 86, 2271–2277. doi: 10.1212/WNL.0000000000002671

Ryan, M., Heverin, M., Doherty, M. A., Davis, N., Corr, E. M., Vajda, A., et al. (2018). Determining the incidence of familiality in ALS: a study of temporal trends in Ireland from 1994 to 2016. *Neurol. Genet.* 4:e239. doi: 10.1212/NXG.0000000000000239

Sabatelli, M., Madia, F., Conte, A., Luigetti, M., Zollino, M., Mancuso, I., et al. (2008). Natural history of young-adult amyotrophic lateral sclerosis. *Neurology* 71, 876–881. doi: 10.1212/01.wnl.0000312378.94737.45

Saxon, J. A., Harris, J. M., Thompson, J. C., Jones, M., Richardson, A. M. T., Langheinrich, T., et al. (2017). Semantic dementia, progressive non-fluent aphasia and their association with amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* 88, 711–712. doi: 10.1136/jnnp-2016-314912

Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., et al. (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. *J. Neurosci.* 27, 2349–2356. doi: 10.1523/JNEUROSCI.5587-06.2007

Shibata, N., Asayama, K., Hirano, A., and Kobayashi, M. (1996). Immunohistochemical study on superoxide dismutases in spinal cords from autopsied patients with amyotrophic lateral sclerosis. *Dev. Neurosci.* 18, 492–498. doi: 10.1159/000111445

Shoesmith, C. L., Findlater, K., Rowe, A., and Strong, M. J. (2007). Prognosis of amyotrophic lateral sclerosis with respiratory onset. *J. Neurol. Neurosurg. Psychiatry* 78, 629–631. doi: 10.1136/jnnp.2006.103564

Snowden, J. S., Rollinson, S., Thompson, J. C., Harris, J. M., Stopford, C. L., Richardson, A. M., et al. (2012). Distinct clinical and pathological characteristics of frontotemporal dementia associated with C9ORF72 mutations. *Brain* 135, 693–708. doi: 10.1093/brain/awr355

Sommerlad, A., Lee, J., Warren, J., and Price, G. (2014). Neurodegenerative disorder masquerading as psychosis in a forensic psychiatry setting. *BMJ Case Rep.* 2014:bcr2013203458. doi: 10.1136/bcr-2013-203458

Strong, M. J., Abrahams, S., Goldstein, L. H., Woolley, S., Mclaughlin, P., Snowden, J., et al. (2017). Amyotrophic lateral sclerosis - frontotemporal spectrum disorder (ALS-FTSD): revised diagnostic criteria. *Amyotroph. Lateral Scler. Frontotemporal. Degener.* 18, 153–174. doi: 10.1080/21678421.2016.1267768

Synofzik, M., Biskup, S., Leyhe, T., Reimold, M., Fallgatter, A. J., and Metzger, F. (2012). Suicide attempt as the presenting symptom of c9orf72 dementia. *Am. J. Psychiatry* 169, 1211–1213. doi: 10.1176/appi.ajp.2012.12060733

Talbot, P. R., Goulding, P. J., Lloyd, J. J., Snowden, J. S., Neary, D., and Testa, H. J. (1995). Inter-relation between "classic" motor neuron disease and frontotemporal dementia: neuropsychological and single photon emission computed tomography study. *J. Neurol. Neurosurg. Psychiatry* 58, 541–547. doi: 10.1136/jnnp.58.5.541

Talman, P., Duong, T., Vucic, S., Mathers, S., Venkatesh, S., Henderson, R., et al. (2016). Identification and outcomes of clinical phenotypes in amyotrophic lateral sclerosis/motor neuron disease: Australian National Motor Neuron Disease observational cohort. *BMJ Open* 6:e012054. doi: 10.1136/bmjopen-2016-012054

Tan, R. H., Shepherd, C. E., Kril, J. J., McCann, H., McGeachie, A., McGinley, C., et al. (2013). Classification of FTLD-TDP cases into pathological subtypes using antibodies against phosphorylated and non-phosphorylated TDP43. *Acta Neuropathol. Commun.* 1:33. doi: 10.1186/2051-5960-1-33

Thakore, N. J., and Pioro, E. P. (2016). Depression in ALS in a large self-reporting cohort. *Neurology* 86, 1031–1038. doi: 10.1212/WNL.0000000000002465

Trojsi, F., Di Nardo, F., Santangelo, G., Siciliano, M., Femiano, C., Passaniti, C., et al. (2017). Resting state fMRI correlates of Theory of Mind impairment in amyotrophic lateral sclerosis. *Cortex* 97, 1–16. doi: 10.1016/j.cortex.2017.09.016

Turner, M. R., Goldacre, R., Talbot, K., and Goldacre, M. J. (2016). Psychiatric disorders prior to amyotrophic lateral sclerosis. *Ann. Neurol.* 80, 935–938. doi: 10.1002/ana.24801

Van Blitterswijk, M., DeJesus-Hernandez, M., Niemantsverdriet, E., Murray, M. E., Heckman, M. G., Diehl, N. N., et al. (2013). Association between repeat sizes

and clinical and pathological characteristics in carriers of C9ORF72 repeat expansions (Xpansize-72): a cross-sectional cohort study. *Lancet Neurol.* 12, 978–988. doi: 10.1016/S1474-4422(13)70210-2

Van Blitterswijk, M., van Es, M. A., Hennekam, E. A., Dooijes, D., van Rheenen, W., Medic, J., et al. (2012). Evidence for an oligogenic basis of amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 21, 3776–3784. doi: 10.1093/hmg/dds199

van der Hulst, E. J., Bak, T. H., and Abrahams, S. (2015). Impaired affective and cognitive theory of mind and behavioural change in amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* 86, 1208–1215. doi: 10.1136/jnnp-2014-309290

van der Zee, J., Gijselinck, I., Dillen, L., Van Langenhove, T., Theuns, J., Engelborghs, S., et al. (2013). A pan-European study of the C9orf72 repeat associated with FTLD: geographic prevalence, genomic instability, and intermediate repeats. *Hum. Mutat.* 34, 363–373. doi: 10.1002/humu.22244

van Es, M. A., Hardiman, O., Chio, A., Al-Chalabi, A., Pasterkamp, R. J., Veldink, J. H., et al. (2017). Amyotrophic lateral sclerosis. *Lancet* 390, 2084–2098. doi: 10.1016/S0140-6736(17)31287-4

Van Mossevelde, S., van der Zee, J., Gijselinck, I., Engelborghs, S., Sieben, A., Van Langenhove, T., et al. (2016). Clinical features of TBK1 carriers compared with C9orf72, GRN and non-mutation carriers in a Belgian cohort. *Brain* 139, 452–467. doi: 10.1093/brain/awv358

Van Mossevelde, S., van der Zee, J., Gijselinck, I., Sleegers, K., De Bleecker, J., Sieben, A., et al. (2017). Clinical evidence of disease anticipation in families segregating a C9orf72 repeat expansion. *JAMA Neurol.* 74, 445–452. doi: 10.1001/jamaneurol.2016.4847

Völlm, B. A., Taylor, A. N., Richardson, P., Corcoran, R., Stirling, J., McKie, S., et al. (2006). Neuronal correlates of theory of mind and empathy: a functional magnetic resonance imaging study in a nonverbal task. *Neuroimage* 29, 90–98. doi: 10.1016/j.neuroimage.2005.07.022

Walhout, R., Schmidt, R., Westeneng, H. J., Verstraete, E., Seelen, M., and van Rheenen, W. (2015). Brain morphologic changes in asymptomatic C9orf72 repeat expansion carriers. *Neurology* 85, 1780–1788. doi: 10.1212/WNL.0000000000002135

Walhout, R., Verstraete, E., Van Den Heuvel, M. P., Veldink, J. H., and Van Den Berg, L. H. (2017). Patterns of symptom development in patients with motor neuron disease. *Amyotroph. Lateral Scler. Frontotemporal. Degener.* 19, 21–28. doi: 10.1080/21678421.2017.1386688

Watson, A., Pribadi, M., Chowdari, K., Clifton, S., Wood, J., Miller, B. L., et al. (2016). C9orf72 repeat expansions that cause frontotemporal dementia are detectable among patients with psychosis. *Psychiatry Res.* 235, 200–202. doi: 10.1016/j.psychres.2015.12.007

Wei, Q., Chen, X., Cao, B., Ou, R., Zhao, B., Wu, Y., et al. (2016). Associations between neuropsychiatric symptoms and cognition in Chinese patients with amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Frontotemporal. Degener.* 17, 358–365. doi: 10.3109/21678421.2016.1154574

Weihl, C. C. (2011). Valosin containing protein associated fronto-temporal lobar degeneration: clinical presentation, pathologic features and pathogenesis. *Curr. Alzheimer Res.* 8, 252–260. doi: 10.2174/156720511795563773

Wijesekera, L. C., Mathers, S., Talman, P., Galtrey, C., Parkinson, M. H., Ganesalingam, J., et al. (2009). Natural history and clinical features of the flail arm and flail leg ALS variants. *Neurology* 12, 1087–1094. doi: 10.1212/01.wnl.0000345041.83406.a2

Wisniewski, H. M., Constantinidis, J., Wegiel, J., Bobinski, M., and Tarnawski, M. (1994). Neurofibrillary pathology in brains of elderly schizophrenics treated with neuroleptics. *Alzheimer Dis. Assoc. Disord.* 8, 211–227. doi: 10.1097/00002093-199408040-00001

Witgert, M., Salamone, A. R., Strutt, A. M., Jawaid, A., Massman, P. J., Bradshaw, M., et al. (2010). Frontal-lobe mediated behavioral dysfunction in amyotrophic lateral sclerosis. *Eur. J. Neurol.* 17, 103–110. doi: 10.1111/j.1468-1331.2009.02801.x

Xi, Z., Yunusova, Y., van Blitterswijk, M., Dib, S., Ghani, M., Moreno, D., et al. (2014). Identical twins with the C9orf72 repeat expansion are discordant for ALS. *Neurology* 83, 1476–1478. doi: 10.1212/WNL.0000000000000886

Yan, J., Deng, H. X., Siddique, N., Fecto, F., Chen, W., Yang, Y., et al. (2010). Frameshift and novel mutations in FUS in familial amyotrophic lateral sclerosis and ALS/dementia. *Neurology* 9, 807–814. doi: 10.1212/WNL.0b013e3181f07e0c

Zhang, F., Wang, G., Shugart, Y. Y., Xu, Y., Liu, C., Wang, L., et al. (2014). Association analysis of a functional variant in ATXN2 with schizophrenia. *Neurosci. Lett.* 562, 24–27. doi: 10.1016/j.neulet.2013.12.001

Zhou, J., and Seeley, W. W. (2014). Network dysfunction in Alzheimer's disease and frontotemporal dementia: implications for psychiatry. *Biol. Psychiatry* 7, 565–573. doi: 10.1016/j.biopsych.2014.01.020

Zou, Z. Y., Zhou, Z. R., Che, C. H., Liu, C. Y., He, R. L., and Huang, H. P. (2017). Genetic epidemiology of amyotrophic lateral sclerosis: a systematic review and meta-analysis. *J. Neurol. Neurosurg. Psychiatry* 7, 540–549. doi: 10.1136/jnnp-2016-315018

Zu, T., Liu, Y., Bañez-Coronel, M., Reid, T., Pletnikova, O., Lewis, J., et al. (2013). RAN proteins and RNA foci from antisense transcripts in C9ORF72 ALS and frontotemporal dementia. *Proc. Natl. Acad. Sci. U.S.A.* 110, E4968–E4977. doi: 10.1073/pnas.1315438110

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.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 05 April 2019
doi: 10.3389/fnins.2019.00302

Notch Signalling in the Hippocampus of Patients With Motor Neuron Disease

Ulises Gómez-Pinedo[1], Lucía Galán[2], Jordi A. Matías-Guiu[2], Vanesa Pytel[1,2], Teresa Moreno[2], Antonio Guerrero-Sola[2] and Jorge Matías-Guiu[1,2]*

[1] Laboratory of Neurobiology, Institute of Neurosciences, IdISSC, Hospital Clínico San Carlos, Universidad Complutense de Madrid, Madrid, Spain, [2] Department of Neurology, Institute of Neurosciences, IdISSC, Hospital Clínico San Carlos, Universidad Complutense de Madrid, Madrid, Spain

Introduction: The Notch signalling pathway regulates neuronal survival. It has some similarities with the APP signalling pathway, and competes with the latter for α- and γ-secretase proteolytic complexes. The objective of this study was to study the Notch signalling pathway in the hippocampi of patients with motor neuron disease.

Methods: We studied biological material from the autopsies of 12 patients with motor neuron disease and 4 controls. We analysed the molecular markers of the Notch and APP signalling pathways, TDP43, tau, and markers of neurogenesis.

Results and Conclusion: Low NICD expression suggests Notch signalling pathway inactivation in neurons. Inactivation of the pathway despite increased Notch1 expression is associated with a lack of α-secretase expression. We observed increased β-secretase expression associated with activation of the amyloid cascade of APP, leading to increases in amyloid-β and AICD peptides and decreased levels of Fe65. Inactivation of the Notch signalling pathway is an important factor in decreased neurogenic response in the hippocampi of patients with amyotrophic lateral sclerosis.

Keywords: ALS, Notch, NICD, APP, ADAM10, ADAM17, BACE1, hippocampal neurogenesis

OPEN ACCESS

Edited by:
Foteini Christidi, National and Kapodistrian University of Athens, Greece

Reviewed by:
Chiara F. Valori, German Center for Neurodegenerative Diseases (DZNE), Germany
Patrizia Longone, Fondazione Santa Lucia (IRCCS), Italy

****Correspondence:***
Ulises Gómez-Pinedo
u.gomez.pinedo@gmail.com

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: *10 December 2018*
Accepted: *15 March 2019*
Published: *05 April 2019*

Citation:
Gómez-Pinedo U, Galán L, Matías-Guiu JA, Pytel V, Moreno T, Guerrero-Sola A and Matías-Guiu J (2019) Notch Signalling in the Hippocampus of Patients With Motor Neuron Disease. Front. Neurosci. 13:302. doi: 10.3389/fnins.2019.00302

INTRODUCTION

The Notch signalling pathway regulates cell migration and growth, synaptic plasticity, and neuronal survival (Ables et al., 2011). Notch proteins are highly conserved transmembrane receptors with such pleiotropic functions as neuronal development and organ homeostasis, and are activated by ligand binding. The ligand-receptor association triggers sequential proteolytic processes via α- and γ-secretases. Proteolysis generates a Notch intracellular domain (NICD) that may translocate to the nucleus. There is an evident parallel between the Notch and amyloid precursor protein (APP) signalling pathways, which compete for proteolytic complexes; an association between both pathways has therefore been suggested.

Notch1, the most extensively studied Notch receptor, is expressed in the cortex and hippocampus, and may be involved in neurodegeneration (Woo et al., 2009). Notch1-deficient mice display memory impairment (Costa et al., 2003). It has been suggested that Notch1 participates in olfactory function (Brai et al., 2014), which is impaired in patients with Alzheimer disease (AD) (Berezovska et al., 1998; Moehlmann et al., 2002; Brai et al., 2016) and in experimental models of

familial AD secondary to presenilin mutations (Okochi et al., 2002). Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting motor neurons in the brain, brainstem, and spinal cord (Rowland and Shneider, 2001). However, as recent anatomical pathology studies of ALS have shown that degeneration affects not only motor areas but also such other structures as the hippocampus (Coan and Mitchell, 2015), we aimed to determine whether the Notch pathway is active in the hippocampi of patients with ALS.

MATERIALS AND METHODS

We studied biological samples from the autopsies of 12 patients with ALS or ALS with frontotemporal dementia (ALS-FTD) who died between 2006 and 2017 and met diagnostic criteria for ALS (Ludolph et al., 2015). Ten patients died due to respiratory insufficiency during the terminal stage of ALS, one patient due to cardiac arrest attributed to bulbar involvement, and the remaining patient due to a concomitant cerebral haemorrhage. We also studied the brains of 4 controls, who died during hospitalisation due to non-neurological diseases and had no history of neurodegenerative disease. Only 10 brains were used for the study of neurogenesis. Patients or their families expressed in writing their consent for the brain to be donated for research. Autopsies were performed within 2–6 h after death, in accordance with our centre's protocol and Spanish national regulations. The procedure, which we described in a previous article (Gómez-Pinedo et al., 2016), is outlined in **Supplementary Material 1**. Briefly, five slides were used per patient and IHC, analysing in each slides 32 fields (per hippocampal zone: CA1, CA2, CA3, and dentate gyrus). ImageJ version 1.46r was used when the unit of measurement was the amount of labelling per field (optical density [OD]). Inclusions were calculated as the number of stained inclusions observed in neurons divided by the mean number of neurons per field (percentage of cells per mm^2). Statistical analysis was performed using the SPSS statistics software, version 20.0. GraphPad Prism version 5.0 was used to plot graphs and to calculate Pearson correlation coefficients between the parameters studied. Data are expressed as mean ± SD. Due to the small size of our sample, means were compared using the non-parametric Mann–Whitney U test. Statistical significance was set at $p < 0.05$.

RESULTS

Notch1 Expression

Notch1 expression was observed at higher levels in patients than in controls (**Figure 1**). Mean labelling per field was 1.315 ± 0.448 OD in patients and 0.725 ± 0.061 OD in controls ($p < 0.02$). **Figure 1** also shows differences between patients: one patient with ALS-FTD showed lower Notch1 expression than controls, 2 patients with ALS showed similar expression to that observed in controls, and the remaining patients displayed clearly greater Notch1 expression. Patients and controls showed different labelling patterns. In controls, labelling was mainly observed in the cytoplasm and dendrites in CA1, especially in the polymorphic layer of the subgranular zone (SGZ). In patients, however, labelling was heterogeneous in all hippocampal areas, being more evident in granular neurons and remarkable in CA1, CA3, the polymorphic layer of CA4, and the SGZ; Notch1 expression was observed in neurons and to a lesser extent in astrocytes and neuronal processes, following a slight synaptic pattern in CA1 and in capillary walls (**Figure 1**).

NICD Expression

NICD expression was variable in patients with ALS, and was observed at lower levels than in controls (**Figure 2**). Mean labelling per field was 0.228 ± 0.070 OD in patients and 1.101 ± 0.101 OD in controls ($p < 0.0001$). NICD expression is inversely related to Notch1 expression and shows homogeneous values, since it behaves similarly in all patients with ALS, including those with ALS-FTD; NICD labelling in patients was lower than that observed in controls. In patients with ALS, NICD labelling was mainly observed in cell nuclei, especially in granular neurons, neurons near the SGZ, and in the polymorphic layer. The SGZ contained astrocytic cells coexpressing GFAP and NICD, especially in patients with greater numbers of extracellular amyloid plaques (**Supplementary Material 2**). Patients with no plaques or isolated plaques showed no NICD labelling, except in isolated astrocytes near the SGZ (**Supplementary Material 2**). In controls, NICD was mainly expressed in neurons of the SGZ and colocalised with GFAP. Labelling was also observed in microglia (Iba1-positive cells) (**Figure 2**).

ADAM10, ADAM17, and BACE1 Expression

ADAM10 expression was 0.199 ± 0.060 OD in patients and 0.462 ± 0.089 OD in controls ($p = 0.0015$); it was lower in all patients with ALS (**Figure 3**). ADAM17 expression was 0.230 ± 0.057 OD in patients and 0.542 ± 0.084 OD in controls ($p = 0.0015$); again, expression was lower in all patients than in controls (**Figure 3**). In patients with ALS, ADAM10 and ADAM17 expression was similar in terms of location, although ADAM10 expression was slightly more marked in the granular layer and less marked in the SGZ. ADAM17 expression was more diffuse, whereas ADAM10 expression was more defined and homogeneous (**Supplementary Material 4**). In controls, expression of both ADAM10 and ADAM17 was more marked in the SGZ (**Supplementary Material 3**), colocalised with GFAP, and was also observed in neurons. BACE1 expression was evaluated with OD and by determining the number of cells labelled by the antibody. BACE1 expression was 0.475 ± 0.114 OD in patients with ALS and 0.243 ± 0.048 OD in controls ($p < 0.0001$). Higher levels were observed in the majority of patients with ALS, however, three patients showed similar levels to those of controls (**Figure 4**). A total of 13.73 ± 2.97 cells per field in patients with ALS and 8.00 ± 2.588 in controls were labelled by anti-BACE1 antibody ($p = 0.0047$). Similarly, the antibody labelled more cells in all patients with ALS, with the exception of the 3 patients showing similar BACE1 expression to those of controls as measured by OD (**Figure 4**).

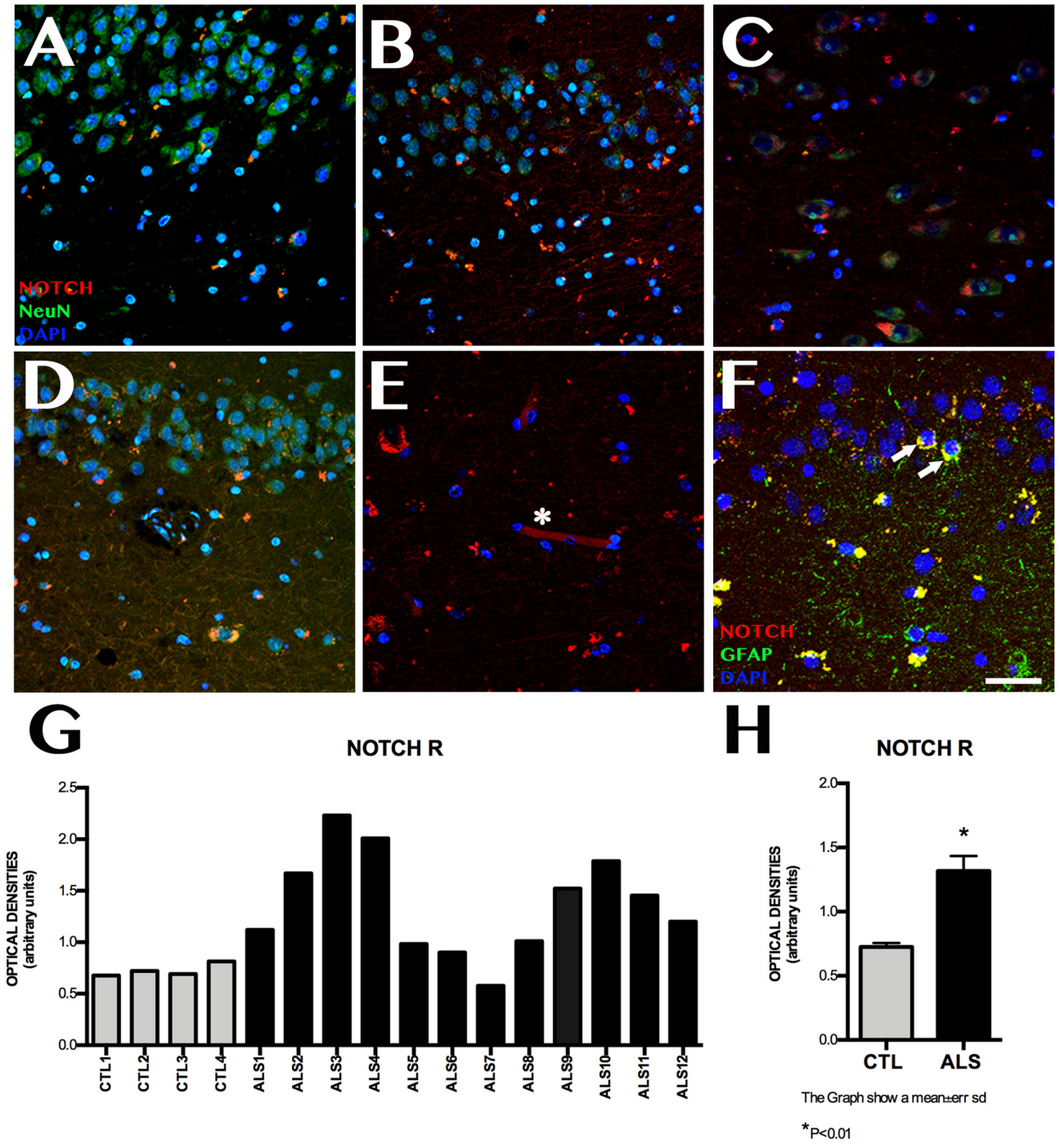

FIGURE 1 | Notch1 expression. Confocal microscopy images from controls and patients with ALS, showing immunohistochemical expression of Notch1. Labelling is heterogeneous in several areas of the hippocampus **(A–D)**, and is more marked in CA1, in the cytoplasm of neurons **(A)**, and in the granular neurons of the dentate gyrus (**E**, asterisks). The subgranular zone shows labelling near capillaries (**E**, asterisk) and faint labelling in GFAP-positive cells from the subgranular zone (**F**, arrows). The graph in G shows quantitative data from the hippocampi (including CA1, CA2, CA3, CA4, and dentate gyrus) of all patients and controls. Values are heterogeneous among patients but generally higher in patients than in controls. The graph in H shows the mean values for patients and controls, which are significantly higher in patients ($p < 0.05$). Images correspond to the following areas: A, CA1 control; B, CA2 ALS; C, CA3 ALS; D, CA1 and subgranular zone ALS; E, CA3; and F, subgranular zone ALS. Scale bar: 50 μm. Graphs express data as means **(G)** and standard deviation **(H)**.

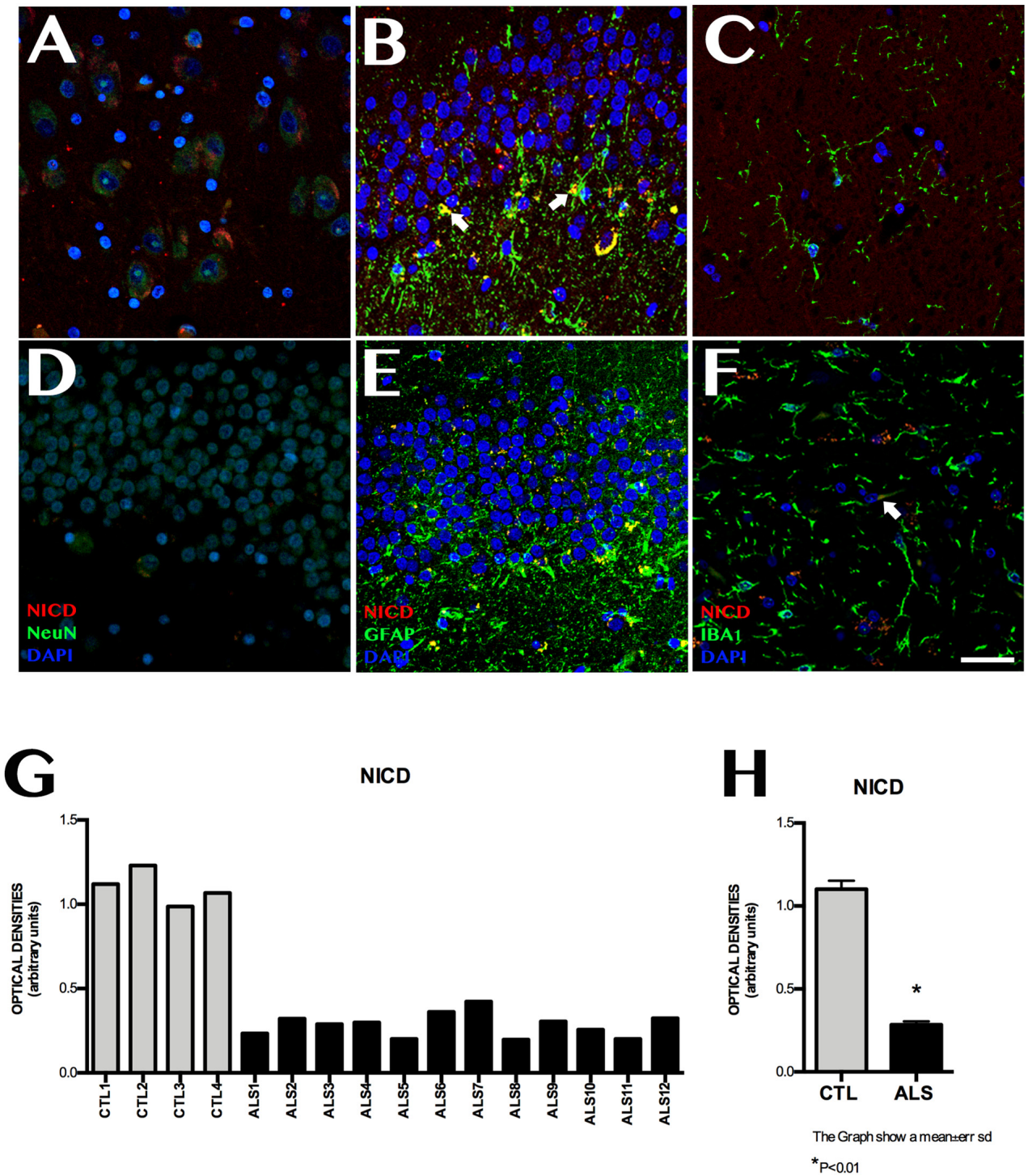

FIGURE 2 | NICD expression in the hippocampi of patients with ALS and controls. Immunohistochemical images of NICD expression obtained by confocal microscopy, showing clear differences in labelling patterns between patients and controls. The most significant differences were observed in the granular layer and the subgranular zone. Controls showed more marked labelling than patients with ALS **(A–C)**, especially in the granular zone, Controls displayed cells (astrocytes) coexpressing GFAP and NICD in the subgranular zone (**B** arrows), whereas patients showed faint labelling in the granular layer **(D,E)**, in addition to Iba1-positive cells (**F**, arrows). The graph in **F** shows quantitative data from the hippocampi (including CA1, CA2, CA3, CA4, and dentate gyrus) of all patients and controls. Values are higher in controls than in patients. The graph in **G** shows significant differences in mean values between patients and controls, with controls showing higher expression ($p < 0.05$). Images **A**, CA3; **B**, **D**, and **E** show the granular layer and subgranular zone, whereas image **C** and **F** shows the CA2. Scale bar: 50 μm. Graphs express data as means **(G)** and standard deviation **(H)**.

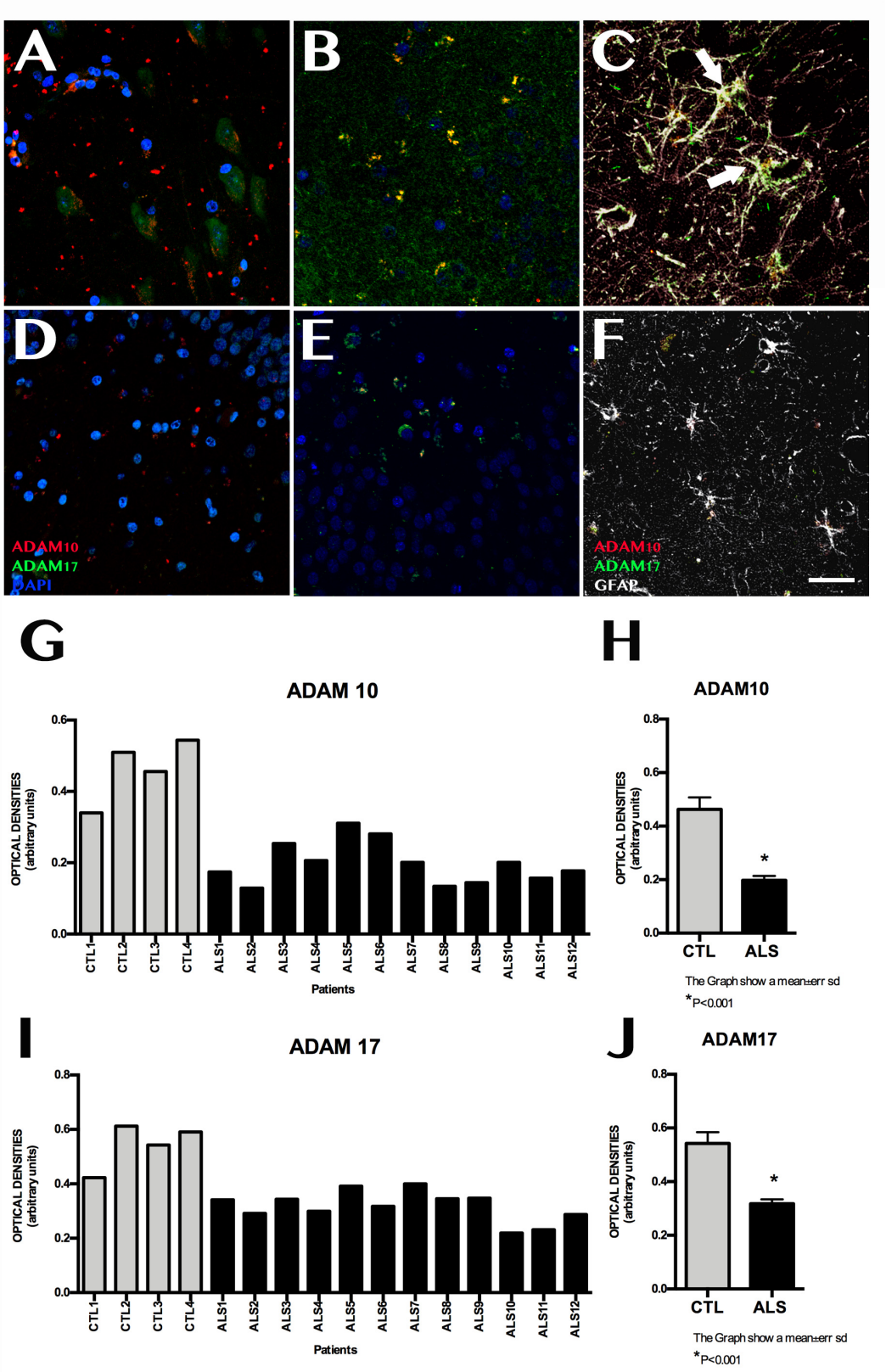

FIGURE 3 | Continued

FIGURE 3 | ADAM10 and ADAM17 expression in the hippocampus. Images of the hippocampus obtained using confocal microscopy after immunohistochemistry against ADAM10 and ADAM17 show lower marker expression in controls **(A–C)** than in patients **(D–F)**. Labelling was mainly observed in the granular layer of the dentate gyrus; the subgranular zone showed weak, diffuse labelling **(D–F)**. Labelling could not be observed in the remaining areas of the hippocampus in some patients. Controls displayed denser labelling, which was observed in the granular layer and subgranular zone, where astrocytes coexpressed ADAM10 and ADAM17 (C, arrows). Quantitative data for ADAM10 and ADAM17 are shown in graphs **(G)** and **(H)**, respectively. Graphs show similar ADAM10 and ADAM17 expression in both patients and controls; this is further confirmed by graphs **(I)** and **(J)**, which show statistically significant differences ($p < 0.05$). Images **A** and **B** correspond to representative areas of the dentate gyrus and the subgranular zone in controls, whereas images **C** and **D** display the same areas in patients. Scale bar: 25 μm. Graphs **E** and **F** express data as means, and graphs **G** and **H** as means ± standard deviation.

APP, Amyloid-β, AICD, and Fe65 Expression

APP expression was 5689 ± 2036 OD in patients and 2544 ± 1209 OD in controls ($p = 0.0076$). APP intracellular domain (AICD) expression was elevated in 5 patients, whereas the remaining 5 showed similar levels to those observed in controls (**Supplementary Material 4**). Amyloid-β (Aβ) expression was elevated in all patients with ALS (38 249 ± 24 449 OD vs. 6003 ± 2231 OD in controls; $p = 0.0127$). AICD expression was 14.10 ± 3.07 OD in patients and 9.2 ± 3.49 OD in controls ($p = 0.0153$). In patients displaying Aβ expression, AICD was observed in astrocytes and microglia surrounding amyloid plaques; it was also expressed in dendrites. Elevated AICD expression was observed in all patients with ALS, with the exception of 3 who showed similar levels to those observed in controls (**Supplementary Material 4**). Fe65 expression was 13.90 ± 3.24 OD in patients and 22.60 ± 4.03 OD in controls ($p = 0.0006$). Decreased Fe65 expression was observed in all patients, with the exception of 3 who showed similar expression to that of controls; these were not the same patients showing increased AICD expression (**Supplementary Material 4**). Ten patients displayed amyloid plaques with varying degrees of labelling. Plaques were frequently diffuse and isolated; only 4 of these patients showed prominent plaques, mainly located in CA1, CA3, and cortical regions.

Expression of Markers of Neurogenesis

Shows data for 10 of the 12 patients with ALS (the remaining 2 patients were not included in this part of the study for technical reasons). Patients showed a marked decrease in proliferative and neurogenic activity in the hippocampus. Proliferation in the SGZ was analysed using the proteins PCNA and Ki67, expressed during cell division. The number of cells labelled with these 2 markers was significantly lower in patients than in controls: 0.47 ± 0.72 cells/μm^2 vs. 2 ± 1.41 cells/μm^2 for PCNA labelling, and 0.29 ± 0.72 cells/μm^2 vs. 1.65 ± 0.96 cells/μm^2 for Ki67 labelling ($p < 0.01$). The study of such immunohistochemical markers of human pluripotent cells as GFAPδ in the dentate gyrus identified fewer labelled cells in patients than in controls: 1.18 ± 0.83 cells/μm^2 vs. 5.7 ± 1.81 cells/μm^2 ($p < 0.01$). The markers DCX, TuJ1, and PSA-NCAM were used to study neuroblast expression in the dentate gyrus. PSA-NCAM expression was reduced in patients with ALS (0.39 ± 0.43 cells/μm^2 vs. 7.3 ± 4.29 cells/μm^2; $p < 0.01$). The other 2 markers could not be viewed since they only labelled small dendritic projections but no somata.

TDP43 and Tau Expression

Cytoplasmic TDP43 expression was evaluated using staining for phosphorylated TDP43; expression was determined at 55.75 ± 5.97 OD in patients vs. 58.8 ± 5.167 OD in controls; differences were not statistically significant. However, the number of TDP43-positive cytoplasmic inclusions per field did show statistically significant differences between patients and controls (20.30 ± 10.32 vs. 1.504 ± 0.993; $p = 0.0004$). All patients displayed TDP43-positive cytoplasmic inclusions. Quantification of phosphorylated tau (phospho-tau) expression revealed levels of 26 106 ± 20 413 OD in patients and 4824 ± 2896 OD in controls ($p = 0.0395$). Only 6 patients showed increased phospho-tau expression; we observed neurofibrillary tangles mainly in CA3 and CA1, and staining of small fibres in CA1 axonal projections. The remaining patients showed similar levels of phospho-tau expression to that of controls.

Correlations Between Molecular Markers

Supplementary Material 5 show the correlations between the various molecular markers. In the **Supplementary Tables 1, 2** describe the correlations between molecular markers of NOTCH pathway and the correlation with NOTCH and adult neurogenesis.

Clinical Correlations

Patients (7 men and 5 women) were aged between 37 and 87 years at symptom onset; onset was spinal in 5 cases and bulbar in 7. Survival times ranged from 4 months to 14 years. Two patients also had dementia; one case was attributed to FTD due to the associated aphasia. Seven patients underwent a genetic study. Three patients showed a *SOD1* mutation, which was pathogenic in only one case. One patient had a pathogenic mutation of the gene coding for TDP43. No patient had more than 20 repeats in *C9ORF72*. No correlations were found between molecular data and such clinical characteristics as age, sex, form of onset, survival time, or presence of any of the genetic variants detected (**Supplementary Table 3**).

DISCUSSION

Notch1 Is Overexpressed, Whereas NICD Is Underexpressed

Notch1 is generated by a convertase enzyme in the Golgi apparatus via S1 cleavage and subsequently transported to the cell membrane, where it is expressed as a heterodimeric transmembrane protein (Lieber et al., 2002). Notch1

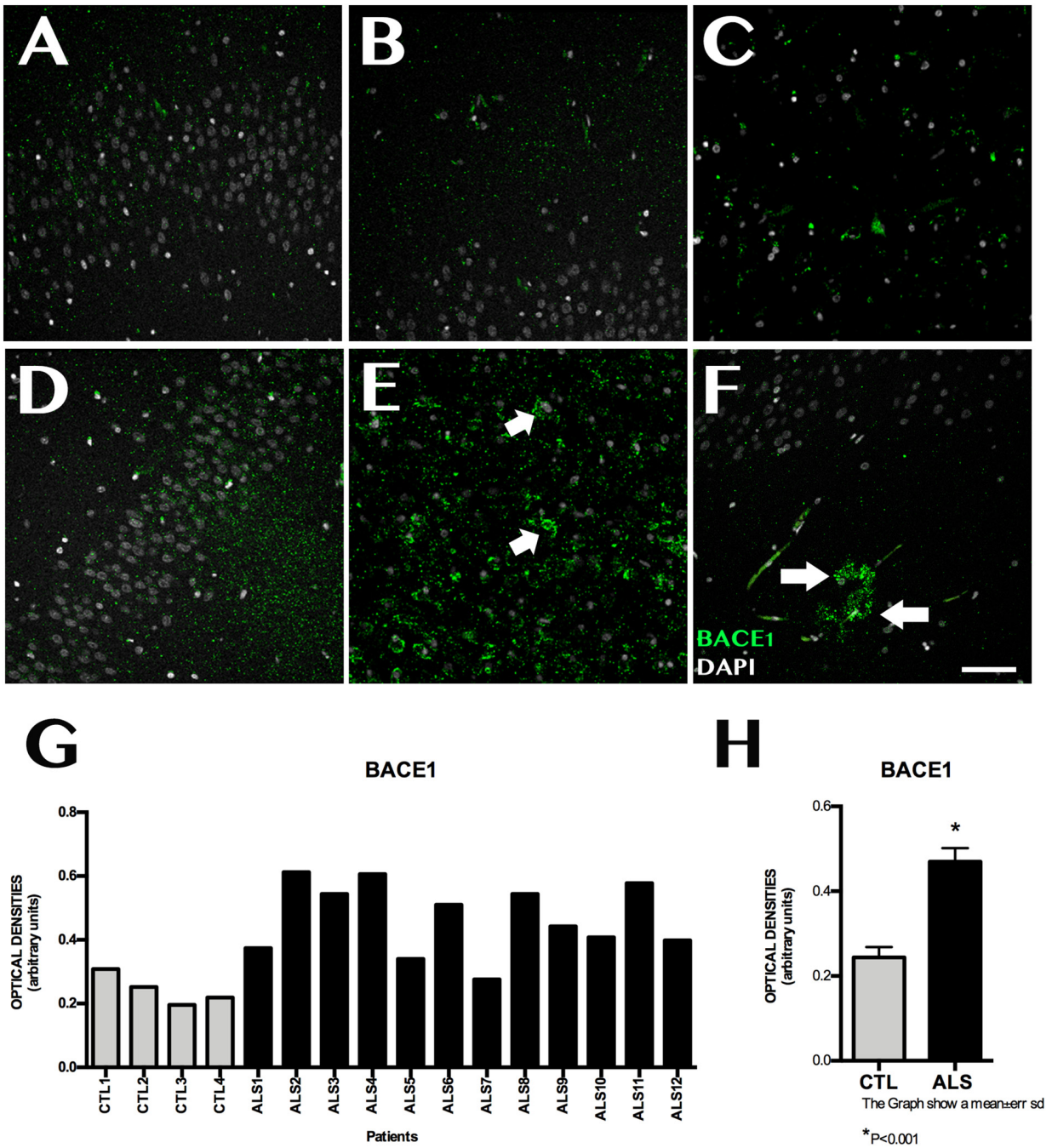

FIGURE 4 | BACE1 expression in the hippocampus. **(A–C,F)** displays the dentate gyrus/subgranular zone and CA1 in controls **(D–F)** shows the increased BACE1 expression in patients, located in the subgranular zone (**E**, arrow) and in areas with amyloid plaques (**F**, arrows). Increased BACE1 expression is observed in nine patients; the remaining three showed similar levels to those observed in controls **(G)**. The graph in **(H)** expresses data as means, and shows significant differences ($p < 0.05$). Image **G** expresses data as means and graph **H** as means ± standard deviation. Scale bar: 50 μm.

overexpression has previously been observed in other neurodegenerative diseases (Nagarsheth et al., 2006; Lathia et al., 2008) activation of the Notch signalling pathway has been found to play a role in ageing and memory (Ables et al., 2011; Alberi et al., 2013). Notch1's role in ALS has previously been observed in several experimental studies, which report conflicting findings. Wang et al. (2015) observed that the Notch signalling pathway is activated *in vitro* models and $SOD1^{G93A}$ mice, and that Notch suppression with a Notch1 signalling inhibitor significantly reduced neuronal apoptosis. Nonneman et al. (2018), in contrast, studied Notch signalling in the spinal cords of $SOD1^{G93A}$ mice and patients with

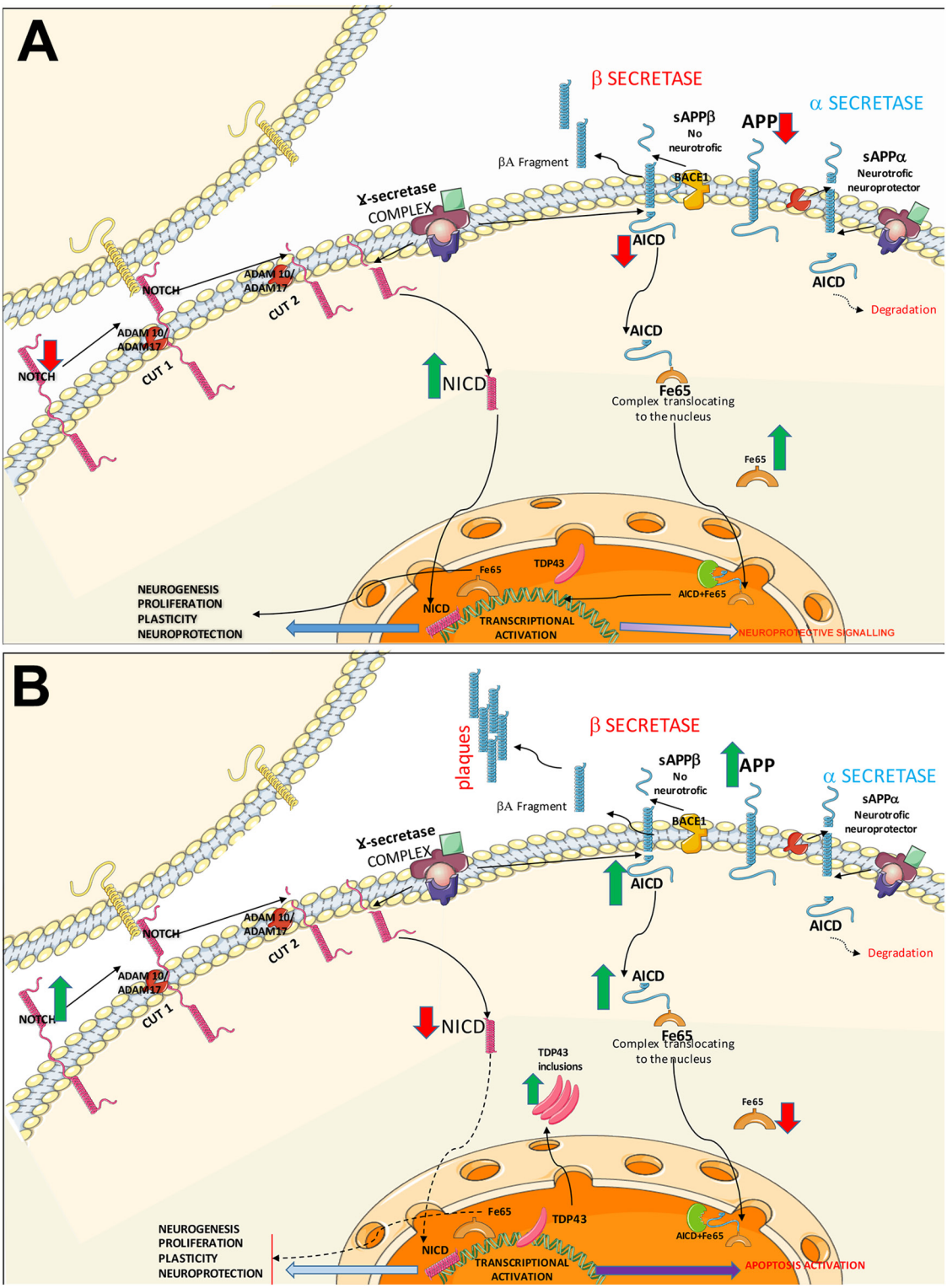

FIGURE 5 | Diagram of the Notch and APP signalling pathways. The diagram shows the possible common mechanisms between both pathways in controls **(A)** and patients with ALS **(B)**. **(A)** Healthy control. In the Notch signalling pathway, Notch1 activation requires direct contact between the membranes of 2 cells. Once the receptor is active, the protein undergoes two consecutive cleavages by α-secretases (ADAM10 and ADAM17), followed by transmembrane cleavage by γ-secretases. NICD is internalised into the nucleus, activating transcription factors and promoting mechanisms of cell plasticity, proliferation, and neurogenesis. The APP signalling pathway may follow two routes. The non-amyloidogenic (neuroprotective) APP pathway: after α-secretase–mediated cleavage, γ-secretases generate

(Continued)

FIGURE 5 | Continued
AICD and decrease APP levels. Cytosolic adaptor protein Fe65 binds to AICD; this complex translocates to the nucleus where it interacts activating signalling to promotes neurite growth and cell plasticity. The second pathway is the amyloidogenic APP pathway, where APP is processed by α-secretase and γ-secretase, forming Aβ. AICD is also generated, which may activate NICD expression via the lysosomal system or interact with Fe65. Both pathways converge in the activation of substrates by α-secretases. **(B)** A possible pathogenic mechanism in ALS is increased Notch1 expression due to poor processing of α-secretases, given the competition between the Notch and APP pathways. This results in poor NICD activation, leading to transcription block and the subsequent decrease in plasticity, proliferation, and neurogenesis. In the APP pathway, the competition for α-secretases (ADAM10, ADAM17) causes β-secretase activation, leading to the formation of Aβ oligomers; Aβ plaque formation in the intracellular space generates AICD, directly affecting Fe65 expression. AICD and Fe65 form a complex that translocates to the nucleus, where it may activate the apoptotic pathway. Increased AICD expression and low Fe65 activity may affect other lysosomal degradation pathways, increasing cytoplasmic TDP43 inclusions.

sporadic ALS, finding increased pathway activation in reactive GFAP-positive astrocytes. Astrocyte-specific inactivation of Jagged-1 in presymptomatic $SOD1^{G93A}$ mice increased the activation of the Notch signalling pathway and accelerated disease progression without affecting disease onset. In a study of *Drosophila*, Yang et al. (2015) observed that dipeptide repeat proteins associated with a repeat expansion in *C9ORF72*, present in patients with FTD and ALS, were accompanied by Notch signalling suppression. We did not observe Notch signalling pathway activation in the hippocampi of our patients: while they displayed increased Notch1 expression, NICD expression was significantly decreased. Our results are consistent with those reported by Ma et al. (2017), who observed decreased NICD signalling in spinal cord motor neurons of SOD mice; this decrease was correlated with disease expression. Disease onset occurred between 90 and 120 days of age, at which time NICD levels progressively decreased in motor neurons. After binding to one of its transmembrane ligands, Notch undergoes sequential cleavage from NICD first by α-secretase and then by γ-secretase; NICD is then internalised into the nucleus (**Figure 5**). Our results suggest that although Notch expression increases, probably in response to increased expression of its ligands (Nonneman et al., 2018). This is consistent with the findings of Ma et al. (2017), who suggest the involvement of some mechanism within the cell that interrupts activation of the pathway. However, our patients showed NICD expression in astrocytes (GFAP-positive cells) and microglia (Iba1-positive cells), suggesting Notch pathway activation in these cells. This observation coincides with the results of Nonneman et al. (2018), who report Notch signalling pathway inactivation in neurons and activation in glial cells. This is consistent with the hypothesis of neuronal loss and gliosis in ALS (Rowland and Shneider, 2001).

APP and Notch1 Expression

In a previous study, we observed amyloid cascade activation in the hippocampi of patients with ALS-FTD, in the form of increased APP and Aβ expression. AICD expression was variable, as the protein was not overexpressed in all patients; we also observed decreased Fe65 expression, suggesting that AICD may have bound to Fe65 and been internalised into the nucleus, as occurs when the APP signalling pathway is activated (Gómez-Pinedo et al., 2016). These results are consistent with our findings from an *in vivo* study of patients with ALS using PET with amyloid tracers (Matías-Guiu et al., 2016). The fact that Notch and APP signalling pathways compete with each other for α- and γ-secretase underscores the need to analyse the link between these pathways. In order to generate AICD, APP undergoes sequential cleavage, first by α- or β-secretase and subsequently by γ-secretase. When cleavage is first mediated by β-secretase, APP generates Aβ peptides, which are oligomerised in the form of aggregates. APP cleavage by γ-secretase generates AICD (**Figure 5**). In this study, we observed increased APP and Aβ expression. Similarly, a rat model of AD showed that soluble $A\beta_{1-42}$ suppresses Notch1 and NICD expression (Zhang et al., 2016). AICD generated by γ-secretase–mediated cleavage is rapidly degraded by the endosomal-lysosomal system. Fe65 stabilises AICD; together, both molecules localise to the nuclear compartment, where they bind the histone acetylase Tip60, forming AFT complexes (Von Rotz et al., 2004). AICD also competes with NICD within the nucleus. A study of human embryonic kidney cells found colocalisation in AFT complexes; NICD can localise to the nucleus together with Fe65 and Tip60 in the absence of AICD (Konietzko et al., 2010).

Fe65 Expression Increases With Notch1 Overexpression

Fe65 binds to AICD; in the nucleus, this complex is involved in regulating the transcription of certain genes, including the gene coding for APP (Cao and Südhof, 2001); *in vitro* studies have shown colocalisation of AICD and TDP43 in the nucleus (Wang et al., 2014). Fe65 is highly expressed in the hippocampus (Kesavapany et al., 2002). Fischer et al. (2005) observed increased Notch1 expression, interaction between APP and Notch1, and NICD binding to Fe65 in the cerebral cortex of adults with Down syndrome, which is associated with enhanced APP production. NICD may colocalise with AICD, Fe65, and Tip60, interrupting the formation of the AFT complex and playing a protective role by inhibiting AFT complex–induced cell death (Kim et al., 2007). Our study shows that decreased Fe65 expression coincides with decreased NICD expression and increased Notch1 expression; this is consistent with the results of a previous study by our research group (Gómez-Pinedo et al., 2016).

Notch Signalling Pathway Inactivation May Explain Decreased Neurogenic Response in the Hippocampus

The hippocampus, one of the classic neurogenic niches of the adult brain, constantly generates neurons throughout life (Palmer et al., 1997; Eriksson et al., 1998; Seri et al., 2004). Granule cells are born in the SGZ of the dentate gyrus; they migrate to the granular layer and integrate into the neural network.

However, these cells are estimated to be less numerous than those born in the subventricular zone. AD (Hollands et al., 2016) and other neurodegenerative diseases (Winner and Winkler, 2015) have been found to be associated with alterations in hippocampal neurogenesis. Our research group has described decreased hippocampal neurogenesis in patients with ALS, which stands in contrast with the increased neurogenesis observed in the subventricular zone in these patients (Galán et al., 2017). The Notch signalling pathway has been linked to hippocampal neurogenesis as the Notch receptor is expressed in neural stem cells (Traiffort and Ferent, 2015). It has been suggested that this pathway may alter the number of neural stem cells by acting on cell survival (Ables et al., 2010), self-renewal (Aguirre et al., 2010), and differentiation (Breunig et al., 2007), and may act as a mechanism of communication between a neural stem cell and its descendants (Semerci et al., 2017). Our results show that expression of markers of proliferation (Ki67), differentiating cells (GFAPδ), and differentiation (PSA-NCAM) is significantly correlated with NICD expression and negatively correlated with Notch1, which suggests that decreased neurogenesis in ALS (Galán et al., 2017) may be associated with Notch signalling pathway inactivation. Interestingly, some drugs that increase Notch signalling have been found to promote hippocampal neurogenesis (Xue et al., 2017).

Expression of α- and β-Secretases

The role of α- and β-secretases comes to the forefront in view of 3 main findings: Notch signalling inactivation despite increased Notch1 expression; decreased hippocampal neurogenesis resulting from Notch signalling inactivation; and APP signalling pathway activation (Gómez-Pinedo et al., 2016). α-Secretase acts on Notch during S2 cleavage in response to ligand binding, which induces a conformational change. The cleaved form that remains bound to the membrane is called NEXT and will be the substrate for γ-secretase–mediated proteolysis (S3 cleavage), whereas the resulting intracellular Notch fragments are short-lived. α-Secretases are membrane-anchored, zinc-dependent members of the A disintegrin and metalloproteinase (ADAM) family. ADAM includes a wide range of proteins with protease and adhesive domains, which play a key role in cell-cell and cell-matrix interactions in various important biological cell processes. The role of α-secretase involves ADAM10 and ADAM17, and to a lesser extent ADAM12 and ADAM9. Little information is available on ADAM molecular expression in ALS. Our study detected decreased ADAM10 and ADAM17 expression, which may explain S2 cleavage inhibition (Groot and Vooijs, 2012). Our results do not allow us to determine the reason for reduced α-secretase expression, although the discovery of some factors involved in ADAM10 regulation permits us to propose several hypotheses. Synapse-associated protein 97 binds to ADAM10, creating a complex that enables its transportation from the endoplasmic reticulum to the cell membrane. Glucagon-like peptide-1 is associated with decreased ADAM10 expression and lower Aβ levels (Saftig and Lichtenthaler, 2015). The role of the TspanC8 subgroup of tetraspanins (including Tspan5, Tspan10, Tspan14, Tspan15, Tspan17, and Tspan33) is especially noteworthy (Dornier et al., 2012; Haining et al., 2012). TspanC8 tetraspanins interact with ADAM10 and regulate the cleavage of ADAM10 substrates. Tspan5, Tspan10, and Tspan14 regulate ADAM10-dependent Notch signalling (Zhou et al., 2014); Tspan15 promotes ADAM10-mediated N-cadherin cleavage; and Tspan14 reduces GPVI cleavage (Noy et al., 2016); changes in tetraspanin expression may alter the action of ADAM10. Another hypothesis suggests competition with other substrates where ADAM10 is also involved, such as the TNFα signalling pathway, which has been observed to be active in ALS (Tortarolo et al., 2017) and cause cell alterations (Olmos and Lladó, 2014); this is probably related to microglial activation, one of the first events to be observed in experimental models of the disease (Beers et al., 2011; Liao et al., 2012; Liu and Wang, 2017; Gómez-Pinedo et al., 2018). The observation of NICD expression in Iba1-positive cells in our patients supports this hypothesis. NICD expression is directly correlated with ADAM10 and ADAM17 expression, whereas Notch1 expression decreases with increased metalloprotease expression; this supports our hypothesis that decreased Notch1 and NICD expression is associated with Notch signalling pathway inactivation. Conversely, we observed increased β-secretase expression (BACE1) (Yan et al., 2001); this was not unexpected given the results of our previous study, which showed amyloidogenic APP pathway activation (Gómez-Pinedo et al., 2016), and other results from experimental studies into the interaction between BACE1 and TDP43 (Herman et al., 2012). It has been suggested that BACE1 may play a role in the Notch signalling pathway since it seems to be involved in maintaining the balance between hippocampal astrogenesis and neurogenesis in mice by regulating the Jag1-Notch pathway (Hu et al., 2013; He et al., 2014). In our study, BACE1 expression was significantly correlated with Notch1 expression and negatively correlated with NICD expression, which supports the hypothesis that the amyloidogenic APP pathway is activated in the hippocampi of patients with ALS, whereas the Notch signalling pathway is not.

Lack of Correlation Between Notch Expression and Cytoplasmic TDP43 and Tau Expression

A previous study by our research group found no correlation between cytoplasmic TDP43 and APP; furthermore, the 2 proteins did not colocalise, although we did find a correlation between TDP43 expression and Aβ expression (Gómez-Pinedo et al., 2016). Zhan et al. (2013) report that TDP-43 upregulates a wide range of Notch target genes, leading to the activation of this cell differentiation pathway *in vivo*. Notch signalling pathway activation has been linked to prion disease (Ishikura et al., 2005), suggesting a common mechanism with ALS, considering that the C-terminal domain of TDP-43 has prion-like characteristics (Zhan et al., 2013). In our study, no correlation was observed between cytoplasmic TDP43 expression and Notch1 and NICD expression in patients; this contradicts the hypothesis that changes in the signalling pathway may be affected by TDP43. Our previous study detected tau overexpression (Gómez-Pinedo et al., 2016); we hypothesised that this finding

may be linked to increased AICD expression resulting from APP pathway activation (Kim et al., 2003; Ghosal et al., 2009). Tau overexpression has also been reported by other researchers (Vintilescu et al., 2016), and the protein's potential role in ALS has recently been reviewed (Moszczynski et al., 2018). Our study found no correlation with Notch, however.

Study Limitations

Our study has a number of limitations, some of which were discussed in our previous article (Gómez-Pinedo et al., 2016). Autopsies were performed 2 to 6 h after death; this time interval is longer than those used in animal studies. Although different tissue samples were used, some of the patients included in this study were included in the previous studies. However, our sample also included new patients, reducing clinical bias: the two previous studies included a large proportion of patients with bulbar-onset ALS, with very short survival times, whereas this study mostly includes patients with spinal-onset ALS, some of whom had long survival times.

CONCLUSION

To our knowledge, this is the first study to analyse the Notch signalling pathway in biological samples taken in tissue samples from the hippocampus from patients with ALS; In a recent paper, Nonneman et al. (2018) found that Notch signalling pathway is activated in the reactive astrocytes in the spinal cord of SOD1hG93A mice, as well as in patients with sporadic ALS and their finding of a upregulation of astrocytic Jagged-1 is in concordance with the our finding of the greater expression of Notch1 in our cases. Given the involvement of the Notch signalling pathway in cell survival, the finding that the pathway is not active in neurons (decreased NICD expression) is consistent with neuronal loss in patients with ALS. Inactivation of this pathway in neurons despite increased Notch1 expression is associated with a lack of α-secretase expression, preventing APP non-amyloidogenic signalling pathway activation; this was described in one of our previous studies. We also observed increased β-secretase expression in association with activation of the amyloid cascade of APP, leading to increased Aβ and AICD expression and decreased Fe65 expression. Inactivation of the Notch signalling pathway is an important factor in neuronal death in ALS and also plays a major role in decreased hippocampal neurogenic response in these patients, although the blockade of expression of Notch1 in oligodendrocyte precursor cells does not influence the evolution of the mutant SOD1hG93A mice (Eykens et al., 2018). Our study contributes to the understanding of molecular changes in cells due to ALS. Lack of α-secretase expression should be analysed in future studies, given its potential therapeutic implications.

ETHICS STATEMENT

All participants or their relatives gave informed consent prior to inclusion in the study (including consent to autopsy). Autopsies were performed according to the procedures established by our hospital's anatomical pathology department. This study was approved by the Clinical Research Ethics Committee of Hospital Clínico San Carlos. Data were managed in accordance with Spanish data protection legislation (Organic Law 15/1999 of 13 December). At the time the study was approved, no consent was necessary for publishing the results.

AUTHOR CONTRIBUTIONS

UG-P and LG contributed manuscript drafting, study concept and design, data acquisition, data analysis and interpretation, statistical analysis. JAM-G contributed manuscript drafting, study concept and design, data analysis and interpretation, statistical analysis. VP performed data analysis and interpretation, statistical analysis. AG-S performed data analysis, critical review of the manuscript. TM data acquisition, critical review of the manuscript. JM-G performed the study concept and design, data analysis and interpretation, study supervision, and manuscript drafting. All authors have read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors wish to thank María Soledad Benito-Martín for her assistance with tissue processing and the Spanish Society of Neurology's translation department for their assistance with the manuscript. The authors also wish to thank Servier Medical Art (https://smart.servier.com) for the use of their material in the preparation of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2019.00302/full#supplementary-material

REFERENCES

Ables, J. L., Breunig, J. J., Eisch, A. J., and Rakic, P. (2011). Not(ch) just development: Notch signalling in the adult brain. *Nat. Rev. Neurosci.* 12, 269–283. doi: 10.1038/nrn3024

Ables, J. L., Decarolis, N. A., Johnson, M. A., Rivera, P. D., Gao, Z., Cooper, D. C., et al. (2010). Notch1 is required for maintenance of the reservoir of adult hippocampal stem cells. *J. Neurosci.* 30, 10484–10492. doi: 10.1523/JNEUROSCI.4721-09.2010

Aguirre, A., Rubio, M. E., and Gallo, V. (2010). Notch and EGFR pathway interaction regulates neural stem cell number and self-renewal. *Nature* 467, 323–327. doi: 10.1038/nature09347

Alberi, L., Hoey, S. E., Brai, E., Scotti, A. L., and Marathe, S. (2013). Notch signaling in the brain: in good and bad times. *Age. Res. Rev.* 12, 801–814. doi: 10.1016/j.arr.2013.03.004

Beers, D. R., Zhao, W., Liao, B., Kano, O., Wang, J., Huang, A., et al. (2011). Neuroinflammation modulates distinct regional and temporal clinical responses in ALS mice. *Brain Behav. Immun.* 25, 1025–1035. doi: 10.1016/j.bbi.2010.12.008

Berezovska, O., Xia, M. Q., and Hyman, B. T. (1998). Notch is expressed in adult brain, is coexpressed with presenilin-1, and is altered in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* 57, 738–745. doi: 10.1097/00005072

Brai, E., Alina Raio, N., and Alberi, L. (2016). Notch1 hallmarks fibrillary depositions in sporadic Alzheimer's disease. *Acta Neuropathol. Commun.* 4:64. doi: 10.1186/s40478-016-0327-2

Brai, E., Marathe, S., Zentilin, L., Giacca, M., Nimpf, J., Kretz, R., et al. (2014). Notch1 activity in the olfactory bulb is odour-dependent and contributes to olfactory behaviour. *Eur. J. Neurosci.* 40, 3436–3449. doi: 10.1111/ejn.12719

Breunig, J. J., Silbereis, J., Vaccarino, F. M., Sestan, N., and Rakic, P. (2007). Notch regulates cell fate and dendrite morphology of newborn neurons in the postnatal dentate gyrus. *Proc. Natl. Acad. Sci. U.S.A.* 104, 20558–20563. doi: 10.1073/pnas.0710156104

Cao, X., and Südhof, T. C. (2001). A transcriptively active complex of APP with Fe65 and histone acetyltransferase Tip60. *Science* 293, 115–120. doi: 10.1126/science.1058783

Coan, G., and Mitchell, C. S. (2015). An assessment of possible neuropathology and clinical relationships in 46 sporadic amyotrophic lateral sclerosis patient autopsies. *Neurodegener. Dis.* 15, 301–312. doi: 10.1159/000433581

Costa, R. M., Honjo, T., and Silva, A. J. (2003). Learning and memory deficits in Notch mutant mice. *Curr. Biol.* 13, 1348–1354. doi: 10.1016/S0960-9822(03)00492-5

Dornier, E., Coumailleau, F., Ottavi, J. F., Moretti, J., Boucheix, C., Mauduit, P., et al. (2012). TspanC8 tetraspanins regulate ADAM10/Kuzbanian trafficking and promote Notch activation in flies and mammals. *J. Cell Biol.* 199, 481–496. doi: 10.1083/jcb.201201133

Eriksson, P. S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A., et al. (1998). Neurogenesis in the adult human hippocampus. *Nat. Med.* 4, 1313–1317. doi: 10.1038/3305

Eykens, C., Nonneman, A., Jensen, C., Iavarone, A., Van Damme, P., Van Den Bosch, L., et al. (2018). Conditional deletion of Id2 or Notch1 in oligodendrocyte progenitor cells does not ameliorate disease outcome in SOD1G93A mice. *Neurobiol. Aging.* 68, 1–4. doi: 10.1016/j.neurobiolaging.2018.03.026

Fischer, D. F., van Dijk, R., Sluijs, J. A., Nair, S. M., Racchi, M., Levelt, C. N., et al. (2005). Activation of the Notch pathway in Down syndrome: cross-talk of Notch and APP. *FASEB J.* 19, 1451–1458. doi: 10.1016/j.neulet.2014.03.075

Galán, L., Gómez-Pinedo, U., Guerrero, A., García-Verdugo, J. M., and Matías-Guiu, J. (2017). Amyotrophic lateral sclerosis modifies progenitor neural proliferation in adult classic neurogenic brain niches. *BMC Neurol.* 17:173. doi: 10.1186/s12883-017-0956-5

Ghosal, K., Vogt, D. L., Liang, M., Shen, Y., Lamb, B. T., and Pimplikar, S. W. (2009). Alzheimer's disease-like pathological features in transgenic mice expressing the APP intracellular domain. *Proc. Natl. Acad. Sci. U.S.A.* 106, 18367–18372. doi: 10.1073/pnas.0907652106

Gómez-Pinedo, U., Galán, L., Yañez, M., Matias-Guiu, J., Valencia, C., Guerrero-Sola, A., et al. (2018). Histological changes in the rat brain and spinal cord following prolonged intracerebroventricular infusion of cerebrospinal fluid from amyotrophic lateral sclerosis patients are similar to those caused by the disease. *Neurologia* 33, 211–223. doi: 10.1016/j.nrl.2016.07.002

Gómez-Pinedo, U., Villar-Quiles, R. N., Galán, L., Matías-Guiu, J. A., Benito-Martin, M. S., Guerrero-Sola, A., et al. (2016). Immununochemical markers of the amyloid cascade in the hippocampus in motor neuron diseases. *Front. Neurol.* 7:195. doi: 10.3389/fneur.2016.00195

Groot, A. J., and Vooijs, M. A. (2012). The role of adams in Notch signaling. *Adv. Exp. Med. Biol.* 727, 15–36. doi: 10.1007/978-1-4614-0899-4_2

Haining, E. J., Yang, J., Bailey, R. L., Khan, K., Collier, R., Tsai, S., et al. (2012). The TspanC8 subgroup of tetraspanins interacts with A disintegrin and metalloprotease 10 (ADAM10) and regulates its maturation and cell surface expression. *J. Biol. Chem.* 287, 39753–39765. doi: 10.1074/jbc.M112.416503

He, W., Hu, J., Xia, Y., and Yan, R. (2014). β-site amyloid precursor protein cleaving enzyme 1 (BACE1) regulates Notch signaling by controlling the cleavage of Jagged 1 (Jag1) and Jagged 2 (Jag2) proteins. *J. Biol. Chem.* 289, 20630–20637. doi: 10.1074/jbc.M114.579862

Herman, A. M., Khandelwal, P. J., Rebeck, G. W., and Moussa, C. E. (2012). Wild type TDP-43 induces neuro-inflammation and alters APP metabolism in lentiviral gene transfer models. *Exp. Neurol.* 235, 297–305. doi: 10.1016/j.expneurol.2012.02.011

Hollands, C., Bartolotti, N., and Lazarov, O. (2016). Alzheimer's disease and hippocampal adult neurogenesis; exploring shared mechanisms. *Front. Neurosci.* 10:178. doi: 10.3389/fnins.2016.00178

Hu, X., He, W., Luo, X., Tsubota, K. E., and Yan, R. (2013). BACE1 regulates hippocampal astrogenesis via the Jagged1-Notch pathway. *Cell Rep.* 4, 40–49. doi: 10.1016/j.celrep.2013.06.005

Ishikura, N., Clever, J. L., Bouzamondo-Bernstein, E., Samayoa, E., Prusiner, S. B., Huang, E. J., et al. (2005). Notch-1 activation and dendritic atrophy in prion disease. *Proc. Natl. Acad. Sci. U.S.A.* 102, 886–891. doi: 10.1073/pnas.0408612101

Kesavapany, S., Banner, S. J., Lau, K. F., Shaw, C. E., Miller, C. C., Cooper, J. D., et al. (2002). Expression of the Fe65 adapter protein in adult and developing mouse brain. *Neuroscience* 115, 951–960. doi: 10.1016/S0306-4522(02)00422-0

Kim, H. S., Kim, E. M., Lee, J. P., Park, C. H., Kim, S., Seo, J. H., et al. (2003). C-terminal fragments of amyloid precursor protein exert neurotoxicity by inducing glycogen synthase kinase-3beta expression. *FASEB J.* 17, 1951–1953. doi: 10.1096/fj.03-0106fje

Kim, S. Y., Kim, M. Y., Mo, J. S., and Park, H. S. (2007). Notch1 intracellular domain suppresses APP intracellular domain-Tip60-Fe65 complex mediated signaling through physical interaction. *Biochim. Biophys. Acta* 1773, 736–746. doi: 10.1016/j.bbamcr.2007.02.001

Konietzko, U., Goodger, Z. V., Meyer, M., Kohli, B. M., Bosset, J., Lahiri, D. K., et al. (2010). Co-localization of the amyloid precursor protein and Notch intracellular domains in nuclear transcription factories. *Neurobiol. Aging* 31, 58–73. doi: 10.1016/j.neurobiolaging.2008.03.001

Lathia, J. D., Mattson, M. P., and Cheng, A. (2008). Notch: from neural development to neurological disorders. *J. Neurochem.* 107, 1471–1481. doi: 10.1111/j.1471-4159.2008.05715.x

Liao, B., Zhao, W., Beers, D. R., Henkel, J. S., and Appel, S. H. (2012). Transformation from a neuroprotective to a neurotoxic microglial phenotype in a mouse model of ALS. *Exp. Neurol.* 237, 147–152. doi: 10.1016/j.expneurol.2012.06.011

Lieber, T., Kidd, S., and Young, M. W. (2002). Kuzbanian-mediated cleavage of *Drosophila* Notch. *Genes Dev.* 16, 209–221. doi: 10.1101/gad.942302

Liu, J., and Wang, F. (2017). Role of neuroinflammation in amyotrophic lateral sclerosis: cellular mechanisms and therapeutic implications. *Front. Immunol.* 8:1005. doi: 10.3389/fimmu.2017.01005

Ludolph, A., Drory, V., Hardiman, O., Nakano, I., Ravits, J., Robberecht, W., et al. (2015). A revision of the El Escorial criteria –. *Amyotroph Lateral Scler Frontotemporal Degener.* 2015, 291–292. doi: 10.3109/21678421.2015.1049183

Ma, X., Drannik, A., Jiang, F., Peterson, R., and Turnbull, J. (2017). Crosstalk between Notch and Sonic hedgehog signaling in a mouse model of amyotrophic lateral sclerosis. *Neuroreport* 8, 141–148. doi: 10.1097/WNR.0000000000000725

Matías-Guiu, J. A., Pytel, V., Cabrera-Martín, M. N., Galan, L., Valles-Salgado, M., Guerrero, A., et al. (2016). Amyloid- and FDG-PET imaging in amyotrophic lateral sclerosis. *Eur. J. Nucl. Med. Mol. Imaging* 43, 2050–2060. doi: 10.1007/s00259-016-3434-1

Moehlmann, T., Winkler, E., Xia, X., Edbauer, D., Murrell, J., Capell, A., et al. (2002). Presenilin-1 mutations of leucine 166 equally affect the generation of the Notch and APP intracellular domains independent of their effect on Abeta 42 production. *Proc. Natl. Acad. Sci. U.S.A.* 99, 8025–8030. doi: 10.1073/pnas.112686799

Moszczynski, A. J., Hintermayer, M. A., and Strong, M. J. (2018). Phosphorylation of threonine 175 tau in the induction of tau pathology in amyotrophic lateral sclerosis-frontotemporal spectrum disorder (ALS-FTSD). A Review. *Front. Neurosci.* 12:259. doi: 10.3389/fnins.2018.00259

Nagarsheth, M. H., Viehman, A., Lippa, S. M., and Lippa, C. F. (2006). Notch-1 immunoexpression is increased in Alzheimer's and Pick's disease. *J. Neurol. Sci.* 244, 111–116. doi: 10.1016/j.jns.2006.01.007

Nonneman, A., Criem, N., Lewandowski, S. A., Nuyts, R., Thal, D. R., Pfrieger, F. W., et al. (2018). Astrocyte-derived Jagged-1 mitigates deleterious

Notch signaling in amyotrophic lateral sclerosis. *Neurobiol. Dis.* 119, 26–40. doi: 10.1016/j.nbd.2018.07.012

Noy, P. J., Yang, J., Reyat, J. S., Matthews, A. L., Charlton, A. E., Furmston, J., et al. (2016). TspanC8 tetraspanins and a disintegrin and metalloprotease 10 (ADAM10) interact via their extracellular regions: evidence for distinct binding mechanisms for different TspanC8 proteins. *J. Biol. Chem.* 291, 3145–3157. doi: 10.1074/jbc.M115.703058

Okochi, M., Steiner, H., Fukumori, A., Tanii, H., Tomita, T., Tanaka, T., et al. (2002). Presenilins mediate a dual intramembranous gamma-secretase cleavage of Notch-1. *EMBO J.* 21, 5408–5416. doi: 10.1093/emboj/cdf541

Olmos, G., and Lladó, J. (2014). Tumor necrosis factor alpha: a link between neuroinflammation and excitotoxicity. *Med. Inflamm.* 2014:861231. doi: 10.1155/2014/861231

Palmer, T. D., Takahashi, J., and Gage, F. H. (1997). The adult rat hippocampus contains primordial neural stem cells. *Mol. Cell Neurosci.* 8, 389–404. doi: 10.1006/mcne.1996.0595

Rowland, L. P., and Shneider, N. A. (2001). Amyotrophic lateral sclerosis. *N. Engl. J. Med.* 344, 1688–1700. doi: 10.1056/NEJM200105313442207

Saftig, P., and Lichtenthaler, S. F. (2015). The alpha secretase ADAM10: a metalloprotease with multiple functions in the brain. *Prog. Neurobiol.* 135, 1–20. doi: 10.1016/j.pneurobio.2015.10.003

Semerci, F., Choi, W. T., Bajic, A., Thakkar, A., Encinas, J. M., Depreux, F., et al. (2017). Lunatic fringe-mediated Notch signaling regulates adult hippocampal neural stem cell maintenance. *Elife* 6:e24660. doi: 10.7554/eLife.24660

Seri, B., García-Verdugo, J. M., Collado-Morente, L., McEwen, B. S., and Alvarez-Buylla, A. (2004). Cell types, lineage, and architecture of the germinal zone in the adult dentate gyrus. *J. Comp. Neurol.* 478, 359–378. doi: 10.1002/cne.20288

Tortarolo, M., Lo Coco, D., Veglianese, P., Vallarola, A., Giordana, M. T., Marcon, G., et al. (2017). Amyotrophic lateral sclerosis, a multisystem pathology: insights into the role of TNFα. *Med. Inflamm.* 2017:2985051. doi: 10.1155/2017/2985051

Traiffort, E., and Ferent, J. (2015). Neural stem cells and Notch signalling. *Med. Sci.* 31, 1115–1125. doi: 10.1051/medsci/20153112015

Vintilescu, C. R., Afreen, S., Rubino, A. E., and Ferreira, A. (2016). The neurotoxic tau45-230 fragment accumulates in upper and lower motor neurons in amyotrophic lateral sclerosis subjects. *Mol. Med.* 22, 477–486. doi: 10.2119/molmed.2016.00095

Von Rotz, R. C., Kohli, B. M., Bosset, J., Meier, M., Suzuki, T., Nitsch, R. M., et al. (2004). The APP intracellular domain forms nuclear multiprotein complexes and regulates the transcription of its own precursor. *J. Cell Sci.* 117, 4435–4448. doi: 10.1242/jcs.01323

Wang, J., Yan, K., Wu, Z. Q., Zheng, C. Y., Xu, R. X., Chen, L. H., et al. (2014). TDP-43 interaction with the intracellular domain of amyloid precursor protein induces p53-associated apoptosis. *Neurosci. Lett.* 569, 131–136. doi: 10.1016/j.neulet.2014.03.075

Wang, S. Y., Ren, M., Jiang, H. Z., Wang, J., Jiang, H. Q., Yin, X., et al. (2015). Notch pathway is activated in cell culture and mouse models of mutant SOD1-related familial amyotrophic lateral sclerosis, with suppression of its activation as an additional mechanism of neuroprotection for lithium and valproate. *Neuroscience* 301, 276–288. doi: 10.1016/j.neuroscience.2015.06.002

Winner, B., and Winkler, J. (2015). Adult neurogenesis in neurodegenerative diseases. *Cold Spring Harb. Perspect. Biol.* 7:a021287. doi: 10.1101/cshperspect.a021287

Woo, H. N., Park, J. S., Gwon, A. R., Arumugam, T. V., and Jo, D. G. (2009). Alzheimer's disease and Notch signaling. *Biochem. Biophys. Res. Commun.* 390, 1093–1097. doi: 10.1016/j.bbrc.2009.10.093

Xue, F., Chen, Y. C., Zhou, C. H., Wang, Y., Cai, M., Yan, W. J., et al. (2017). Risperidone ameliorates cognitive deficits, promotes hippocampal proliferation, and enhances Notch signaling in a murine model of schizophrenia. *Pharmacol. Biochem. Behav.* 163, 101–109. doi: 10.1016/j.pbb.2017.09.010

Yan, R., Han, P., Miao, H., Greengard, P., and Xu, H. (2001). The transmembrane domain of the Alzheimer's β-secretase (BACE1) determines its late Golgi localization and access to β-amyloid precursor protein (APP) substrate. *J. Biol. Chem.* 276, 36788–36796. doi: 10.1074/jbc.M104350200

Yang, D., Abdallah, A., Li, Z., Lu, Y., Almeida, S., and Gao, F. B. (2015). FTD/ALS-associated poly(GR) protein impairs the Notch pathway and is recruited by poly(GA) into cytoplasmic inclusions. *Acta Neuropathol.* 130, 525–535. doi: 10.1007/s00401-015-1448-6

Zhan, L., Hanson, K. A., Kim, S. H., Tare, A., and Tibbetts, R. S. (2013). Identification of genetic modifiers of TDP-43 neurotoxicity in *Drosophila*. *PLoS One* 8:e57214. doi: 10.1371/journal.pone.0057214

Zhang, S., Wang, P., Ren, L., Hu, C., and Bi, J. (2016). Protective effect of melatonin on soluble Aβ1-42-induced memory impairment, astrogliosis, and synaptic dysfunction via the Musashi1/Notch1/Hes1 signaling pathway in the rat hippocampus. *Alzheimers Res. Ther.* 8:40. doi: 10.1186/s13195-016-0206-x

Zhou, J., Fujiwara, T., Ye, S., Li, X., and Zhao, H. (2014). Downregulation of Notch modulators, tetraspanin 5 and 10, inhibits osteoclastogenesis in vitro. *Calcif. Tissue Int.* 95, 209–217. doi: 10.1007/s00223-014-9883-2

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.

frontiers in Psychiatry

REVIEW
published: 12 April 2019
doi: 10.3389/fpsyt.2019.00221

Sleep Disorders in Huntington's Disease

Radoslawa Herzog–Krzywoszanska[1]* *and Lukasz Krzywoszanski*[2]

[1] General Psychology Unit, Chair of Psychology, Faculty of Pedagogy, Pedagogical University of Krakow, Krakow, Poland,
[2] Neurocognitive Psychology Unit, Chair of Psychology, Faculty of Pedagogy, Pedagogical University of Krakow, Krakow, Poland

Huntington's chorea (Huntington's disease, HD) is a genetic disorder caused by autosomal dominant mutation, leading to progressive neurodegenerative changes in the central nervous system. Involuntary movements such as chorea occur typically in HD patients, accompanied by progressive cognitive and psychiatric disturbances. Other common symptoms of HD are circadian and sleep abnormalities, which are observed from the earliest stages of the disease or even before the occurrence of clinical symptoms. The most common sleep problems reported by HD patients include insomnia, difficulties in falling asleep, frequent nocturnal awakenings, and excessive daytime sleepiness. Also, specific changes in sleep architecture have been identified in HD. In this paper, we review studies on sleep and circadian rhythm disorders in HD. We outline findings concerning sleep patterns and disturbances of circadian rhythms in HD patients, as well as the role of psychiatric disorders and motor disorders in HD patients' sleep problems. We also discuss problems related to the different methods of diagnosing sleep disorders in HD. Furthermore, the adverse effects of medication used for the treatment of core HD symptoms as one of the sources of sleep disturbances in HD are emphasized. In conclusion, the diversity and complexity of the determinants of sleep and circadian rhythm disorders in HD are highlighted. Finally, the relevance of effective treatment to improve patients' functioning and quality of life as well as the potential relief of their cognitive and emotional symptoms is addressed.

Keywords: Huntington's disease, neurodegenerative disease, sleep disorders, circadian rhythm disturbances, melatonin

OPEN ACCESS

Edited by:
Foteini Christidi, National and Kapodistrian University of Athens, Greece

Reviewed by:
Nicholas-Tiberio Economou, National and Kapodistrian University of Athens, Greece
Alba Di Pardo, Mediterranean Neurological Institute (IRCCS), Italy

***Correspondence:**
Radoslawa Herzog–Krzywoszanska, radoslawa.herzog-krzywoszanska@up.krakow.pl

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Psychiatry

Received: *15 December 2018*
Accepted: *26 March 2019*
Published: *12 April 2019*

Citation:
Herzog-Krzywoszanska R and Krzywoszanski L (2019) Sleep Disorders in Huntington's Disease. Front. Psychiatry 10:221. doi: 10.3389/fpsyt.2019.00221

INTRODUCTION

Huntington's chorea (Huntington's disease, HD) is a genetic disorder caused by autosomal dominant mutation, leading to progressive neurodegenerative changes in the central nervous system. The disease is caused by a dynamic mutation of the HTT gene, located on the short arm of the fourth chromosome. In the unaffected population, the number of CAG repeats in the HTT gene that encodes the huntingtin (HTT) protein varies from 6 to 35 (1). The mutant version of the gene contains from 36 to 250 repeats of this nucleotide sequence; as a result, a glutamine string present in the amino acid sequence of huntingtin encoded by the HTT gene is excessively elongated. A gradual accumulation of deposits of misfolded huntingtin and other proteins as well as the death of neurons in various areas of the brain are observed during the course of the disease. Neurodegenerative changes in HD primarily occur in the striatum and globus pallidus, but also in the cerebral cortex, cerebellum, amygdala, thalamus, and hypothalamus (2–8). The age of onset of HD symptoms strongly correlates with the number of CAG trinucleotide repeats in HTT. A higher number of CAG sequence repeats

in the mutation carrier causes earlier onset of the disease, more severe cognitive impairments, severe progression of degenerative changes, and worse prognosis (9). The disease usually manifests in people aged 35–50, and it causes death within 15–20 years (10). It is estimated that HD frequency in Europe is 5–10 cases per 100,000 inhabitants (11).

Three main types of disorders described in HD are motor, cognitive, and psychiatric (12, 13). The most common motor symptoms in HD include chorea—uncontrolled movements of the head, neck, and limbs that may impede daily activities. Patients have difficulties maintaining balance and often stumble and fall. The clinical picture includes other motor symptoms such as bradykinesia, muscle rigidity, spasticity, myoclonus, dystonia, tics, ataxia, and athetotic movements (14).

The most common cognitive deficits in HD include attention (15, 16) and executive function disorders manifested by difficulties in taking decisions, planning and execution of complex activities, reduced flexibility of thinking and behavior, as well as a tendency to perseveration (17, 18). HD also involves implicit memory and learning disorders (19).

HD patients also develop psychiatric disorders that often occur before the onset of full-blown HD. Low mood and depression are revealed in a considerable number of the patients (20, 21), while anxiety disorders, dysphoria, emotional lability, apathy, and arousal are more rare (22, 23). In some patients, schizophrenia-like psychosis and paranoid symptoms have been observed (24).

The common symptoms of HD also include sleep disturbances (25). Up to 90% of patients report sleep problems that are evaluated as important by over half of them (26). Information about sleep disorders is also provided by spouses and caregivers. The number of CAG repeats in most studies did not correlate with the occurrence of sleep disorders (27); however, they were partially correlated with the duration of the disease and the severity of clinical symptoms (28). Studies have demonstrated that some sleep disorders are found in the very early phase of HD and even in carriers of the mutation at the premorbid stage (27, 29, 30). Many studies also show a high incidence of sleep disorders in other neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), or spinocerebellar ataxia (31–33).

Lack of sleep causes a decrease in mental alertness, irritability, attention disturbances, deceleration of reactions, and reduced ability for logical thinking. Sleep disorders considerably affect patients' life activities, and their negative impact can be manifested especially in people with neurodegenerative disorders, who experience serious difficulties in everyday functioning due to the illness (29, 34, 35).

In this paper, we review the literature on sleep disorders and circadian rhythm disturbances in HD patients. We performed a search of online electronic databases (PubMed, MEDLINE, Scopus, and Google Scholar) that was most recently updated on October 10, 2018, followed by analysis of reference lists for additional articles.

SLEEP PATTERN DISORDERS IN HD

A typical sleep pattern in adults includes non-rapid eye movement sleep (NREM) divided into three stages (N1, N2, and N3): the sleep gradually deepens from N1 to N3, followed by rapid eye movement sleep (REM), during which suppression of postural muscle tone (atonia) is normally observed. NREM lasts about 60–90 min, REM lasts about 10–15 min, and the whole sleep cycle consisting of NREM and REM is repeated four or five times during a night's sleep.

There is no homogenous pattern of sleep disorders in patients with HD (36) and problems can occur in various areas. The literature usually reports insomnia, increased sleep onset latency, decrease in total sleep time, frequent nocturnal awakenings (27, 28, 37–40), REM sleep disorders (27, 38, 41), increased motor activity during sleep (27), decreased sleep efficiency (37, 38, 41, 42), and excessive daytime sleepiness (43). The problems mainly concern impaired maintenance of sleep: patients frequently experience awakenings that are accompanied by electroencephalographic abnormalities (27, 38, 41). Some studies also show that awakenings may be accompanied by anxiety and choreiform movements (44). Reduced N3 stage and an increased sleep spindle density are observed in HD patients compared to healthy controls (28). It was revealed that HD patients spend less time in the deep sleep phase, as indicated by the higher percentage of the first stage of sleep compared to the control group (27). Compared to healthy subjects, individuals with HD also spend more time in the NREM stage than in the REM stage (37), which may be related to REM sleep disturbances in HD.

Reports presenting research concerning sleep disorders during the REM stage in HD patients are ambiguous. Some authors report a lack, shortening, or delay of the REM stage (27, 37, 38), but no REM sleep disorders were detected in other studies on HD patients (28).

In the study conducted by Arnulf et al. (27), reduced duration of the REM sleep stage was noted in mutation carriers without core symptoms, as well as in patients in the first stage of the disease, for whom it intensified with progression. These authors also claim that reduced REM sleep may precede chorea and provide an early marker of the disease. According to Arnulf et al. (27), reduced REM sleep atonia in HD patients with decreased duration of REM sleep and reduced eye movement density (38) indicates that REM sleep executive systems, which are mainly located in the brainstem, are damaged in HD. This postulate is consistent with findings showing atrophy and degenerative changes in the brainstem in HD (45, 46). Another cause of REM sleep disturbance may be the dependence of REM sleep mechanisms on cerebral blood flow and oxygen metabolism, both of which are impaired in HD (27).

HD patients also demonstrate behavioral disorders related to the REM sleep stage (RBD, REM sleep behavior disorder) (43). Skeletal muscle atonia is one of the characteristic features of REM sleep, and its absence can lead to sudden body movements, e.g., hitting, kicking, tossing, and turning during sleep; these phenomena are accompanied by realistic dreams, often provoking a sense of danger (47, 48).

Some authors (27) suggest that in HD a mutant form of huntingtin may accumulate in areas that control muscle atonia during REM sleep, thus leading to their failure. These areas include the dorsolateral pons, the locus coeruleus, the reticular formation of the medulla, the pedunculopontine tegmental nucleus, and the hypothalamus. Cell loss in the locus coeruleus

(49) and in the hypothalamus (45, 50, 51) in HD has been confirmed in studies.

RBD carries a risk of self-injury or injury to other persons in the bed. Therefore, systematic interviews with HD patients and their caregivers about sudden movements and aggressive actions during their sleep may be useful in medical care. A significant role can be played by counseling on RBD, securing the sleep environment and pharmacological treatment.

CIRCADIAN RHYTHM DISTURBANCES IN HD

Changes in the sleep–wake cycle, such as difficulty in falling asleep and maintaining sleep in HD, are considered to be a manifestation of circadian rhythm sleep disorder (CRSD) (52). Some authors argue that the REM sleep delay in HD patients could also be a symptom of circadian dysfunction (26, 53). Moreover, circadian rhythm disorders may underlie excessive sleepiness in HD. Excessive daytime sleepiness (EDS) involves a strong subjective feeling of sleepiness, difficulty in maintaining wakefulness, and a tendency to fall asleep at the wrong time or in the wrong place. The most severe form of excessive sleepiness is sleep attacks, i.e., sudden, uncontrolled incidences of falling asleep, both while performing monotonous tasks and during intensified activity such as riding a bike: a short sleep lasting a few minutes is followed by sudden awakening with amnesia of the episode (54). The results of studies on the prevalence of EDS in HD patients vary depending on the applied research method (e.g., interview with the patient, the results of the Epworth Sleepiness Scale, or multiple sleep latency tests). In some research, daytime sleepiness in the HD group was similar to that in the control group (55), whereas in the study by Videnovic et al. (43), the frequency of EDS occurrence was high and affected 50% of the patients. Higher EDS results were correlated with depression; therefore, it is believed that effective treatment of EDS in HD should take into account the coexisting depression (43). EDS may also be caused by sleep disordered breathing, but only two studies have shown abnormalities of the sleep respiration in few HD patients (56, 57), while no differences between the HD patients and control subjects with respect to sleep respiratory variables have been found in other studies (58, 59).

The circadian rhythm in mammals is regulated by the suprachiasmatic nucleus of the hypothalamus (SCN), which controls melatonin synthesis in the pineal gland. Some researchers (60, 61) have described changes in circadian melatonin secretion in HD, both in the early and late stages of the disease. In another study, mean daytime melatonin levels did not differ between the patients with HD and controls, but the evening rise in melatonin level in HD patients was delayed compared to individuals without neurological disorders (61, 62). A more recent study (63) found reduced 24-h averaged plasma melatonin concentration, flattening of the circadian rhythm of melatonin secretion, and greater spread of melatonin onset time in premanifest HD and moderate HD patients compared with control subjects.

Circadian abnormalities were manifested in an HD animal model in transgenic R6/2 mice in which daytime activity was increased and nighttime activity was reduced (64, 65). Disturbed night–day activity in R6/2 mice worsened with the progression of the disease, finally leading to complete disruption of the behavior. Circadian sleep–wake cycle disturbances in R6/2 mice might be a result of the disruption of circadian rhythm regulation by SCN. SCN is a neural stimulator of the sleep–wake rhythm, and its activity is based on the cyclic expression of genes crucial for the internal clock—the so-called clock genes. Abnormal expression of mammalian Period2 (mPer2) and brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein-1 (BMAL1) clock genes in SCN, as well as in the striatum and motor cortex, was observed in R6/2 mice. The authors (64) claim that the data from the studies on R6/2 mice suggest that abnormalities in SCN activity leading to sleep disturbances can also be observed in HD patients. An effect on the circadian regulation mechanism of the pathological processes that occur in HD has not yet been recognized. It is known, however, that HD is associated with serious damage to the hypothalamus, where the SCN is located (50, 66). The normalization of the circadian rhythm slows the increase of cognitive impairments in animal models of HD and thus potentially may also have a significant effect on the inhibition of the rate of disease progression and improvement of life quality in patients with HD (67).

PSYCHIATRIC DISORDERS AND SLEEP DISORDERS IN HD

Difficulties with falling asleep, shortness of sleep, and frequent awakenings may be the consequences of psychiatric disorders such as depression, mania, and anxiety, all of which affect many patients with HD. Depression with suicidal thoughts and tendencies develops in about 30% of people with HD and is often manifested even before the occurrence of motor symptoms (20, 21). According to some studies, the risk of depression and the rates of attempted suicide are higher in the period prior to the clinical HD phase and at its beginning (68). It is assumed that in most cases, depression in HD is endogenous and can result from damage to the medial caudate nucleus, from which projections go to the prefrontal and orbitofrontal areas. Research shows that depression in HD patients is associated with a reduction in glucose metabolism in the prefrontal and orbitofrontal cortex (69). Research on HD animal models also indicates a potential role of hypothalamus dysfunction as the neurobiological basis of depression in HD (70).

However, in addition to biological factors, psychosocial factors also play an important role in the development of depression in HD, which is an incurable disease that leads to disability and a significant reduction in quality of patients' life. Coping with the disease and the associated stress may significantly affect the incidence of depression and anxiety symptoms in patients with HD. Studies have shown that the genetic diagnosis itself is a strong stressor that can lead to suicidal attempts and psychiatric disorders requiring hospitalization, not only in people who are diagnosed as defective gene carriers, but also among those in whom the result was negative (71).

Much clinical evidence indicates a strong relationship between depression and sleep disorders among different groups of patients

with extrapyramidal system diseases, e.g., PD (72). Videnovic et al.'s (43) study shows more frequent sleep disturbances in HD patients with coexisting depression than in HD patients without depression symptoms.

In the study of Aziz et al. (73), sleep disorders reported by HD patients were associated with depression but did not correlate with the severity of motor, behavioral, and cognitive symptoms that are typical of HD. In contrast, a delayed sleep phase was associated with both depression and the deterioration of cognitive and everyday functioning. In another study (74), subjective reports of sleep problems were associated with a greater severity of depressive symptoms but were not associated with neurocognitive symptoms in premanifest and symptomatic HD individuals.

Some sleep disorder symptoms noted in HD patients, such as sleep onset latency, shortness of sleep, and frequent awakenings, are consistent with the symptoms of sleep disorders observed in depression. However, there are also significant differences between the pattern of sleep disorders in HD and sleep disturbances in depression, especially regarding REM sleep. Depression is characterized by a decreased latency of REM sleep, increased REM sleep, and increased number and density of rapid eye movements (75), whereas increased REM sleep latency, reduced REM sleep, and a decreased density of rapid eye movement are observed in HD (27, 37, 38). The similarity of some symptoms and differences in other symptoms of sleep disorders in HD and in depression most likely result from heterogeneous causes of sleep disorders in HD. They can be both a consequence of damage to brain areas responsible for sleep processes, e.g., the brainstem in reference to REM sleep and endogenous depression, and the result of an emotional reaction to stressful situations.

The course and quality of sleep in HD patients can also be adversely affected by arousal resulting from mania or anxiety disorders (24, 76). As much as 10% of HD patients may experience mania or hypomania episodes, and over 50% experience anxiety (21). Excessive arousal may be the basis for a pattern of sleep disorders that occurs in some HD patients, manifested by difficulties in maintaining sleep, lack of compensatory sleep during the day, and falling asleep later compared to people not suffering from insomnia (27).

A significant relationship between sleep disorders and psychiatric disorders emphasizes the need for their early detection and treatment in HD. Effective treatment of psychiatric disorders should also lead to sleep normalization.

MOTOR DISORDERS AND SLEEP DISORDERS IN HD

One of the causes of sleep problems in HD patients may be the involuntary movements that are one of the core symptoms of the disease (77, 78). It was originally believed that chorea ceases during sleep, but some studies have disproved this theory (37, 79). Patients are often not aware of their nocturnal movements, and information about their movements during sleep is obtained from their spouses or caregivers (72). As the disease progresses, further extrapyramidal symptoms develop, including bradykinesia, rigidity, myoclonus, and dystonic movements (involuntary movements of the limbs or the entire body). Rigidity and bradykinesia make it difficult to change position during sleep, and painful cramps and dyskinesias are the cause of awakening (73).

Studies demonstrate increased motor activity during sleep (55, 80, 81), including periodic limb movements (37, 82) and behavioral disorders during REM sleep (27) in HD patients compared to subjects not suffering from HD. More movements during sleep occurred in HD patients compared to patients with PD (79). Some HD patients suffer from restless legs syndrome (RLS), in which, due to unpleasant sensations, the patients move their legs to relieve the symptoms, as well as the syndrome of periodic limb movements in sleep (PLMS), characterized by brief, repetitive, and stereotyped limb movement during sleep. Both of these syndromes are associated with difficulties in falling asleep and frequent awakenings, which usually lead to constant fatigue, irritability, deterioration of concentration, and sleepiness during day (83). Comella (83) suggests that there is a genetic association between RLS and HD, but some studies have not revealed restless legs syndrome among examined HD patients (27). Evers and Stögbauer (84) hypothesize that dopaminergic transmission in HD may be reduced in parts of the basal ganglia, which are of particular importance for RLS. According to them, another explanation for the relationship between HD and RLS may be reduced sensitivity of the dopamine D1 and D2 receptors in HD patients with RLS. On the basis of their results, Savva et al. (85) postulate that RLS should be regarded as one of the early symptoms of HD that results from the neurodegenerative changes that occur in the disease.

METHODS FOR DIAGNOSING SLEEP DISORDERS IN HD

The most commonly used methods used for screening and diagnosis of sleep disorders in HD are self-report tools designed for sleep assessment in the general population, such as the Pittsburgh Sleep Quality Index (PSQI), IRLS, The Berlin questionnaire, and the Epworth Sleepiness Scale (ESS) (86). However, methods for sleep assessment in the general population may not evaluate the characteristic symptoms of sleep disorders of people with HD due to the specificity of the disease. Despite the prevalence of sleep and circadian rhythm disorders in HD, the Unified Huntington Disease Rating Scale (UHDRS), which is designed to assess the functioning of HD patients, does not include questions about sleep or the circadian rhythm (87). Research results indicate that in motor disorders, including HD, non-motor symptoms such as sleep disorders are often not reported by the patient or family (82, 88), which may impede the detection and treatment of sleep disorders. For both refining diagnoses and expanding the scientific knowledge about sleep disorders in HD, it seems advisable to include sleep questions in existing assessment tools and creating new HD-specific sleep scales. The detailed self-report questionnaire for assessment of sleep problems specific for HD patients, designed similarly to the questionnaires for assessment of sleep problems in PD patients,

was developed recently (29). This questionnaire contains 45 questions grouped into four subcategories: quality of sleep (e.g., difficulty falling asleep or maintaining sleep), motor activity (e.g., painful muscle cramps in the arms or legs causing waking at night), abnormal nocturnal behavior (e.g., acting out dreams, injury to self or others while dreaming), and other aspects of disturbed sleep (e.g., nocturia, numbness, paresthesia, sleep apnea, and daytime somnolence).

The use of objective methods that provide data based on physical phenomena such as polysomnography and actigraphy could be potentially beneficial in the diagnosis of sleep disorders in HD, but it is associated with a number of difficulties. Polysomnography is considered a gold-standard diagnostic tool, but the availability of this method is significantly limited by the fact that it is complex, time-consuming, and expensive. Actigraphy is designed to assess the state of wakefulness and sleep on the basis of patients' physical activity, but in the case of diseases with uncontrolled movements like HD, the test results may not be reliable. Studies using activity monitors show that during sleep, more movements and increased activity occur in HD patients compared to controls, which may mean that they either wake up at nighttime or make involuntary movements during sleep (55, 80). Fish et al. (79) observed that involuntary movements during sleep have a different character from those performed during waking—they are shorter and more fragmented. In combination with patients' declarations that they did not wake up or did so only occasionally (55), the results support the hypothesis that nocturnal HD activity is associated with involuntary movements during sleep rather than waking up. In addition, low concordance of actigraphy results with EEG records and sleep diaries was demonstrated in the sleep study in HD patients carried out by Townhill et al. (81).

Contradictory results indicating overestimation of total sleep time by activity monitors in seven HD patients was reported in the study of Maskevich et al. (89), but the subjects in the analyzed sample were in the presymptomatic and early phase of the disease and had very little chorea. Those discrepancies indicate the need for caution in the interpretation of results obtained in studies of sleep and circadian rhythms in HD patients using activity monitors. However, Adams et al. (90) suggest that further development and improvement of accelerator-based research methods will allow them to be used to accurately monitor and diagnose sleep disorders in HD.

Piano et al. (91) investigated the coherence of self-report and laboratory-based sleep assessment in 30 HD patients; the concordance of subjective sleep evaluation based on two self-report measures, the Pittsburgh Sleep Quality Index and the Huntington Disease Sleep Questionnaire, was modest (Cohen $\kappa = 0.375$). Moreover, the results in both self-report measures demonstrated poor concordance with the Sleep Efficiently Index determined in a full-night laboratory-based video-polysomnographic recording (Cohen $\kappa = 0.062$ and 0.143, respectively) (91).

Proper diagnosis of sleep disorders in HD may require skillful combination of results from many different methods and integration of data from different sources as well as careful consideration of the specificity of this disease. The currently available methods should be considered imperfect as they require further development and improvement. There is also a need to develop diagnostic standards and guidelines for diagnosis of sleep disorders in HD.

PHARMACOTHERAPY AND SLEEP DISORDERS IN HD

No systematic research has been conducted regarding the treatment of sleep disorders in HD patients; thus, the evidence base for the pharmacological treatment of HD is insufficient (92). The treatment is further complicated by the fact that many medications administered to HD patients to alleviate motor and psychiatric symptoms may change sleep architecture. Appropriate selection of drugs is also important because alleviating motor symptoms and depression themselves can improve sleep quality in HD patients.

Medications used in HD that are commonly associated with sleep disturbances are tetrabenazine, clonazepam, diazepam, riluzole, quetiapine, dosulepin, olanzapine, and venlafaxine. Drugs that may increase patients' activation include amantadine, sodium valproate, and L-DOPA (52, 93). Antidepressants (especially venlafaxine) should not be used in HD patients suffering from RBD because they can aggravate the symptoms. In HD patients with RBD, it is possible to administer clonazepam, but in some cases, it is not well tolerated by HD patients and can cause respiratory depression (27). All dopaminergic drugs, including L-DOPA, could have sedative effects that may lead to excessive sleepiness (94). This undesirable side effect can be mitigated by dose adjustment or changing the drugs administered to patients. If given at night, some nondopaminergic drugs with sedative side effects that are used in chorea treatment, such as olanzapine, may help HD patients with insomnia (95).

In general, drugs that can interfere with sleep should be used with caution. In cases in which there is no opportunity to change medication, stimulant drugs should be given early in the day and drugs with sedative side effects should be given in the evening.

Apart from preventing negative effects on sleep related to drugs used in HD patients, some pharmacological agents can be used in order to improve the quality of sleep. The application of short-acting benzodiazepines for insomnia in HD patients may provide improvement; however, these medications need to be used with caution because of their potential side effects. Mirtazapine, which is a noradrenergic and specific serotonergic antidepressant, is recommended for HD patients suffering simultaneously from depression and sleep disorders (96). If the HD patient has daytime sleepiness, treatment with stimulants such as modafinil can be beneficial; its effect has mainly been evaluated in PD patients, in whom it improved daytime sleepiness and cognitive functions, was well tolerated, and did not impair motor functions (53). One study evaluating the effects of modafinil on HD patients showed (97) that it did not improve cognitive function or mood, but increased alertness.

Melatonin may also be beneficial in treatment because it promotes sleep and normalizes the circadian rhythm. Research has shown its effectiveness in improving the quality of sleep in AD

patients (98) and PD patients (99, 100). In HD, delayed melatonin secretion rhythm and decreasing melatonin levels may contribute to progressive neurodegeneration. Therefore, the use of melatonin or its receptors' agonists (ramelteon, agomelatine) could not only improve sleep quality but also enhance neuroprotection in HD patients (50). Regulation of the sleep–wake rhythm slows the increase of cognitive impairments in animal models of HD and thus potentially may also have a significant effect on the inhibition of the rate of disease progression and improvement of life quality in patients with HD (67).

Sleep quality in HD patients can also be improved by changing sleep behaviors. It is important to determine bedtime and wake time, avoid naps during the day, do regular physical exercise, maintain a proper diet, and limit the intake of caffeine, tobacco, and alcohol. In HD patients, relaxation training methods and cognitive-behavioral therapy can also be applied.

CONCLUSIONS

Different patterns of sleep disturbances are observed in HD patients: insomnia, difficulties in falling asleep, frequent nocturnal awakenings, and excessive daytime sleepiness are the most common sleep problems reported by patients with HD. In several HD studies, specific changes in sleep architecture and in circadian melatonin secretion were identified in laboratory testing.

Sleep disorders in HD have diverse and complex determinants, the most significant of which includes damage to brain areas that are responsible for the proper sleep pattern and circadian rhythm regulation. Sleep and circadian rhythm disorders in HD might also be associated with psychiatric disorders, especially depression, mania, and anxiety disorders. Another group of factors related to sleep disturbances in HD are involuntary movements and increased motor activity during sleep, and other motor disturbances. Moreover, the pattern of sleep disorders in HD is often altered by the many drugs used to alleviate the core symptoms of the disease, a substantial number of which may adversely affect sleep.

Therefore, studies on sleep disorders in HD are associated with numerous difficulties, as is also the case with other neurodegenerative diseases. However, identification of sleep disorders and more comprehensive recognition of their causes are necessary to ensure adequate care and treatment for patients. Disorders of sleep and the circadian rhythm in patients with HD can contribute to exacerbation of symptoms such as irritability and anxiety, as well as cognitive disorders that can interfere with daily functioning more than the motor symptoms. The effective treatment of sleep disorders in HD patients may play an important role in improving the quality of their life, helping them cope with everyday problems, and relieving the cognitive and emotional symptoms. It should also have a great impact on the quality of sleep of caregivers and thus create the opportunity to delay patients' institutionalization. More detailed knowledge of sleep problems in HD can also provide more profound insight into the nature of the neurodegenerative processes that occur in the disease (52).

Various methods are used for diagnosis of sleep disorders in HD patients. The most frequently used are self-reporting tools designed to assess sleep in the general population. However, the results obtained with these methods show low consistency with the results of laboratory sleep assessment, which makes their use in HD patients limited. Moreover, the proper diagnosis of sleep disorders in HD may require consideration of the specificity of the symptoms of this disease. The use of objective methods such as polysomnography and actigraphy might have potential benefits in the diagnosis of sleep disorders in HD patients, but the availability of polysomnography is limited because it is complex, time-consuming, and expensive, and the results of actigraphy may not be reliable in the case of diseases with uncontrolled movements, such as HD. Integration of data from various sources and results obtained from many different methods should allow the most comprehensive diagnosis of sleep disorders and circadian rhythm disorders in HD patients. The need to develop diagnostic standards and guidelines for the diagnosis of sleep disorders in HD should be considered one of the important challenges in improving the care of HD patients.

AUTHOR CONTRIBUTIONS

RH conceived and designed the study. RH and LK performed literature search and selection. LK created a database of literature relevant to the study. RH and LK wrote sections of the manuscript. Both authors contributed to manuscript revision and read and approved the submitted version.

REFERENCES

1. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* (1993) 72:971–83. doi: 10.1016/0092-8674(93)90585-E
2. Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP. Neuropathological classification of Huntington's disease. *J Neuropathol Exp Neurol* (1985) 44:559–77. doi: 10.1097/00005072-198511000-00003
3. Macdonald V, Halliday G. Pyramidal cell loss in motor cortices in Huntington's disease. *Neurobiol Dis* (2002) 10:378–86. doi: 10.1006/nbdi.2002.0528
4. Macdonald V, Halliday GM, Trent RJ, McCusker EA. Significant loss of pyramidal neurons in the angular gyrus of patients with Huntington's disease. *Neuropathol Appl Neurobiol* (1997) 23:492–5. doi: 10.1111/j.1365-2990.1997.tb01326.x
5. Jeste DV, Barban L, Parisi J. Reduced Purkinje cell density in Huntington's disease. *Exp Neurol* (1984) 85:78–86. doi: 10.1016/0014-4886(84)90162-6
6. Kremer HPH. Chapter 17 The hypothalamic lateral tuberal nucleus: normal anatomy and changes in neurological diseases. In: Swaab DF, Hofman MA, Mirmiran M, Ravid R, Van Leeuwen FW, editors. *Progress in brain research*. Amsterdam, New York: Elsevier (1992) p. 249–61. doi: 10.1016/S0079-6123(08)64576-8
7. Kremer HPH, Roos RAC, Dingjan GM, Bots GTAM, Bruyn GW, Hofman MA. The hypothalamic lateral tuberal nucleus and the characteristics of neuronal loss in Huntington's disease. *Neurosci Lett* (1991) 132:101–4. doi: 10.1016/0304-3940(91)90443-W
8. Heinsen H, Rüb U, Bauer M, Ulmar G, Bethke B, Schüler M, et al. Nerve cell loss in the thalamic mediodorsal nucleus in Huntington's disease. *Acta Neuropathologica* (1999) 97:613–22. doi: 10.1007/s004010051037

9. Kieburtz K, MacDonald M, Shih C, Feigin A, Steinberg K, Bordwell K, et al. Trinucleotide repeat length and progression of illness in Huntington's disease. *J Med Genet* (1994) 31:872–4. doi: 10.1136/jmg.31.11.872
10. Bates GP. History of genetic disease: the molecular genetics of Huntington disease - a history. *Nat Rev Genet* (2005) 6:766–73. doi: 10.1038/nrg1686
11. Warby SC, Visscher H, Collins JA, Doty CN, Carter C, Butland SL, et al. HTT haplotypes contribute to differences in Huntington disease prevalence between Europe and East Asia. *Eur J Hum Genet* (2011) 19:561–6. doi: 10.1038/ejhg.2010.229
12. Roos RA. Huntington's disease: a clinical review. *Orphanet J Rare Dis* (2010) 5:40. doi: 10.1186/1750-1172-5-40
13. Walker FO. Huntington's disease. *Lancet* (2007) 369:218–28. doi: 10.1016/S0140-6736(07)60111-1
14. Martin JB, Gusella JF. Huntington's disease. Pathogenesis and management. *N Engl J Med* (1986) 315:1267–76. doi: 10.1056/NEJM198611133152006
15. Filoteo JV, Delis DC, Roman MJ, Demadura T, Ford E, Butters N, et al. Visual attention and perception in patients with Huntington's disease: comparisons with other subcortical and cortical dementias. *J Clin Exp Neuropsychol* (1995) 17:654–67. doi: 10.1080/01688639508405156
16. Thompson JC, Poliakoff E, Sollom AC, Howard E, Craufurd D, Snowden JS. Automaticity and attention in Huntington's disease: when two hands are not better than one. *Neuropsychologia* (2010) 48:171–8. doi: 10.1016/j.neuropsychologia.2009.09.002
17. Diamond R, White RF, Myers RH, Mastromauro C, Koroshetz WJ, Butters N, et al. Evidence of presymptomatic cognitive decline in Huntington's disease. *J Clin Exp Neuropsychol* (1992) 14:961–75. doi: 10.1080/01688639208402547
18. Duff K, Beglinger LJ, Theriault D, Allison J, Paulsen JS. Cognitive deficits in Huntington's disease on the Repeatable Battery for the Assessment of Neuropsychological Status. *J Clin Exp Neuropsychol* (2010) 32:231–8. doi: 10.1080/13803390902926184
19. Herzog-Krzywoszańska R. *Ukryta wiedza: rola jąder podstawy w utajonym uczeniu się [Tacit knowledge: the role of basal ganglia in implicit learning]*. Krakow: Jagiellonian University Press (2014).
20. Slaughter JR, Martens MP, Slaughter KA. Depression and Huntington's disease: prevalence, clinical manifestations, etiology, and treatment. *CNS Spectr* (2001) 6:306–326. doi: 10.1017/S109285290002201X
21. Paulsen JS, Nehl C, Hoth KF, Kanz JE, Benjamin M, Conybeare R, et al. Depression and stages of Huntington's disease. *J Neuropsychiatry Clin Neurosci* (2005) 17:496–502. doi: 10.1176/appi.neuropsych.17.4.496
22. Marshall J, White K, Weaver M, Flury Wetherill L, Hui S, Stout JC, et al. Specific psychiatric manifestations among preclinical Huntington disease mutation carriers. *Arch Neurol* (2007) 64:116. doi: 10.1001/archneur.64.1.116
23. Snowden JS, Craufurd D, Thompson J, Neary D. Psychomotor, executive, and memory function in preclinical Huntington's disease. *J Clin Exp Neuropsychol* (2002) 24:133–45. doi: 10.1076/jcen.24.2.133.998
24. Rosenblatt A, Leroi I. Neuropsychiatry of Huntington's disease and other basal ganglia disorders. *Psychosomatics* (2000) 41:24–30. doi: 10.1016/S0033-3182(00)71170-4
25. Abbott SM, Videnovic A. Chronic sleep disturbance and neural injury: links to neurodegenerative disease. *Nat Sci Sleep* (2016) 8:55–61. doi: 10.2147/NSS.S78947
26. Goodman AOG, Barker RA. How vital is sleep in Huntington's disease? *J Neurol* (2010) 257:882–97. doi: 10.1007/s00415-010-5517-4
27. Arnulf I, Nielsen J, Lohmann E, Schieffer J, Wild E, Jennum P, et al. Rapid eye movement sleep disturbances in Huntington disease. *Arch Neurol* (2008) 65:482–8. doi: 10.1001/archneur.65.4.482
28. Wiegand M, Möller AA, Lauer CJ, Stolz S, Schreiber W, Dose M, et al. Nocturnal sleep in Huntington's disease. *J Neurol* (1991) 238:203–8. doi: 10.1007/BF00314781
29. Goodman AOG, Rogers L, Pilsworth S, McAllister CJ, Shneerson JM, Morton AJ, et al. Asymptomatic sleep abnormalities are a common early feature in patients with Huntington's disease. *Curr Neurol Neurosci Rep* (2011) 11:211–7. doi: 10.1007/s11910-010-0163-x
30. Lazar AS, Panin F, Goodman AOG, Lazic SE, Lazar ZI, Mason SL, et al. Sleep deficits but no metabolic deficits in premanifest Huntington's disease. *Ann Neurol* (2015) 78:630–48. doi: 10.1002/ana.24495
31. Bliwise DL. Sleep disorders in Alzheimer's disease and other dementias. *Clin Cornerstone* (2004) 6:16–28. doi: 10.1016/S1098-3597(04)90014-2
32. Ondo WG, Dat Vuong K, Khan H, Atassi F, Kwak C, Jankovic J. Daytime sleepiness and other sleep disorders in Parkinson's disease. *Neurology* (2001) 57:1392–6. doi: 10.1212/WNL.57.8.1392
33. Tuin I, Voss U, Kang J-S, Kessler K, Rub U, Nolte D, et al. Stages of sleep pathology in spinocerebellar ataxia type 2 (SCA2). *Neurology* (2006) 67:1966–72. doi: 10.1212/01.wnl.0000247054.90322.14
34. Wulff K, Gatti S, Wettstein JG, Foster RG. Sleep and circadian rhythm disruption in psychiatric and neurodegenerative disease. *Nat Rev Neurosci* (2010) 11:589–99. doi: 10.1038/nrn2868
35. Diago EB, Martínez-Horta S, Lasaosa SS, Alebesque AV, Pérez-Pérez J, Kulisevsky J, et al. Circadian rhythm, cognition, and mood disorders in Huntington's disease. *J Huntingtons Dis* (2018) 7:193–8. doi: 10.3233/JHD-180291
36. Happe S, Trenkwalder C. Movement disorders in sleep: gilles de la Tourette syndrome, Huntington' s disease, and dystonia. *Somnologie* (2002) 6:63–67. doi: 10.1046/j.1439-054X.2002.02181.x
37. Hansotia P, Wall R, Berendes J. Sleep disturbances and severity of Huntington's disease. *Neurology* (1985) 35:1672–4. doi: 10.1212/WNL.35.11.1672
38. Silvestri R, Raffaele M, De Domenico P, Tisano A, Mento G, Casella C, et al. Sleep features in Tourette's syndrome, neuroacanthocytosis and Huntington's chorea. *Neurophysiol Clin* (1995) 25:66–77. doi: 10.1016/0987-7053(96)81034-3
39. Goodman AOG, Morton AJ, Barker RA. Identifying sleep disturbances in Huntington's disease using a simple disease-focused questionnaire. *PLoS Curr* (2010) 2:RRN1189. doi: 10.1371/currents.RRN1189
40. Moser AD, Epping E, Espe-Pfeifer P, Martin E, Zhorne L, Mathews K, et al. A survey-based study identifies common but unrecognized symptoms in a large series of juvenile Huntington's disease. *Neurodegener Dis Manag* (2017) 7:307–15. doi: 10.2217/nmt-2017-0019
41. Wiegand M, Möller AA, Schreiber W, Lauer C, Krieg JC. Brain morphology and sleep EEG in patients with Huntington's disease. *Eur Arch Psychiatry Clin Neurosci* (1991) 240:148–52. doi: 10.1007/BF02190755
42. Mena-Segovia J, Cintra L, Prospéro-García O, Giordano M. Changes in sleep-waking cycle after striatal excitotoxic lesions. *Behav Brain Res* (2002) 136:475–81. doi: 10.1016/S0166-4328(02)00201-2
43. Videnovic A, Leurgans S, Fan W, Jaglin J, Shannon KM. Daytime somnolence and nocturnal sleep disturbances in Huntington disease. *Parkinsonism Relat Disord* (2009) 15:471–4. doi: 10.1016/j.parkreldis.2008.10.002
44. Nance MA, Westphal B, Nugent S. Diagnosis of patients presenting to a Huntington disease (HD) clinic without a family history of HD. *Neurology* (1996) 47:1578–80. doi: 10.1212/WNL.47.6.1578
45. Petersén Å, Björkqvist M. Hypothalamic-endocrine aspects in Huntington's disease. *Eur J Neurosci* (2006) 24:961–7. doi: 10.1111/j.1460-9568.2006.04985.x
46. Politis M, Pavese N, Tai YF, Tabrizi SJ, Barker RA, Piccini P. Hypothalamic involvement in Huntington's disease: an *in vivo* PET study. *Brain* (2008) 131:2860–9. doi: 10.1093/brain/awn244
47. Olson EJ, Boeve BF, Silber MH. Rapid eye movement sleep behaviour disorder: demographic, clinical and laboratory findings in 93 cases. *Brain* (2000) 123:331–9. doi: 10.1093/brain/123.2.331
48. Schenck CH, Mahowald MW. REM sleep behavior disorder: clinical, developmental, and neuroscience perspectives 16 years after its formal identification in SLEEP. *Sleep* (2002) 25:120–38. doi: 10.1093/sleep/25.2.120
49. Zweig RM, Ross CA, Hedreen JC, Peyser C, Cardillo JE, Folstein SE, et al. Locus coeruleus involvement in Huntington's disease. *Arch Neurol* (1992) 49:152–6. doi: 10.1001/archneur.1992.00530260052019
50. Aziz NA. Hypothalamic dysfunction and neuroendocrine and metabolic alterations in Huntington disease: clinical consequences and therapeutic implications. *Rev Neurosci* (2007) 18:223–51. doi: 10.1515/REVNEURO.2007.18.3-4.223
51. Petersén Å, Gabery S. Hypothalamic and limbic system changes in Huntington's disease. *J Huntingtons Dis* (2012) 1:5–16. doi: 10.3233/JHD-2012-120006
52. Morton AJ. Circadian and sleep disorder in Huntington's disease. *Exp Neurol* (2013) 243:34–44. doi: 10.1016/j.expneurol.2012.10.014
53. Adler CH, Caviness JN, Hentz JG, Lind M, Tiede J. Randomized trial of modafinil for treating subjective daytime sleepiness in patients with Parkinson's disease. *Mov Disord* (2003) 18:287–93. doi: 10.1002/mds.10390

54. Paus S, Brecht HM, Köster J, Seeger G, Klockgether T, Wüllner U. Sleep attacks, daytime sleepiness, and dopamine agonists in Parkinson's disease. *Mov Disord* (2003) 18:659–67. doi: 10.1002/mds.10417
55. Hurelbrink CB, Lewis SJG, Barker RA. The use of the Actiwatch–Neurologica® system to objectively assess the involuntary movements and sleep–wake activity in patients with mild–moderate Huntington's disease. *J Neurol* (2005) 252:642–7. doi: 10.1007/s00415-005-0709-z
56. Antczak J, Witkowski G, Rakowicz M, Ziora-Jakutowicz K. Huntington's disease and sleep related breathing disorders. *Hygeia Public Health* (2013) 48:449–55. Available at: http://www.h-ph.pl/pdf/hyg-2013/hyg-2013-4-449.pdf.
57. Banno K, Hobson DE, Kryger MH. Long-term treatment of sleep breathing disorder in a patient with Huntington's disease. *Parkinsonism Relat Disord* (2005) 11:261–4. doi: 10.1016/j.parkreldis.2005.01.005
58. Bollen EL, Den Heijer JC, Ponsioen C, Kramer C, Van der Velde EA, Van Dijk JG, et al. Respiration during sleep in Huntington's chorea. *J Neurol Sci* (1988) 84:63–8. doi: 10.1016/0022-510X(88)90174-8
59. Cuturic M, Abramson RK, Vallini D, Frank EM, Shamsnia M. Sleep patterns in patients with Huntington's disease and their unaffected first-degree relatives: a brief report. *Behav Sleep Med* (2009) 7:245–54. doi: 10.1080/15402000903190215
60. Alders J, Smits M, Kremer B, Naarding P. The role of melatonin in sleep disturbances in end-stage Huntington's disease. *J Neuropsychiatry Clin Neurosci* (2009) 21:226–7. doi: 10.1176/appi.neuropsych.21.2.226
61. Aziz NA, Pijl H, Frölich M, Schröder-van der Elst JP, Bent C, Roelfsema F, et al. Delayed onset of the diurnal melatonin rise in patients with Huntington's disease. *J Neurol* (2009) 256:1961–5. doi: 10.1007/s00415-009-5196-1
62. van Wamelen DJ, Roos RA, Aziz NA. Therapeutic strategies for circadian rhythm and sleep disturbances in Huntington disease. *Neurodegener Dis Manag* (2015) 5:549–59. doi: 10.2217/nmt.15.45
63. Kalliolia E, Silajdžić E, Nambron R, Hill NR, Doshi A, Frost C, et al. Plasma melatonin is reduced in Huntington's disease. *Mov Disord* (2014) 29:1511–5. doi: 10.1002/mds.26003
64. Kudo T, Schroeder A, Loh DH, Kuljis D, Jordan MC, Roos KP, et al. Dysfunctions in circadian behavior and physiology in mouse models of Huntington's disease. *Exp Neurol* (2011) 228:80–90. doi: 10.1016/j.expneurol.2010.12.011
65. Rudenko O, Tkach V, Berezin V, Bock E. Detection of early behavioral markers of Huntington's disease in R6/2 mice employing an automated social home cage. *Behav Brain Res* (2009) 203:188–99. doi: 10.1016/j.bbr.2009.04.034
66. Kassubek J, Juengling FD, Kioschies T, Henkel K, Karitzky J, Kramer B, et al. Topography of cerebral atrophy in early Huntington's disease: a voxel based morphometric MRI study. *J Neurol Neurosurg Psychiatry* (2004) 75:213–20. doi: 10.1136/jnnp.2002.009019
67. Pallier PN, Maywood ES, Zheng Z, Chesham JE, Inyushkin AN, Dyball R, et al. Pharmacological imposition of sleep slows cognitive decline and reverses dysregulation of circadian gene expression in a transgenic mouse model of Huntington's disease. *J Neurosci Res* (2007) 27:7869–78. doi: 10.1523/JNEUROSCI.0649-07.2007
68. Julien CL, Thompson JC, Wild S, Yardumian P, Snowden JS, Turner G, et al. Psychiatric disorders in preclinical Huntington's disease. *J Neurol Neurosurg Psychiatry* (2007) 78:939–43. doi: 10.1136/jnnp.2006.103309
69. Mayberg HS, Starkstein SE, Peyser CE, Brandt J, Dannals RF, Folstein SE. Paralimbic frontal lobe hypometabolism in depression associated with Huntington's disease. *Neurology* (1992) 42:1791–7. doi: 10.1212/WNL.42.9.1791
70. Hult Lundh S, Nilsson N, Soylu R, Kirik D, Petersen A. Hypothalamic expression of mutant huntingtin contributes to the development of depressive-like behavior in the BAC transgenic mouse model of Huntington's disease. *Hum Mol Genet* (2013) 22:3485–97. doi: 10.1093/hmg/ddt203
71. Almqvist EW, Bloch M, Brinkman R, Craufurd D, Hayden MR. A worldwide assessment of the frequency of suicide, suicide attempts, or psychiatric hospitalization after predictive testing for Huntington disease. *Am J Hum Genet* (1999) 64:1293–304. doi: 10.1086/302374
72. Brotini S, Gigli GL. Epidemiology and clinical features of sleep disorders in extrapyramidal disease. *Sleep Med* (2004) 5:169–79. doi: 10.1016/j.sleep.2003.10.012
73. Aziz NA, Anguelova GV, Marinus J, Lammers GJ, Roos RAC. Sleep and circadian rhythm alterations correlate with depression and cognitive impairment in Huntington's disease. *Parkinsonism Relat Disord* (2010) 16:345–50. doi: 10.1016/j.parkreldis.2010.02.009
74. Baker CR, Domínguez D JF, Stout JC, Gabery S, Churchyard A, Chua P, et al. Subjective sleep problems in Huntington's disease: a pilot investigation of the relationship to brain structure, neurocognitive, and neuropsychiatric function. *J Neurol Sci* (2016) 364:148–53. doi: 10.1016/j.jns.2016.03.021
75. Berger M, Riemann D. REM sleep in depression-an overview. *J Sleep Res* (1993) 2:211–23. doi: 10.1111/j.1365-2869.1993.tb00092.x
76. Anderson KE, Marshall FJ. Behavioral symptoms associated with Huntington's disease. In: Anderson KE, Weiner WJ, Lang AE, editors. *Behavioral neurology of movement disorders advances in neurology*. Philadelphia: Lippincott Williams & Wilkins (2005) p. 197–208.
77. Mano T, Shiozawa Z, Sobue I. Extrapyramidal involuntary movements during sleep. In: Broughton RJ, ed. *Henri Gastaut and the Marseilles School's Contribution to the Neurosciences: Proceedings of the 25th and Final Colloque de Marseille, Electroencephalogr Clin Neurophysiol Suppl*. Amsterdam: Elsevier Biomedical Press (1982) 35:431–42.
78. Neutel D, Tchikviladzé M, Charles P, Leu-Semenescu S, Roze E, Durr A, et al. Nocturnal agitation in Huntington disease is caused by arousal-related abnormal movements rather than by rapid eye movement sleep behavior disorder. *Sleep Med* (2015) 16:754–9. doi: 10.1016/j.sleep.2014.12.021
79. Fish DR, Sawyers D, Allen PJ, Blackie JD, Lees AJ, Marsden CD. The effect of sleep on the dyskinetic movements of Parkinson's disease, Gilles de la Tourette syndrome, Huntington's disease, and torsion dystonia. *Arch Neurol* (1991) 48:210–4. doi: 10.1001/archneur.1991.00530140106023
80. Morton AJ. Disintegration of the sleep-wake cycle and circadian timing in Huntington's disease. *J Neurosci Res* (2005) 25:157–63. doi: 10.1523/JNEUROSCI.3842-04.2005
81. Townhill J, Hughes AC, Thomas B, Busse ME, Price K, Dunnett SB, et al. Using Actiwatch to monitor circadian rhythm disturbance in Huntington' disease: a cautionary note. *J Neurosci Methods* (2016) 265:13–8. doi: 10.1016/j.jneumeth.2016.01.009
82. Piano C, Losurdo A, Della Marca G, Solito M, Calandra-Buonaura G, Provini F, et al. Polysomnographic findings and clinical correlates in Huntington disease: a cross-sectional cohort study. *Sleep* (2015) 38:1489–95. doi: 10.5665/sleep.4996
83. Comella CL. Sleep disorders in Parkinson's disease: an overview. *Mov Disord* (2007) 22 Suppl 1:367–73. doi: 10.1002/mds.21682
84. Evers S, Stögbauer F. Genetic association of Huntington's disease and restless legs syndrome? A family report. *Mov Disord* (2003) 18:225–7. doi: 10.1002/mds.10341
85. Savva E, Schnorf H, Burkhard PR. Restless legs syndrome: an early manifestation of Huntington's disease? *Acta Neurologica Scandinavica* (2009) 119:274–6. doi: 10.1111/j.1600-0404.2008.01091.x
86. Kurtis MM, Balestrino R, Rodriguez-Blazquez C, Forjaz MJ, Martinez-Martin P. A review of scales to evaluate sleep disturbances in movement disorders. *Front Neurol* (2018) 9:1–20. doi: 10.3389/fneur.2018.00369
87. Rodríguez-Blázquez C, Forjaz MJ, Kurtis MM, Balestrino R, Martinez-Martin P. Rating scales for movement disorders with sleep disturbances: a narrative review. *Front Neurol* (2018) 9:435. doi: 10.3389/fneur.2018.00435
88. Chaudhuri KR, Prieto-Jurcynska C, Naidu Y, Mitra T, Frades-Payo B, Tluk S, et al. The nondeclaration of nonmotor symptoms of Parkinson's disease to health care professionals: an international study using the nonmotor symptoms questionnaire. *Mov Disord* (2010) 25:704–9. doi: 10.1002/mds.22868
89. Maskevich S, Jumabhoy R, Dao PDM, Stout JC, Drummond SPA. Pilot validation of ambulatory activity monitors for sleep measurement in Huntington's disease gene carriers. *J Huntingtons Dis* (2017) 6:249–53. doi: 10.3233/JHD-170251
90. Adams JL, Dinesh K, Xiong M, Tarolli CG, Sharma S, Sheth N, et al. Multiple wearable sensors in Parkinson and Huntington disease individuals: a pilot study in clinic and at home. *Digit Biomark* (2017) 1:52–63. doi: 10.1159/000479018
91. Piano C, Della Marca G, Losurdo A, Imperatori C, Solito M, Calandra-Buonaura G, et al. Subjective assessment of sleep in Huntington disease: reliability of sleep questionnaires compared to polysomnography. *Neurodegener Dis* (2017) 17:330–7. doi: 10.1159/000480701
92. Pidgeon C, Rickards H. The pathophysiology and pharmacological treatment of Huntington disease. *Behav Neurol* (2013) 26:245–53. doi: 10.3233/BEN-2012-120267

93. Frank S. Treatment of Huntington's disease. *Neurotherapeutics* (2014) 11:153–60. doi: 10.1007/s13311-013-0244-z
94. Ferreira J, Galitzky M, Montastruc J, Rascol O. Sleep attacks and Parkinson's disease treatment. *Lancet* (2000) 355:1333–4. doi: 10.1016/S0140-6736(00)02119-X
95. Mason SL, Barker RA. Advancing pharmacotherapy for treating Huntington's disease: a review of the existing literature. *Expert Opin Pharmacother* (2016) 17:41–52. doi: 10.1517/14656566.2016.1109630
96. Schiefer J, Werner CJ, Reetz K. Compensation in the course of Huntington's disease - More than just a hypothesis? *Degener Neurol Neuromuscul Dis* (2015) 5:37–50. doi: 10.1016/j.ebiom.2015.09.024
97. Blackwell AD, Paterson NS, Barker RA, Robbins TW, Sahakian BJ. The effects of modafinil on mood and cognition in Huntington's disease. *Psychopharmacology* (2008) 199:29–36. doi: 10.1007/s00213-008-1068-0
98. Srinivasan V, Pandi-Perumal S, Cardinali D, Poeggeler B, Hardeland R. Melatonin in Alzheimer's disease and other neurodegenerative disorders. *Behav Brain Funct* (2006) 2:15. doi: 10.1186/1744-9081-2-15
99. Dowling GA, Mastick J, Colling E, Carter JH, Singer CM, Aminoff MJ. Melatonin for sleep disturbances in Parkinson's disease. *Sleep Med* (2005) 6:459–66. doi: 10.1016/j.sleep.2005.04.004
100. Mayo JC, Sainz RM, Tan D-X, Antolín I, Rodríguez C, Reiter RJ. Melatonin and Parkinson's disease. *Endocrine* (2005) 27:169–78. doi: 10.1385/ENDO:27:2:169

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.

frontiers in Psychiatry

CASE REPORT
published: 02 May 2019
doi: 10.3389/fpsyt.2019.00270

Schizophrenia and Hereditary Polyneuropathy: *PMP22* Deletion as a Common Pathophysiological Link?

*Dominique Endres[1,2], Simon J. Maier[1,2], Christiane Ziegler[2], Kathrin Nickel[1,2], Anne N. Riering[2], Benjamin Berger[3], Johann Lambeck[3], Miriam Fritz[3], Birgitta Gläser[4], Friedrich Stock[5], Michael Dacko[6], Thomas Lange[6], Irina Mader[7,8], Katharina Domschke[2] and Ludger Tebartz van Elst[1,2]**

[1] Section for Experimental Neuropsychiatry, Department of Psychiatry and Psychotherapy, Medical Center—University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, [2] Department of Psychiatry and Psychotherapy, Medical Center—University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, [3] Department of Neurology, Medical Center—University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, [4] Institute of Human Genetics, Medical Center—University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, [5] Institute of Human Genetics, University Hospital Münster, Münster, Germany, [6] Department of Radiology, Medical Physics, Medical Center—University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, [7] Department of Neuroradiology, Medical Center—University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, [8] Department of Radiology, Clinic Schön Vogtareuth, Vogtareuth, Germany

OPEN ACCESS

Edited by:
Francesca Trojsi,
Università degli Studi della Campania Luigi Vanvitelli Caserta, Italy

Reviewed by:
Antonio Lucio Teixeira, University of Texas Health Science Center at Houston, United States
Daniele Coraci, Università Cattolica del Sacro Cuore, Italy
Raffaele Dubbioso, Azienda Ospedaliera Universitaria Federico II, Italy

***Correspondence:**
Ludger Tebartz van Elst
tebartzvanelst@uniklinik-freiburg.de

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Psychiatry

Received: 23 December 2018
Accepted: 09 April 2019
Published: 02 May 2019

Citation:
Endres D, Maier SJ, Ziegler C, Nickel K, Riering AN, Berger B, Lambeck J, Fritz M, Gläser B, Stock F, Dacko M, Lange T, Mader I, Domschke K and Tebartz van Elst L (2019) Schizophrenia and Hereditary Polyneuropathy: PMP22 Deletion as a Common Pathophysiological Link? Front. Psychiatry 10:270. doi: 10.3389/fpsyt.2019.00270

Background: Schizophrenic disorders are common and debilitating due to their symptoms, which can include delusions, hallucinations, and other negative symptoms. Organic forms can result from various cerebral disorders. In this paper, we discuss a potential association between schizophrenia and hereditary polyneuropathies (PNPs).

Case presentation: We present the case of a 55-year-old female patient with chronically paranoid–hallucinatory schizophrenia, severe cognitive deficits since the age of 30, and comorbid repeated focal pressure neuropathies beginning at age 20. At the age of 35, genetic testing revealed a deletion on chromosome 17p12 covering the peripheral myelin protein 22 gene (*PMP22*), which led to the diagnosis of hereditary neuropathy with liability to pressure palsy (HNPP). Cerebral magnetic resonance imaging showed internal atrophy, magnetic resonance spectroscopy found alteration of the glutamate and myo-inositol levels in the anterior cingulate cortex, neuropsychological testing showed deficits in working memory and psychomotor speed, and electrophysiological testing detected signs of sensorimotor demyelinating PNP (accentuated in the legs).

Conclusion: There may be an association between schizophrenia and HNPP. In observational studies, the deletion of interest (chromosome 17p12) was nearly 10 times more common in schizophreniform patients than in controls. This potential association could be pathophysiologically explained by the role of PMP22, which is mainly expressed in the peripheral nervous system. However, *PMP22* mRNA and protein can also be found in the brain. PMP22 seems to play an important role in regulating cell growth and myelination, functions that are disturbed in schizophrenia. Such a connection obviously cannot be clarified on the basis of one case.

Future studies should analyze whether patients with HNPP exhibit increased rates of psychotic disorders, and patients with schizophrenia and repeated focal pressure neuropathies should be examined for the *PMP22* mutation. Alternatively, the co-occurrence of schizophrenia and HNPP could be coincidental.

Keywords: hereditary polyneuropathy, schizophrenia, psychosis, *PMP22*, hereditary neuropathy with liability to pressure palsy

BACKGROUND

Schizophrenia is a common disorder with a prevalence rate of about 1% (1). The clinical presentation is characterized by hallucinations, delusions, loss of self-boundaries, disorganized thinking and speech, cognitive deficits, lack of motivation, and social withdrawal (1). Secondary, organic forms can result from various cerebral disorders that are caused by genetic (22q11 deletion syndrome, cerebrotendinous xanthomatosis, Niemann–Pick type C, etc.), immunological (limbic encephalitis, anti-NMDA-R encephalitis, Hashimoto encephalopathy, etc.), infectious (neuroborreliosis, neurosyphilis, etc.), epileptic (paraepileptic psychosis, etc.), traumatic (traumatic brain injury), or neurodegenerative (frontotemporal dementia, etc). factors (2, 3).

The familial aggregation of schizophrenia is well established (1, 4). A variety of single-nucleotide polymorphisms (SNPs) in hundreds to thousands of genes have been implicated in the pathophysiology of the endogenous variants of schizophrenia (5). In these complex genetic forms, single genes only have a small effect size, and environmental factors are further important modulators. In contrast, secondary genetic forms are either monogenetic or oligogenetic: here, only one or a small number of genetic variants are important for the expression of clinical characteristics, such as copy number variants (CNVs), which are defined by deletions, duplications, or insertions of deoxyribonucleic acid (DNA) fragments, and chromosome aberrations (6, 7). In these forms, genetic variation in single genes has a high effect size, and environmental factors are considered to be less important (3). Secondary monogenic or oligogenic forms may potentially also occur in the context of hereditary polyneuropathies (hPNPs) (8). However, the association between schizophrenia and hPNPs is largely unknown. The hPNPs include isolated hPNPs (hereditary sensory neuropathy, hereditary sensory and autonomic neuropathy, and hereditary neuropathy with liability to pressure palsy or HNPP) and hPNPs in the context of systemic disorders (e.g., acute intermittent porphyria and Fabry's disease). These systemic disorders are characterized by a monogenic or oligogenic background similar to that of secondary schizophrenia.

Searching PubMed for "hereditary polyneuropathy AND (schizophrenia OR psychosis)" yielded only eight results (as of 23 December 2018). One observational study discussed an association between hereditary spastic paraparesis and schizophrenia (8). In addition, an association between hereditary spastic paraplegia and psychosis in a female patient due to dysmorphic changes in her corpus callosum has been described independently (9). No results were returned when the same literature research was performed using "HNPP AND (schizophrenia OR psychosis)". A nonsystematic literature search also showed that there might be a link between transthyretin-associated polyneuropathy (PNP) and schizophrenia or depression (10). Charcot–Marie–Tooth disorder, which is caused by a duplication of the *PMP22* gene, and coincident psychosis were reported in monozygotic twins (11, 12).

CASE PRESENTATION

We present the case of a 55-year-old female Caucasian patient trained as an occupational therapist who has suffered from chronic paranoid–hallucinatory schizophrenia since the age of 30. She continuously showed positive symptoms with superimposed exacerbations. At the age of 34, she was forced to retire early from her career due to her illness. Her delusions included the idea that she had sinned and needed to die, and she perceived diverse signs as confirmation of these delusions. She suffered from auditory hallucinations (voices from God, the devil, and her dead partner or mother), visual hallucinations (visions of angels), and a loss of self-boundaries (believing that other people could read her thoughts). Negative symptoms included a lack of motivation, flattened mood, and social withdrawal. Cognitive impairment has been observed since the onset of psychotic symptoms, with inattention and increasing deficits in working memory.

Intermittently, the patient abused alcohol (at least four beers per day) and benzodiazepines, but no illegal drugs. Her consumption of these substances increased during psychotic exacerbations with social withdrawal. The early death of her life partner reinforced this withdrawal. Since the onset of the disease, she had attempted suicide 10 times. Therefore, there were frequent inpatient stays in different psychiatric hospitals. Neither various neuroleptic treatments with average or high doses of aripiprazole, amisulpride, clozapine, haloperidol, perazine, pimozide, quetiapine, and risperidone, nor anticonvulsive treatment with valproate as an augmentation strategy led to full remission. Under different combination treatments, the described symptoms persisted at a reduced level.

At age 20, the patient developed clinical signs of HNPP. Initially, she was quickly fatigued and showed transient hypoesthesia of the left arm and foot. She developed transient foot dorsi-flexor paresis twice on the right side. The symptoms occurred after mechanical pressure on the corresponding body regions. In the further course of the disease, she developed transient left brachial plexus paresis at the age of 32 and again at the age of 35. When she was 35, genetic testing revealed a deletion on chromosome 17p12

involving the peripheral myelin protein 22 gene (*PMP22*), confirming the diagnosis of HNPP. The most recent neurological examination showed a discrete foot dorsi-flexor paresis on the right side (Medical Research Council score M4), absent Achilles tendon reflexes on both sides, slight pallhypesthesia of the lower extremity accentuated on the right side (5/8 on the right versus 6/8 on the left), and an ataxic and unsteady gait.

Developmental, Somatic, and Family History

The patient's developmental history was negative for *in utero* or birth complications, febrile convulsions, inflammatory brain diseases, and cerebral contusions. There was no evidence of any neurodevelopmental disorder. In the first two decades, the patient's premorbid personality showed dependent traits. Her somatic medical history included only hypothyroidism, which was diagnosed at age 43. Her family history was positive for schizophrenia and HNPP. Her father's half-brother suffered from schizophrenia. The patient's older brother (transient hypesthesia of parts of one hand; side and explicit location unclear) and father (paresis of the shoulder abductors) potentially also suffered from HNPP (no genetic diagnostics performed, clinical reports not available). Her younger brother and her mother were healthy.

Basic Investigations and Magnetic Resonance Spectroscopy

The serum, cerebrospinal fluid, and cerebral magnetic resonance imaging (cMRI) investigations did not show any evidence of an immunological cause for schizophrenia or PNPs. The electrophysiological tests revealed sensorimotor, demyelinating PNP (accentuated in the legs). Neurosonography showed bilateral enlargement of the median and ulnar nerve at typical sides of entrapment syndromes. Neuropsychological testing showed considerable deficits in working memory and a mild deficit in psychomotor speed. The electroencephalography (EEG) showed rare intermittent slowing. The cMRI showed generalized atrophy (**Figure 1**). Magnetic resonance spectroscopy (MRS) was performed in the dorsolateral prefrontal cortex (DLPFC), the dorsal anterior cingulate cortex (dACC), and the pregenual anterior cingulate cortex (pACC) using a MEGA PRESS sequence (repetition time = 1,500 ms, echo time = 68 ms, flip angle = 90°) and the acquired spectra were quantified with the software LCModel. Glutamate concentrations in the dACC and pACC were relatively high and outside the 90% reference intervals. The myo-inositol concentrations in the pACC were relatively low and within the 90% reference interval (**Figure 2**). The DLPFC measurement displayed poor spectral quality and was therefore not analyzed. The diagnostic findings are summarized in **Table 1**.

Genetic Investigations: Cytogenetic and Array Analysis

MLPA (multiplex ligation-dependent probe amplification) analysis revealed a heterozygous deletion of the *PMP22* gene on chromosome 17p12. To delineate the extent of this deletion, we additionally performed conventional karyotyping and array CGH (comparative genomic hybridization).

Conventional R-banded karyotypes from the patient were analyzed according to standard protocols with a resolution of approximately 500 bphs and revealed a structurally and numerically normal female karyotype (46,XX) in all 28 metaphases examined. Furthermore, the genomic DNA of the patient was examined by microarray analysis (CytoSureTM constitutional v3 array 180k; Oxford Gene Technology) according to the manufacturer's instructions. After hybridization, the array was scanned with the SureScan microarray scanner (Agilent); the results were analyzed using CytoSure interpret software v.4.9 (Oxford Gene Technology) and the Genome Reference Consortium human genome GRCh37 (hg19). Molecular karyotyping revealed a heterozygous deletion of approximately 1.33 Mb (125 contiguous oligonucleotides) out of the chromosomal region 17p12 (karyotype after the International System for Human Cytogenetic Nomenclature, 2016: arr[GRCh37] 17p12(14111972_15442257)x1). The deletion encompasses i.a. the genes *COX10*, *HS3ST3B1*, *PMP22*, *TEKT2*, and *CDRT4*. A deletion of that extent in 17p12 is found in nearly 80% of patients with HNPP (13). Further chromosomal deletions or duplications that might have etiologically contributed to our patient's disease were not detected.

Differential Diagnosis

The patient's psychiatric symptoms were compatible with paranoid–hallucinatory schizophrenia (ICD-10: F20.0). The PNPs in combination with the deletion of the *PMP22* gene led to the diagnosis of HNPP (ICD-10: G60.0). Due to the potential secondary schizophrenia in the context of HNPP, a psychotic

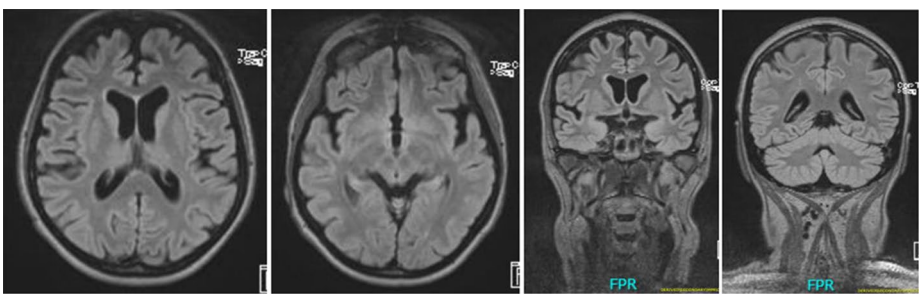

FIGURE 1 | Cerebral magnetic resonance (cMRI) imaging showing generalized atrophy with perisylvian accentuation.

TABLE 1 | Current diagnostic findings.

Serum analyses	• Blood cell count, electrolytes, liver/kidney values, HbA1c, cobalamin, folic acid, and immunofixation were normal. • Thyroid-stimulating hormone (TSH), triiodothyronine, and thyroxine levels were in normal ranges. Autoantibodies against thyroglobulin and thyroid peroxidase and against TSH receptor were not increased. • Screening for infections (borreliosis, lues, and HIV) was negative. • No antibodies against intracellular onconeural antigens (Yo, Hu, CV2/CRMP5, Ri, Ma1/2, SOX1) or intracellular synaptic antigens (GAD, amphiphysin) were found. • Screening for antinuclear antibodies (ANA), anti-neutrophil cytoplasmic antibodies (ANCA), antiphospholipid antibodies (APA), and rheumatoid factor (RF) was negative.	
Cerebrospinal fluid analyses	• Normal white blood cell count (1/µl; reference < 5/µl). • Increased protein concentration (615 mg/L; reference < 450 mg/L), but normal age-corrected albumin quotient: 7; age-dependent reference < 8×10^{-3}). • No CSF-specific oligoclonal bands; IgG Index not increase (0.45; reference ≤ 0.7). • Antibodies against neuronal cell surface antigens (*NMDAR, AMPA-R, GABA-B-R, VGKC-complex [LGI1, Caspr2]*) were negative.	
Cerebral magnetic resonance imaging	• Slight generalized atrophy with perisylvian accentuation. • Very mild microangipathic white matter lesions.	
Magnetic resonance spectroscopy	• dACC: High glutamate concentrations. • pACC: High glutamate and low myo-inositol concentrations. • DLPFC: Not analyzed due to bad spectral quality.	
Electroencephalography (including a hyperventilation episode)	• Alpha-rhythm, rare intermittent slowing, no epileptiform activity.	
Electrophysiological measurements	• Visual evoked potentials: normal. • Motor nerve conduction study (NCS): — Right ulnar nerve: increased distal motor latency, borderline distal nerve conduction velocity, significant reduction of the nerve conduction velocity in the cubital tunnel, F-waves not reproducible. — Right tibial nerve: increased distal motor latency, increased F-wave latency. — Left peroneal nerve: distal CMAP strongly reduced, proximal CMAP not reproducible, so nerve conduction velocity not measurable. • Sensory NCS: — Left radial nerve (superficial ramus): normal amplitude, reduced nerve conduction velocity. — Left sural nerve: sNAP not reproducible.	
Neurosonography	• Median nerve bilaterally enlarged in carpal tunnel (CSA carpal tunnel 19–21 mm^2, CSA forearm 7 mm^2, wrist-to-forearm ratio 2.7–3). • Ulnar nerve bilaterally enlarged in cubital tunnel (CSA cubital tunnel 12–17 mm^2, CSA upper arm 6–7 mm^2, humerus-to-elbow ratio 1.7–2.8).	
Genetic testing	• MLPA analysis: heterozygous deletion of the *PMP22* gene. • Karyotyping: normal female karyotype 46,XX. • DNA microarray (array CGH): heterozygous microdeletion on chromosome 17p12 (1.33 Mb—125 contiguous oligonucleotides)	
Neuropsychological tests and *z* values*	• Working memory (digit span—WAIS-IV)	–2.0**
	• Verbal learning (VLMT)	–0.9
	• Verbal memory (VLMT)	–0.1
	• Working speed (digit symbol—WAIS-V)	–1.0**
	• Phonematic fluency (RWT)	0.4
	• Semantic fluency (RWT)	–0.7

CSA, cross-sectional area

**Neuropsychological assessment was interrupted repeatedly due to the patient's spontaneous delusional utterances and experiences. This might reduce the validity of test results.*

***Below average.*

disorder with delusions due to a known physiological condition might ultimately be diagnosed (ICD-10: F06.2).

DISCUSSION

In this paper, we present the case of a female patient with chronic paranoid–hallucinatory schizophrenia with poor response to therapy and comorbid HNPP.

HNPP, *PMP22* Deletion, and Central Nervous System Involvement

HNPP is an autosomal-dominant, peripheral neuropathy characterized by repeated and transient episodes of focal pressure neuropathies at compression-exposed sites (e.g., brachial plexus, sciatic nerve) (14). Our patient suffered from repeated foot flexor paresis and twice experienced brachial plexus paresis. In HNPP patients, nerve conduction study (NCS) often reveals demyelinating PNP with nerve suffering located predominantly in entrapment sites (15). In our patient, NCS showed findings compatible with sensorimotor, demyelinating PNPs with an entrapment syndrome in the right cubital tunnel. Histologically, sural nerve biopsies of HNPP patients typically display tomaculae, which are redundantly overfolded layers in the myelin coat (16). Genetically, a 1.5-Mb deletion on chromosome 17p12, including the *PMP22* gene, is found in most cases (17). Molecular karyotyping in our patient showed a heterozygous deletion of 1.33 Mb.

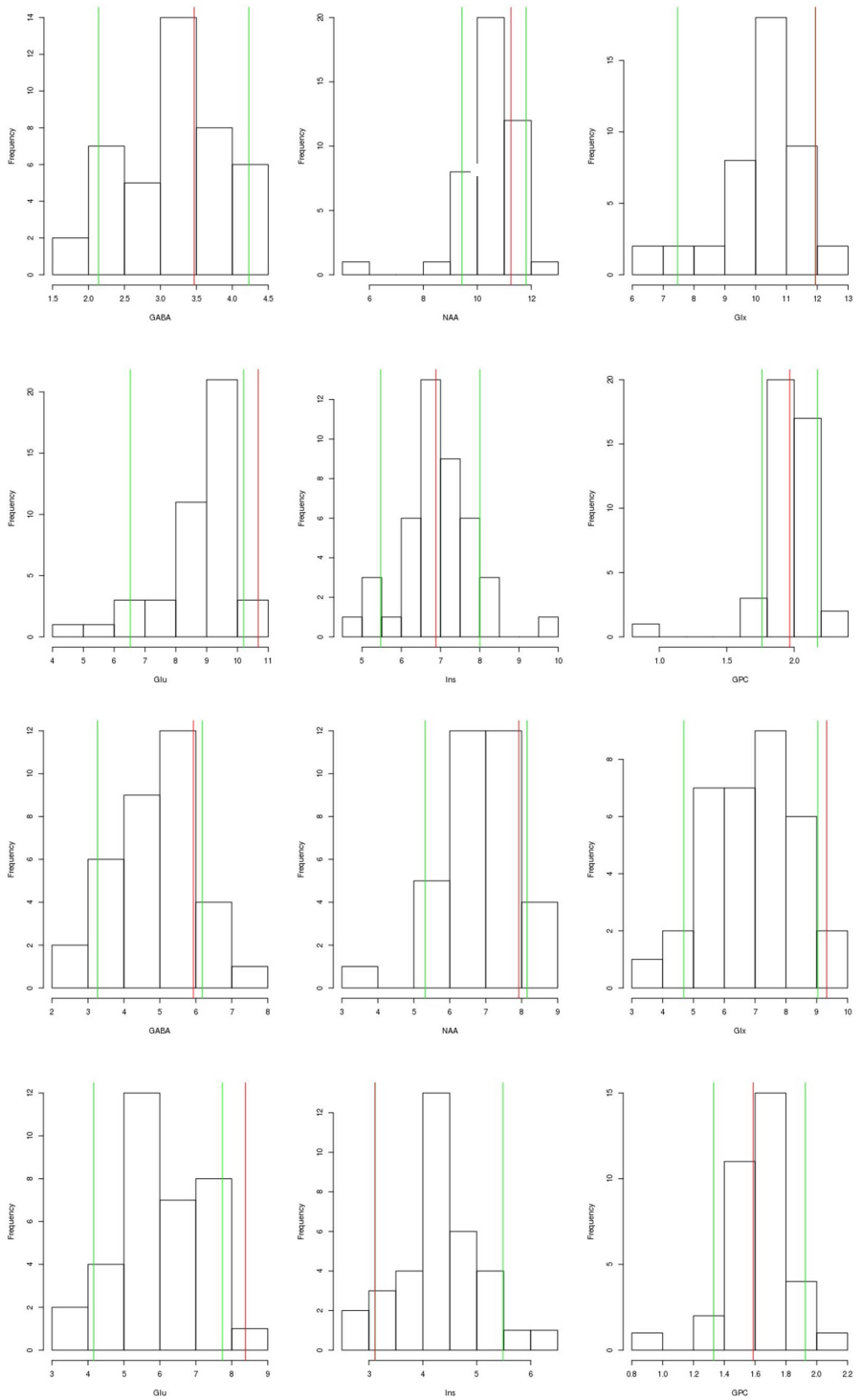

FIGURE 2 | Magnetic resonance spectroscopy (MRS) findings: We present the findings of the dorsal anterior cingulate cortex (dACC; the two upper rows) and the pregenual anterior cingulate cortex (pACC; lower rows). The patient's values (red line) are compared to the values of a healthy control group measured in another study (N = 43, mean age: 35, gender ratio: 14 females/29 males). The green line shows the 90% reference interval of the control group. For Glx in the dACC and Ins in the pACC the green (90% reference interval) and the red line (patient's values) are superimposed, therefore only the red line is visible. The measured metabolite concentrations have been corrected for MRS voxel composition (content of gray matter, white matter, and cerebrospinal fluid), as well as for the influence of age and differences in the signal-to-noise ratio of the MRS measurements. Abbreviations: GABA, gamma-aminobutyric acid including coedited macromolecules; NAA, N-acetylaspartate; Glx, glutamate and glutamine; Glu, glutamate; Ins, myo-inositol; GPC, glycerophosphorylcholine.

The *PMP22* gene is mainly expressed in the peripheral nervous system (PNS); however, mRNA and protein can also be found in the central nervous system (CNS) (18). Recent studies suggested the important involvement of the CNS in most patients with *PMP22* deletion (18, 19). This is supported by prolonged latencies of visual evoked potentials, neurochemical alterations with decreased *N*-acetylaspartate (NAA) and creatine (Cre) concentrations, white matter (WM) volume reduction detected by cMRI, cognitive impairment (in 70% of patients), and fractional anisotropy alteration in several WM regions (e.g., in the columns of the fornix) (18–20). A large family study described CNS involvement and WM lesions predominantly in the subcortical frontal WM (21). Therefore, in line with the studies described, we hypothesize that our patient's schizophreniform symptoms may represent CNS involvement. This consideration is clinically supported by an insufficient therapy response to neuroleptics, severe cognitive deficits, EEG slowing, internal brain atrophy, and neurometabolic alterations (high glutamate in the dACC and pACC and low myo-inositol in the pACC). Compared with the patient group from Chanson and colleagues, we also found brain atrophy and neuropsychological deficits; however, in the presented case, NAA concentration and visual evoked potentials were normal, and DTI measurements were not performed (18). A causal relationship between the poor response to therapy and the *PMP22* deletion remains speculative and is not proven by the case report. However, our hypothesis is also supported by several epidemiological and pathophysiological ideas, which we discuss in detail in the following paragraphs along with potential limitations.

PMP22 Deletion in Patients With Schizophrenia

Copy number variation in the chromosomal region 17p12 has repeatedly been implicated in schizophrenia (22–25). In a study analyzing the involvement of rare CNVs in 471 patients with schizophrenia, a deletion on 17p12 was found in two patients but not in controls. Therefore, the authors reanalyzed the data from two recent, large CNV studies of schizophrenia and found a *PMP22* deletion in 6 out of 4,618 (0.13%) patients and 6 out of 36,092 (0.017%) controls (26–28). The data demonstrate that the described 17p12 deletion can be found nearly 10 times more often in schizophrenic patients compared to healthy controls (28). Moreover, there is at least one case report presenting a patient with schizophrenia in combination with mental retardation and *PMP22* deletion without hPNPs (29).

Pathophysiological Considerations

PMP22 is a small, hydrophobic membrane glycoprotein that is mostly expressed by Schwann cells and comprises 2–5% of PNS myelin proteins in humans (16, 30). *PMP22* mRNA and protein were also detected in the CNS, specifically in most parts of the brain (especially the corpus callosum) and the spinal cord. Changes in *PMP22* mRNA may explain myelin abnormalities because it plays a role in the regulation of cell growth (even in the absence of protein), which was first described in fibroblasts (30, 31). Reductions in *PMP22* mRNA have been observed in the hippocampus and anterior cingulate cortex in the postmortem brains of patients with schizophrenia (32). The PMP22 protein was restricted to a few areas [anterior horn and pia mater of the spinal cord, preganglionic sympathetic neurons; (33)]. In summary, *PMP22* mRNA and protein are important for the regulation of cell growth and the maintenance of myelin integrity and therefore ensure the propagation of action potential (16). Heterozygous deletion of the *PMP22* gene results in a loss-of-function phenotype (16) and altered myelination, as found in patients with schizophrenia (34).

Clinical Importance of Case Studies and Limitations

It is important to note that the co-occurrence of schizophrenia and HNPP could be coincidental, which is supported by the absence of schizophreniform symptoms in our patient's brother and father, who both very likely suffered from HNPP. However, genetic analyses of the patient's brother and father were not performed to our knowledge. So, we can only speculate whether the discrepancy in psychiatric symptoms between our patient and her brother and father could be due to a variable expression and an incomplete penetrance, respectively, of the genetic effect of the *PMP22* deletion. Besides environmental factors, further genetic variants might have contributed to our patient's disease, particularly since the presently identified chromosomal deletion additionally encompasses *COX10*, *HS3ST3B1*, *TEKT2*, and *CDRT4*. However, the detected radiological and neuropsychological findings can also be found in schizophrenia; therefore, they are not clearly associated with the PMP22 deletion (34).

Case studies reporting such potential associations are essential to inspire further clinical trials (35). Retrospective studies analyzing psychiatric comorbidity in patients with HNPP could demonstrate whether there is a relevant association between the disorders and confirm or refute the pathophysiological role of *PMP22* deletion in a subgroup of patients with schizophrenia.

CONCLUSION

There may be an association between schizophrenia and HNPP, which could be explained by the role of *PMP22* in regulating cell growth and myelination. If such an association existed, which, of course, cannot be clarified from one case, then it might explain the poor control of psychiatric symptoms in our patient. But *PMP22* deletion does not necessarily mean causality for the emergence of psychotic symptoms. For further clarification, patients with psychotic disorders and repeated transient focal neurological symptoms (which could be explained by pressure neuropathies) should be examined for *PMP22* gene variation. Future studies should analyze whether

patients with HNPP exhibit increased rates of schizophrenia. Alternatively, the co-occurrence of schizophrenia and HNPP could be coincidental.

ETHICS STATEMENT

The patient has given her signed, written informed consent for this case report, including the images presented, to be published.

AUTHOR CONTRIBUTIONS

DE and LT treated the patient. DE performed the data research and wrote the paper. SM, KN, MD, TL, and IM performed and interpreted the magnetic resonance spectroscopy. FS and BG performed the genetic testing. CZ and KD supported the interpretation of genetic findings. BB, JL, and MF performed and interpreted the electrophysiological measurements, neurosonography, neurological examination, and CSF measurements. IM performed and interpreted the cMRI. AR performed the neuropsychological testing. All authors were critically involved in the theoretical discussion and composition of the manuscript. All authors read and approved the final version of the manuscript.

FUNDING

The article processing charge was funded by the German Research Foundation (DFG) and the University of Freiburg in the funding program Open Access Publishing.

REFERENCES

1. Owen MJ, Sawa A, Mortensen PB. Schizophrenia. *Lancet* (2016) 2388(10039):86–97. doi: 10.1016/S0140-6736(15)01121-6
2. Lishman A. *Lishman's organic psychiatry—a textbook of neuropsychiatry*. Fourth Edition: Wiley-Blackwell (2009).
3. Tebartz van Elst L. *Vom Anfang und Ende der Schizophrenie: Eine neuropsychiatrische Analyse des Schizophreniekonzepts*. Stuttgart: Kohlhammer (2017).
4. Rees E, Kirov G, Walters JT, Richards AL, Howrigan D, Kavanagh DH, et al. Analysis of exome sequence in 604 trios for recessive genotypes in schizophrenia. *Transl Psychiatry* (2015) 215:e607. doi: 10.1038/tp.2015.99
5. Ripke S, O'Dushlaine C, Chambert K, Moran JL, Kähler AK, Akterin S, et al. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. *Nat Genet* (2013) 45(10):1150–9. doi: 10.1038/ng.2742
6. Grayton HM, Fernandes C, Rujescu D, Collier DA. Copy number variations in neurodevelopmental disorders. *Prog Neurobiol* (2012) 99(1):81–91. doi: 10.1016/j.pneurobio.2012.07.005
7. Marshall CR, Howrigan DP, Merico D, Thiruvahindrapuram B, Wu W, Greer DS, et al. Contribution of copy number variants to schizophrenia from a genome-wide study of 41,321 subjects. *Nat Genet* (2017) 49(1):27–35. doi: 10.1038/ng.3725
8. McMonagle P, Hutchinson M, Lawlor B. Hereditary spastic paraparesis and psychosis. *Eur J Neurol* (2006) 13(8):874–9. doi: 10.1111/j.1468-1331.2006.01379.x
9. Osmolak AM, Wallenberg RB, Caplan JP. Hereditary spastic paraplegia and psychosis: connected by the corpus callosum? *Psychosomatics* (2012) 53(1):81–4. doi: 10.1016/j.psym.2011.05.004
10. Fleming CE, Nunes AF, Sousa MM. Transthyretin: more than meets the eye. *Prog Neurobiol* (2009) 89(3):266–76. doi: 10.1016/j.pneurobio.2009.07.007
11. Manyam NV, Cowell HR, Katz L. Charcot–Marie–Tooth disease and schizophrenia in identical twins. *JAMA* (1979) 241(1):54–5. doi: 10.1001/jama.1979.03290270044020
12. Birouk N, Gouider R, Le Guern E, Gugenheim M, Tardieu S, Maisonobe T, et al. Charcot-Marie-Tooth disease type 1A with 17p11.2 duplication. Clinical and electrophysiological phenotype study and factors influencing disease severity in 119 cases. *Brain* (1997) 120(Pt 5):813–23. doi: 10.1093/brain/120.5.813
13. Chance PF, Abbas N, Lensch MW, Pentao L, Roa BB, Patel PI, et al. Two autosomal dominant neuropathies result from reciprocal DNA duplication/deletion of a region on chromosome 17. *Hum Mol Genet* (1994) 3(2):223–8. doi: 10.1093/hmg/3.2.223
14. Luigetti M, Del Grande A, Conte A, Lo Monaco M, Bisogni G, Romano A, et al. Clinical, neurophysiological and pathological findings of HNPP patients with 17p12 deletion: a single-centre experience. *J Neurol Sci* (2014) 341(1-2):46–50. doi: 10.1016/j.jns.2014.03.046
15. Hong YH, Kim M, Kim HJ, Sung JJ, Kim SH, Lee KW. Clinical and electrophysiologic features of HNPP patients with 17p11.2 deletion. *Acta Neurol Scand* (2003) 108(5):352–8. doi: 10.1034/j.1600-0404.2003.00132.x
16. Li J, Parker B, Martyn C, Natarajan C, Guo J. The PMP22 gene and its related diseases. *Mol Neurobiol* (2013) 47(2):673–98. doi: 10.1007/s12035-012-8370-x
17. Chance PF, Alderson MK, Leppig KA, Lensch MW, Matsunami N, Smith B, et al. DNA deletion associated with hereditary neuropathy with liability to pressure palsies. *Cell* (1993) 1572(1):143–51. doi: 10.1016/0092-8674(93)90058-X
18. Chanson JB, Echaniz-Laguna A, Blanc F, Lacour A, Ballonzoli L, Kremer S, et al. Central nervous system abnormalities in patients with PMP22 gene mutations: a prospective study. *J Neurol Neurosurg Psychiatry* (2013) 84(4):392–7. doi: 10.1136/jnnp-2012-303725
19. Brandt AU, Meinert-Bohn E, Rinnenthal JL, Zimmermann H, Mikolajczak J, Oberwahrenbrock T. Afferent visual pathway affection in patients with PMP22 deletion-related hereditary neuropathy with liability to pressure palsies. *PLoS One* (2016) 11(10):e0164617. doi: 10.1371/journal.pone.0164617
20. Wang WW, Song CL, Huang L, Song QW, Liang ZH, Wei Q, et al. DTI study of cerebral normal-appearing white matter in hereditary neuropathy with liability to pressure palsies (HNPP). *Medicine (Baltimore)* (2015) 94(43):e1909. doi: 10.1097/MD.0000000000001909
21. Sanahuja J, Franco E, Rojas-García R, Gallardo E, Combarros O, Begué R, et al. Central nervous system involvement in hereditary neuropathy with liability to pressure palsies: description of a large family with this association. *Arch Neurol* (2005) 62(12):1911–4. doi: 10.1001/archneur.62.12.1911
22. Magri C, Sacchetti E, Traversa M, Valsecchi P, Gardella R, Bonvicini C, et al. New copy number variations in schizophrenia. *PLoS One* (2010) 135(10):e13422. doi: 10.1371/journal.pone.0013422
23. Vassos E, Collier DA, Holden S, Patch C, Rujescu D, St Clair D, et al. Penetrance for copy number variants associated with schizophrenia. *Hum Mol Genet* (2010) 119(17):3477–81. doi: 10.1093/hmg/ddq259
24. Crespi BJ, Crofts HJ. Association testing of copy number variants in schizophrenia and autism spectrum disorders. *J Neurodev Disord* (2012) 304(1):15. doi: 10.1186/1866-1955-4-15
25. Grozeva D, Conrad DF, Barnes CP, Hurles M, Owen MJ, O'Donovan MC, et al. WTCCC. Independent estimation of the frequency of rare CNVs in the UK population confirms their role in schizophrenia. *Schizophr Res* (2012) 135(1-3):1–7. doi: 10.1016/j.schres.2011.11.004
26. Stefansson H, Rujescu D, Cichon S, Pietiläinen OP, Ingason A, Steinberg S, et al. Large recurrent microdeletions associated with schizophrenia. *Nature* (2008) 11455(7210):232–6. doi: 10.1038/nature07229
27. International Schizophrenia Consortium. Rare chromosomal deletions and duplications increase risk of schizophrenia. *Nature* (2008) 455(7210):237–41. doi: 10.1038/nature07239

28. Kirov G, Grozeva D, Norton N, Ivanov D, Mantripragada KK, Holmans P. International Schizophrenia Consortium; Wellcome Trust Case Control Consortium, Craddock N, Owen MJ, O'Donovan MC. Support for the involvement of large copy number variants in the pathogenesis of schizophrenia. *Hum Mol Genet* (2009) 1518(8):1497–503. doi: 10.1093/hmg/ddp043
29. Kunugi H, Ozeki Y, Mizuguchi T, Hirabayashi N, Ogawa M, Ohmura N, et al. A case of schizophrenia with chromosomal microdeletion of 17p11.2 containing a myelin-related gene PMP22. *Open Psychiatry J* (2008) 2:1–4. doi: 10.2174/1874354400802010001
30. Naef R, Suter U. Many facets of the peripheral myelin protein PMP22 in myelination and disease. *Microsc Res Tech* (1998) 41(5):359–71. doi: 10.1002/(SICI)1097-0029(19980601)41:5<359::AID-JEMT3>3.0.CO;2-L
31. Snipes GJ, Suter U, Welcher AA, Shooter EM. Characterization of a novel peripheral nervous system myelin protein (PMP-22/SR13). *J Cell Biol* (1992) 117(1):225–38. doi: 10.1083/jcb.117.1.225
32. Dracheva S, Davis KL, Chin B, Woo DA, Schmeidler J, Haroutunian V. Myelin-associated mRNA and protein expression deficits in the anterior cingulate cortex and hippocampus in elderly schizophrenia patients. *Neurobiol Dis* (2006) 21(3):531–40. doi: 10.1016/j.nbd.2005.08.012
33. Ohsawa Y, Murakami T, Miyazaki Y, Shirabe T, Sunada Y. Peripheral myelin protein 22 is expressed in human central nervous system. *J Neurol Sci* (2006) 15247(1):11–5. doi: 10.1016/j.jns.2006.03.004
34. Davis KL, Stewart DG, Friedman JI, Buchsbaum M, Harvey PD, Hof PR, et al. White matter changes in schizophrenia: evidence for myelin-related dysfunction. *Arch Gen Psychiatry* (2003) 60(5):443–56. doi: 10.1001/archpsyc.60.5.443
35. Albrecht J, Meves A, Bigby M. Case reports and case series from *Lancet* had significant impact on medical literature. *J Clin Epidemiol* (2005) 58(12):1227–32. doi: 10.1016/j.jclinepi.2005.04.003

Conflicts of Interest Statement: BB received travel grants and/or training expenses from Bayer Vital GmbH, Ipsen Pharma GmbH, Norvartis, Biogen GmbH, and Genzyme, as well as lecture fees from Ipsen Pharma GmbH, Alexion Pharma GmbH, Merck, Sanofi Genzyme, and Roche. IM received lecture fees from UCB Pharma GmbH, Germany. LTvE received advisory boards, lectures, or travel grants within the last 3 years: Eli Lilly, Janssen-Cilag, Novartis, Shire, UCB, GSK, Servier, Janssen, and Cyberonics.

The remaining 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.

frontiers in Neuroscience

BRIEF RESEARCH REPORT
published: 10 May 2019
doi: 10.3389/fnins.2019.00440

Cognitive Syndromes and C9orf72 Mutation Are Not Related to Cerebellar Degeneration in Amyotrophic Lateral Sclerosis

Monica Consonni[1], Eleonora Dalla Bella[1], Anna Nigri[2], Chiara Pinardi[2], Greta Demichelis[2], Luca Porcu[3], Cinzia Gellera[4], Viviana Pensato[4], Stefano F. Cappa[5,6], Maria Grazia Bruzzone[2], Giuseppe Lauria[1,7] and Stefania Ferraro[2]*

[1] 3rd Neurology Unit and Motor Neuron Diseases Centre, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy, [2] Neuroradiology Department, Fondazione IRCCS Istituto Neurologico "Carlo Besta," Milan, Italy, [3] Laboratory of Methodology for Clinical Research, Oncology Department, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy, [4] Unit of Genetics of Neurodegenerative and Metabolic Diseases, Motor Neuron Diseases Centre, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy, [5] Institute for Advanced Study-IUSS Pavia, Pavia, Italy, [6] IRCCS Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy, [7] Department of Biomedical and Clinical Sciences "Luigi Sacco", University of Milan, Milan, Italy

OPEN ACCESS

Edited by:
Foteini Christidi,
National and Kapodistrian University of Athens, Greece

Reviewed by:
Michael Hornberger,
University of East Anglia, United Kingdom
Mattia Siciliano,
Second University of Naples, Italy

***Correspondence:**
Monica Consonni
monica.consonni@istituto-besta.it;
monicaconsonni@libero.it

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: 15 February 2019
Accepted: 17 April 2019
Published: 10 May 2019

Citation:
Consonni M, Dalla Bella E, Nigri A, Pinardi C, Demichelis G, Porcu L, Gellera C, Pensato V, Cappa SF, Bruzzone MG, Lauria G and Ferraro S (2019) Cognitive Syndromes and C9orf72 Mutation Are Not Related to Cerebellar Degeneration in Amyotrophic Lateral Sclerosis. Front. Neurosci. 13:440. doi: 10.3389/fnins.2019.00440

Objective: The notion that cerebellar pathology may contribute to cognitive impairment in ALS, especially in patients with C9orf72 repeated expansion, has been inconsistently reported. This study aimed exploring the relationship between cerebellar involvement, cognitive impairment and C9orf72 repeated expansion of patients with ALS.

Methods: Quantitative *in vivo* assessment of cerebellar lobules has been investigated in 66 non-demented patients with ALS and 28 healthy controls (HCs). Pathologic C9orf72 repeated expansion was found in 13 patients. Mild cognitive and/or behavioral impairment was diagnosed in 22 C9orf72 negative ALS patients. Measures of cortical volume (CV) and cortical thickness (CT) of cerebellar lobules of all participants were used for Principal Component Analysis (PCA) to identify clusters of lobular measures highly correlated with each other. PCA outcomes were used for between group comparisons and correlation analyses with neuropsychological and clinical features.

Results: Disease severity measured with ALS functional rating scale and index of disease progression rate significantly correlated with CV reduction of the second PCA cluster loading CV measures of anterior lobules. In all patients, cognitive impairment, measured with verbal fluency, was related to CV reduction of the third cluster comprising posterior lobules. No specific cortical thinning or volume reduction of cerebellar clustering patterns could be detected in ALS subgroups.

Conclusion: Our data show that specific patterns of subregional cerebellar involvement are associated with physical disability or cognitive impairment in ALS, in line with the topographic organization of the cerebellum. However, there was no specific correlation between cerebellar degeneration and cognitive syndromes or C9orf72 mutations.

Keywords: cerebellum, amyotrophic lateral sclerosis, cortical thickness, cortical volume, cognitive impairment

INTRODUCTION

The human cerebellum has been long recognized as playing an essential role in motor control and coordination and it has also become increasingly aligned with cognitive and affective processing (Schmahmann and Sherman, 1998; Buckner, 2013). Functional neuroimaging and connectivity studies in healthy humans suggest a topographic organization in the cerebellum: sensorimotor functions are represented in anterior lobules I–V and inferior lobule VIII, while cognitive processing is supported by posterior lobules (Stoodley and Schmahmann, 2009). Cerebellar abnormalities have been found not only in primary cerebellar injury or degeneration, but also in many psychiatric and neurological diseases without ataxia, including Amyotrophic Lateral Sclerosis (ALS) (Gellersen et al., 2017; Carass et al., 2018).

ALS is a fatal multisystemic neurodegenerative disorder mainly characterized by progressive loss of upper and lower motor neurons with cognitive and/or behavioral disturbance in at least half of cases. ALS-associated cognitive syndrome includes impairment of executive function, often with perseveration, distractibility or inattention, language deficit and social cognition impairment (Strong et al., 2017). Personality change might also occur with apathy, blunting of affect or disinhibited and inappropriate behavior (Strong et al., 2017). In a subset (15%) of patients these cognitive and neuropsychiatric changes are severe enough to fulfill criteria for the behavioral variant of frontotemporal dementia (bvFTD) (Phukan et al., 2012). ALS patients with the C9orf72 hexanucleotide repeat expansion (C9+ALS) had significantly more co-morbid bvFTD features than those without (Byrne et al., 2012). Cognitive and neuropsychiatric features of ALS have largely been attributed to changes in the frontotemporal and insular cortex (Tsujimoto et al., 2011; Agosta et al., 2016; Westeneng et al., 2016; Consonni et al., 2018a,b; Christidi et al., 2018a), although increasing evidence suggest hippocampus and subcortical region degeneration also plays a role (Bede et al., 2013b; Machts et al., 2015; Westeneng et al., 2016), especially in C9+ALS patients (Bede et al., 2013a; Agosta et al., 2017; Floeter et al., 2018; Floeter and Gendron, 2018).

Evidence of cerebellar involvement in ALS is less commonly reported (Prell and Grosskreutz, 2013). Structural changes of the cerebellum in ALS have been described as regional white matter changes (Bede et al., 2015) and decrease gray matter volume (Thivard et al., 2007; Gellersen et al., 2017; Christidi et al., 2018a,b). Cerebellar changes in ALS and FTD have been specifically linked to the C9orf72 repeat expansion (Mackenzie et al., 2013), raising the question whether cerebellar degeneration is an important feature of C9+ALS.

In order to explore the relationship between cerebellar involvement, cognitive impairment and C9orf72 repeated expansion of patients with ALS, quantitative *in vivo* assessment of cerebellar lobules has been investigated in a large sample of non-demented ALS patients stratified on the basis of cognitive and/or behavioral involvement and C9orf72 mutation.

MATERIALS AND METHODS

Participants

Sixty-six non-demented patients diagnosed with probable or definite ALS, according to El Escorial revised criteria (Brooks et al., 2000) and 28 healthy control (HC) volunteers were included in the study. Exclusion criteria were comorbid frontotemporal dementia (Rascovsky et al., 2011), Alzheimer's disease (NIA-AA), evidence of another neurologic condition affecting cognition (e.g., head trauma, hydrocephalus, and vascular disease), drug or alcohol abuse, mental retardation, primary psychiatric disorders, and other severe medical conditions. The review board of the Fondazione IRCCS Istituto Neurologico Carlo Besta of Milan approved the study. Each subject was enrolled after giving written informed consent.

ALS Subgroups

Patients were assigned to subgroups on the basis of the genetic screening for C9orf72 mutations and on the basis of the presence of cognitive (ALSci) and/or behavioral (ALSbi) impairment as defined by recent guidelines (Strong et al., 2017). Accordingly, a cognitive screening protocol was administer to all participants (Consonni et al., 2016; **Table 1**). Our cohort was composed of 13 patients with a pathological C9orf72 expansion (C9+ALS) and 53 non-affected C9orf72 patients (C9-ALS). Among C9-ALS patients, 31 patients had normal cognitive profile (C9-ALScn) and 22 had ALSci and/or ALSbi (C9-ALSimp).

Magnetic Resonance Imaging

All patients and healthy participants underwent a magnetic resonance imaging (MRI) session within a week of neuropsychological assessment, on a Philips Achieva 3.0 T. The MRI protocol included a 3D T1-weighted sequence (FFE, 240 sagittal slices, TR = 9.9 ms, TE = 4.6 ms, matrix 240 × 240, voxel size = 1 × 1 × 1 mm3, and flip angle = 8). In order to have a quantitative evaluation of cerebellum lobules representing the current state of the art, we used the CERES automated cerebellum parcellation algorithm (Romero et al., 2017). With this tool, the MRI images are denoised, corrected for inhomogeneity, rigid-body registered to Montreal Neurological Institute (MNI) template, cropped around the cerebellum area, and normalized to the MNI cropped cerebellum atlas. An automatic multi-atlas patch-based segmentation was then applied to obtain cerebellar cortical thickness (CT) and volume (CV), expressed in percentage of total intracranial volume, for each lobule (Manjón and Coupe, 2016).

Cerebellar Cluster Identification

CT and CV measures over twelve cerebellar lobules were obtained. Principal Component Analysis (PCA) was then applied as an unbiased method to reduce the number of variables for subsequent between-group and correlation analyses. PCA with oblimin rotation was used to identify the clustering pattern (of highly correlated variables) of cerebellar CT and CV measures of all subjects. For this purpose, for each CT and CV measure, *z*-scores were calculated by subtracting the mean CT and CV of

TABLE 1 | Demographic, clinical and neuropsychological data (mean ± standard deviation).

	C9-ALScn *N* = 31	C9-ALSimp *N* = 22	C9+ALS *N* = 13	HC *N* = 28	Group comparisons
Demographic data					
Age (yrs.)	58.22 ± 9.4	60.81 ± 11.7	57.76 ± 8.5	57.28 ± 10.1	n.s.
Education (yrs.)	11.29 ± 3.8	11.45 ± 4.1	8.53 ± 3.2	11.82 ± 3.7	C9+ALS < HC*
Male / Female	16 / 15	11 / 11	4 / 9	12 / 16	n.s.
Handedness (EHI)	21.60 ± 3.9	20.00 ± 6.13	21.33 ± 6.1	22.04 ± 1.8	n.s.
Clinical data					
ALSFRS-R	39.22 ± 5.6	37.95 ± 7.6	39.00 ± 6.09	–	n.s.
Disease duration (mos.)	16.81 ± 12.0	18.77 ± 13.5	13.15 ± 7.3	–	n.s.
Progression rate index	0.65 ± 0.4	0.63 ± 0.4	0.91 ± 0.8	–	n.s.
Bulbar onset (yes / no)	3 / 28	7 / 15	3 / 10	–	n.s.
Neuropsychological data					
ALSci / ALSbi / ALScbi	0 / 0 / 0	17 / 1 / 4	8 / 2 / 0	–	C9+ALS > C9–ALS*
Phonemic fluency index	4.83 ± 1.9	6.90 ± 4.16	8.25 ± 7.9	4.17 ± 2.0	C9-ALSimp > HC*
Object naming (% correct)	95.72 ± 3.8	86.34 ± 10.2	89.09 ± 13.1	94.76 ± 4.9	C9-ALSimp < HC*** C9-ALSimp < C9-ALScn***
Emotion attribution (SET)	4.89 ± 1.2	3.38 ± 1.56	3.36 ± 2.0	4.89 ± 1.1	C9-ALSimp < HC** C9-ALSimp < C9-ALScn**
Stroop effect	19.02 ± 8.1	31.31 ± 24.1	25.08 ± 9.3	20.25 ± 7.9	C9-ALSimp < C9-ALScn*
Delayed recall RAVLT	10.00 ± 2.5	7.62 ± 2.7	7.72 ± 3.28	10.36 ± 2.6	C9-ALSimp < HC** C9-ALSimp < C9-ALScn**
FBI tot	1.96 ± 2.5	4.00 ± 6.7	2.03 ± 3.4	–	n.s.

*ALSbi, ALS patients fulfilling Strong criteria for behavioral impairment; ALSci, ALS patients fulfilling Strong criteria for cognitive impairment; ALScbi, ALS patients fulfilling criteria for ALSci and ALSbi; ALSFRS-R, Revised ALS Functional Rating Scale; FBI, Frontal Behavioral Inventory; RAVLT, Rey Auditory Verbal Learning Test; SET, Story-based Empathy Task; and ns, not significant difference ($p > 0.05$). ***$p < 0.001$; **$p < 0.005$; *$p < 0.05$.*

TABLE 2 | Pattern matrix of PCA results for cerebellar lobar values of Cortical Thickness (CT) of 94 subjects [66 patients with ALS and 24 healthy controls (HCs)].

	Rotated cluster loadings				
	Cluster 1	Cluster 2	Cluster 3	Cluster 4	KMO value
CTz Lobule IX	**0.882**	0.100	−0.165	0.181	0.752
CTz Lobule VIIIB	**0.854**	0.210	−0.246	0.398	0.746
CTz Lobule VIIIA	**0.704**	0.672	−0.120	0.220	0.725
CTz Lobule VIIB	0.255	**0.894**	0.004	−0.049	0.595
CTz Lobule CRUSII	0.053	**0.840**	−0.472	0.003	0.645
CTz Lobule VI	0.113	0.112	**−0.858**	0.144	0.822
CTz Lobule CRUS I	0.050	0.404	**−0.830**	0.048	0.693
CTz Lobule V	0.509	−0.160	**−0.767**	0.373	0.763
CTz Lobule IV	0.472	−0.184	**−0.745**	0.490	0.728
CTz Lobule I&II	0.221	−0.083	−0.114	**0.882**	0.685
CTz Lobule III	0.130	−0.195	−0.213	**0.861**	0.612
CTz Lobule X	0.247	0.138	−0.110	**0.454**	0.700
Eigenvalues	3.929	2.295	1.757	1.122	
% of variance	32.74	19.13	14.64	9.35	

The loading values in bold indicate the elements that contribute the greatest variability to PCA clusters. ALScn, ALS patients with a cognitive and behavioral normal profile; ALSimp, ALS patients fulfilling Strong criteria for behavioral and/or cognitive impairment; C9+ALS, ALS patients with C9orf72 repeat expansions; and HC, Healthy Controls.

HC subjects from the patient's CT and CV, and then dividing the difference by the HC group standard deviation. All variables were normally distributed and had Kaiser-Meyer-Olkin (KMO) values higher than 0.5. Clusters with eigenvalues over the Kaiser criterion of one were retained. Cluster scores were calculated with the Anderson-Rubin method and used for between group and correlation analyses (Field, 2013).

Statistics

The Kolmogorov–Smirnov test was used to test the normality of the distribution of demographic, clinical, neuropsychological and neuroimaging data. Fisher Exact test, Kruskal–Wallis and Mann–Whitney *U* tests were used to test between group differences (i.e., C9-ALSimp, C9-ALScn, C9+ALS, and HC). The variability of cerebellar cluster loadings of groups was analyzed by ANCOVA, with age as covariates. Simple contrasts were then used to test specific group comparisons. Partial correlation analyses (correcting for age and education) were used to explore the relationship of cerebellar cluster scores with neuropsychological and clinical profiles in all patients as a whole group. Significant correlations were then bias corrected and accelerated bootstrap 95% confidence intervals (CIs) were computed with 1000 bootstrap equally sized samples obtained by randomly resampling replacement from the original data. Partial correlations with $p < 0.05$ and CIs not crossing "0" were reported. IBM SPSS Statistics (version 21) was used to perform analyses.

RESULTS

Clinical, Demographic and Neuropsychological Data

Descriptive statistics are summarized in **Table 1**. C9-ALScn, C9-ALSimp, C9+ALS, and HC groups did not differ in to age, handedness, gender, but education ($H = 9.019$, $p = 0.029$). The C9+ALS group had a slightly lower education level than the HC group ($U = 93.0$, $p = 0.012$). Disease duration, functional

disability assessed with the ALSFRS-R, progression and type of onset were similar across ALS subgroups. HC and C9-ALScn groups had similar neuropsychological performances. The occurrence of cognitive and/or behavioral impairment is higher for C9+ALS patients than C9-ALS patients (77% vs. 41%; p = 0.031). The C9-ALSimp group compared with HC and C9-ALScn groups had lower performances in all cognitive domains (**Table 1**).

Cerebellar Clustering Patterns

CTz. The KMO measure tested the sample adequacy of 12 cerebellar lobular CTz values for PCA (KMO = 0.708). The rotation converged on 23 iterations. Four clusters had eigenvalues over Kaiser criterion of one and in combination explained 78.41% of the variance (**Table 2**). Cluster 1 included CTz values of lobule VIIIA, VIIIB, and IX; Cluster 2 included CTz values of Crus II and VIIB, Cluster 3 loadings were CTz values of lobule IV, V, VI, and Crus I; cluster 4 included lobule I&II and III and X.

CVz. The KMO measure tested the sample adequacy of 12 cerebellar lobular CV for PCA (KMO = 0.671). The rotation converged on 10 iterations. Four clusters had eigenvalues over Kaiser criterion of one and in combination explained 62.13% of the variance (**Table 3**). Cluster 1 included CVz of lobule VIIIB, IX, and X; Cluster 2 included CVz of anterior lobules (I&II, III, IV, and V); Cluster 3 loadings were CVz values of CrusII, VIIB and VIIIA; cluster 4 included CVz of lobule VI and Crus I.

We did not find group differences in cluster scores of CTz and CVz measures (**Table 4**). Correlation analyses revealed that only CVz scores of the second and third cluster were related to functional disability measured with the ALSFRS-R in all patients (r = 0.283, p = 0.024, CIs: 0.043–0.468; r = 0.281, p = 0.026, CIs: 0.053–0.467). Cluster 2 CVz scores were also related to the progression rate (r = −0.248, p = 0.050, CIs: −0.087 – −0.403). Cluster 4 CVz scores were inversely related to the phonemic fluency index (r = −0.335, p = 0.010, CIs: −0.157 – −0.535).

TABLE 3 | Pattern matrix of PCA results for cerebellar lobar values of Cortical Volumes (CV) of 94 subjects (66 patients with ALS and 24 HCs).

	Rotated cluster loadings				
	Cluster 1	**Cluster 2**	**Cluster 3**	**Cluster 4**	**KMO value**
CVz Lobule IX	**0.826**	−0.079	0.283	0.155	0.661
CVz Lobule VIIIB	**0.727**	0.391	0.153	0.108	0.690
CVz Lobule X	**0.651**	−0.054	0.324	0.494	0.779
CVz Lobule III	−0.035	**0.788**	0.264	0.040	0.655
CVz Lobule V	0.201	**0.684**	0.126	0.506	0.685
CVz Lobule I&II	0.057	**0.602**	0.002	−0.014	0.528
CVz Lobule IV	0.487	**0.498**	0.119	0.364	0.702
CVz Lobule CRUSII	0.298	0.066	**0.831**	−0.016	0.609
CVz Lobule VIIB	−0.092	0.130	**0.773**	0.284	0.668
CVz Lobule VIIIA	0.351	0.119	**0.692**	0.115	0.702
CVz Lobule VI	0.006	0.255	0.174	**0.809**	0.601
CVz Lobule CRUS I	0.339	−0.095	0.070	**0.697**	0.677
Eigenvalues	3.226	1.653	1.408	1.166	
% of variance	26.88	13.77	11.73	9.71	

The loading values in bold indicate the elements that contribute the greatest variability to PCA clusters. ALScn, ALS patients with a cognitive and behavioral normal profile; ALSimp, ALS patients fulfilling Strong criteria for behavioral and/or cognitive impairment; C9+ALS, ALS patients with C9orf72 repeat expansions; and HC, Healthy Controls.

DISCUSSION

We tested the relationship between cerebellar degeneration, cognitive syndromes and C9orf72 mutation in ALS patients. The notion that cerebellum contributes to non-motor disorders, such as impairments in language, attention and social cognition is well recognized (Buckner, 2013). Consistently, we performed a quantitative *in vivo* assessment of cerebellar lobular CT and CV in a large sample of non-demented ALS patients stratified on the basis of cognitive and/or behavioral impairment and C9orf72 repeat expansion. To this aim, we used an unbiased reduction method, the PCA, which allows projecting data into a lower-dimensional space thus reducing the type I error and identifying clusters of highly correlated variables with each other (Field, 2013).

We identified 4 clusters of regional cerebellar CT and demonstrated their scores were similar across ALS subgroups

TABLE 4 | Clustering pattern of cerebellar lobules (mean cluster score ± standard deviation).

	ALScn *N* = 31	**ALSimp *N* = 22**	**C9+ALS *N* = 13**	**HC *N* = 28**	**Group comparisons**
Cortical thickness – cluster scores					
1. Lobule VIIIA, VIIIB, IX	−0.278 ± 0.96	0.362 ± 0.75	−0.253 ± 1.27	0.141 ± 0.99	n.s.
2. Lobule Crus II, VIIB	−0.004 ± 1.14	0.076 ± 0.82	−0.531 ± 1.16	0.191 ± 0.83	n.s.
3. Lobule IV, V, VI, Crus I	−0.272 ± 0.89	−0.063 ± 0.80	0.463 ± 1.43	0.136 ± 0.95	n.s.
4. Lobule I&II, III, X	−0.122 ± 1.07	0.353 ± 0.88	0.125 ± 0.99	−0.200 ± 0.96	n.s.
Cortical volumes – cluster scores					
1. Lobule VIIIB, IX, X	0.038 ± 0.86	−0.191 ± 0.94	0.263 ± 1.44	−0.014 ± 0.95	n.s.
2. Lobule I&II, III, IV, V	0.064 ± 0.97	−0.086 ± 1.29	−0.014 ± 1.11	0.003 ± 0.72	n.s.
3. Lobule Crus II, VIIB, VIIIA	−0.042 ± 0.96	−0.323 ± 1.03	0.004 ± 0.99	0.299 ± 0.98	n.s.
4. Lobule VI, Crus I	0.201 ± 0.92	−0.306 ± 0.80	0.006 ± 0.87	0.014 ± 1.23	n.s.

n.s., not significant differences ($p > 0.05$).

and HC individuals. Moreover, the lack of significant correlations between cerebellar CT values, neuropsychological performances and clinical features in patients suggests that the cerebellar cortical thinning has a poor sensibility to detect cognitive impairment in ALS. Cognitive and neuropsychiatric features of ALS seem to be associated instead with thinning in frontotemporal and insular cortex (Schuster et al., 2014; Agosta et al., 2016; Consonni et al., 2018a,b).

By means of lobar CV measures, we identified four clustering patterns. The clustering of the cerebellum respected lobular anatomical boundaries and functional organization. Cluster 1 included posterior regions (VIIIB-IX, X), cluster 2 the sensory-motor anterior regions (I-V), cluster 3 (CrusII-VIIIA), and 4 (VI-CrusI) included mainly cognitive posterior lobules. Contrary to our expectation, no significant group differences in cluster scores were found, but, as explorative analysis, a weak but significant correlation between CV measures of the fourth cluster and phonemic fluency indexes of ALS patients. Verbal fluency is a widely recognized marker of dysexecutive impairment in ALS (Strong et al., 2017). Specifically, poor verbal fluency was related to reduced CV of lobule VI and CrusI. This is consistent with previous studies documenting that lobules VI and Crus I are involved in executive functions, such as working memory, planning, organizing, and strategy formation (Stoodley and Schmahmann, 2009). According to a meta-analysis of voxel-based morphometry in cerebellum (Gellersen et al., 2017), the largest cluster of gray matter reduction in ALS spanned parts of the vermis and neighboring regions in left lobule VI, Crus I, and Crus II. This suggests that cerebellar involvement in ALS may be related to progressive cognitive impairment. However, as in other studies (Schönecker et al., 2018), we did not find differences in cerebellar volume within ALS cognitive subgroups, albeit specific patterns of cerebellum atrophy in ALS-FTD patients have been previously reported (Tan et al., 2014; Omer et al., 2017; Christidi et al., 2018a,b). We could not exclude that discrepancies between our findings and those reported by others are attributed to differences in sample sizes and patients' clinical features, as well as different methodological (e.g., Surface-Based Analysis vs. Voxel-Based Morphometry) and statistical approach and whether the whole cerebellum or focal cerebellar regions were addressed.

We also found that severe motor disability and high rate of disease progression were slightly related to CV reduction of the sensory-motor cerebellar lobules. This is consistent with the evidence of gradually progressive cerebellar gray matter degeneration throughout disease progression in ALS (Bede and Hardiman, 2017). A recent study showed reduced gray matter volume (lobule IV and V) in ALS patients with no cognitive defect and posterior (VIII) cerebellar involvement in ALS patients with cognitive impairment (Christidi et al., 2018a). This is in line with our correlation analyses, confirming that specific patterns of subregional involvement are differently associated with cognitive and motor impairment in ALS (Tan et al., 2014).

Unexpectedly, our investigation of the cerebellar subregions did not revealed atrophy clusters specific for C9+ALS patients, which is consistent with recent findings (Floeter et al., 2016; Schönecker et al., 2018) but not with others (Westeneng et al., 2016; Agosta et al., 2017). Since cerebellar involvement in C9orf72 carriers have been found among FTD-ALS spectrum (Bede et al., 2013a; Irwin et al., 2013; Floeter et al., 2016), it is conceivable that it may reflect a signature of ALS dementia other than a signature of the C9orf72 hexanucleotide repeat (Bede et al., 2013a). Further studies deserve larger C9+ALS samples stratified on the basis of the degree of cognitive impairment to address this issue.

The strength of the presented study was the tentative to systematically explore the cognitive contributions of cerebellar atrophy in selected cohorts of patients with ALS. Our data show that cerebellar degeneration is not specific for non-demented patients with ALS and cognitive syndromes or C9orf72 mutations. However, the relatively small number of mutation carriers and the lack of ALS-FTD patients included limit the strength for firm conclusion. Indeed, we could not exclude that overall disease progression both in terms of progressive physical disability and progressive cognitive impairment might be related to cerebellar involvement, in line with its topographic organization.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The review board of the Fondazione IRCCS Istituto Neurologico Carlo Besta of Milan approved the study. Each subject was enrolled after giving written informed consent.

AUTHOR CONTRIBUTIONS

MC, EDB, AN, CP, CG, and SF collected the data. AN, CP, GD, MB, and SF acquired and the analyzed neuroimaging data. MC, SF, and SC designed the study. MC and LP performed the statistical analyses. MC, EDB, SC, MB, GL, and SF gave important intellectual content in data interpretation. MC, EDB, LP, and AN wrote a draft of the manuscript. MC, SC, GL, and SF revised the final version of the manuscript for intellectual content. MC, EDB, AN, CP, GD, LP, CG, VP, SC, MB, GL, and SF read and approved the final version.

FUNDING

This work has been supported by Fondazione Regionale per la Ricerca Biomedica (Regione Lombardia), project nr. 2015-0023.

ACKNOWLEDGMENTS

We thank neurologists of the Fondazione IRCCS Istituto Neurologico Carlo Besta of Milan, Patrizia Dacci, Daniele Cazzato, Susanna Usai, Ettore Salsano, and Davide Pareyson for referring some patients; Valentina E.C. Gessa for the neuropsychological assessment of some patients.

REFERENCES

Agosta, F., Ferraro, P. M., Riva, N., Spinelli, E. G., Chiò, A., Canu, E., et al. (2016). Structural brain correlates of cognitive and behavioral impairment in MND. *Hum. Brain Mapp.* 37, 1614–1626. doi: 10.1002/hbm.23124

Agosta, F., Ferraro, P. M., Riva, N., Spinelli, E. G., Domi, T., Carrera, P., et al. (2017). Structural and functional brain signatures of C9orf72 in motor neuron disease. *Neurobiol. Aging* 57, 206–219. doi: 10.1016/j.neurobiolaging.2017.05.024

Bede, P., Bokde, A. L., Byrne, S., Elamin, M., McLaughlin, R. L., Kenna, K., et al. (2013a). Multiparametric MRI study of ALS stratified for the C9orf72 genotype. *Neurology* 81, 361–369. doi: 10.1212/WNL.0b013e31829c5eee

Bede, P., Elamin, M., Byrne, S., McLaughlin, R. L., Kenna, K., Vajda, A., et al. (2013b). Basal ganglia involvement in amyotrophic lateral sclerosis. *Neurology* 81, 2107–2115. doi: 10.1212/01.wnl.0000437313.80913.2c

Bede, P., Elamin, M., Byrne, S., McLaughlin, R. L., Kenna, K., Vajda, A., et al. (2015). Patterns of cerebral and cerebellar white matter degeneration in ALS. *J. Neurol. Neurosurg. Psychiatry* 86, 468–470.

Bede, P., and Hardiman, O. (2017). Longitudinal structural changes in ALS: a three time-point imaging study of white and gray matter degeneration. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 19, 232–241. doi: 10.1080/21678421.2017.1407795

Brooks, B. R., Miller, R. G., Swash, M., Munsat, T. L., and World Federation of Neurology Research Group on Motor Neuron Diseases. (2000). El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.* 1, 293–299.

Buckner, R. L. (2013). The cerebellum and cognitive function: 25 years of insight from anatomy and neuroimaging. *Neuron* 80, 807–815. doi: 10.1016/j.neuron.2013.10.044

Byrne, S., Elamin, M., Bede, P., Shatunov, A., Walsh, C., Corr, B., et al. (2012). Cognitive and clinical characteristics of patients with amyotrophic lateral sclerosis carrying a C9orf72 repeat expansion: a population-based cohort study. *Lancet Neurol.* 11, 232–240. doi: 10.1016/S1474-4422(12)70014-5

Carass, A., Cuzzocreo, J. L., Han, S., Hernandez-Castillo, C. R., Rasser, P. E., Ganz, M., et al. (2018). Comparing fully automated state-of-the-art cerebellum parcellation from magnetic resonance images. *Neuroimage* 183, 150–172. doi: 10.1016/j.neuroimage.2018.08.003

Christidi, F., Karavasilis, E., Riederer, F., Zalonis, I., Ferentinos, P., Velonakis, G., et al. (2018a). Gray matter and white matter changes in non-demented amyotrophic lateral sclerosis patients with or without cognitive impairment: a combined voxel-based morphometry and tract-based spatial statistics whole-brain analysis. *Brain Imaging Behav.* 12, 547–563.

Christidi, F., Karavasilis, E., Velonakis, G., Rentzos, M., Zambelis, T., Zouvelou, V., et al. (2018b). Motor and extra-motor gray matter integrity may underlie neurophysiologic parameters of motor function in amyotrophic lateral sclerosis: a combined voxel-based morphometry and transcranial stimulation study. *Brain Imaging Behav.* 12, 1730–1741. doi: 10.1007/s11682-018-9841-0

Consonni, M., Catricalà, E., Dalla Bella, E., Gessa, V. C., Lauria, G., and Cappa, S. F. (2016). Beyond the consensus criteria: multiple cognitive profiles in amyotrophic lateral sclerosis? *Cortex* 81, 162–167. doi: 10.1016/j.cortex.2016.04.014

Consonni, M., Cappa, S. F., Dalla Bella, E., Contarino, V. E., and Lauria, G. (2018a). Cortical correlates of behavioural change in amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* 90, 380–386.

Consonni, M., Contarino, V. E., Catricalà, E., Dalla Bella, E., Pensato, V., Gellera, C., et al. (2018b). Cortical markers of cognitive syndromes in amyotrophic lateral sclerosis. *Neuroimage Clin.* 19, 675–682. doi: 10.1016/j.nicl.2018.05.020

Field, A. (2013). *Discovering Statistics Using IBM SPSS Statistics*, 4th Edn. London: Sage publication.

Floeter, M. K., Bageac, D., Danielian, L. E., Braun, L. E., Traynor, B. J., and Kwan, J. Y. (2016). Longitudinal imaging in C9orf72 mutation carriers: relationship to phenotype. *Neuroimage Clin.* 12, 1035–1043.

Floeter, M. K., Danielian, L. E., Braun, L. E., and Wu, T. (2018). Longitudinal diffusion imaging across the C9orf72 clinical spectrum. *J. Neurol. Neurosurg. Psychiatry* 89, 53–60. doi: 10.1136/jnnp-2017-316799

Floeter, M. K., and Gendron, T. F. (2018). Biomarkers for amyotrophic lateral sclerosis and frontotemporal dementia associated with hexanucleotide expansion mutations in C9orf72. *Front. Neurol.* 9:1063. doi: 10.3389/fneur.2018.01063

Gellersen, H. M., Guo, C. C., O'Callaghan, C., Tan, R. H., Sami, S., and Hornberger, M. (2017). Cerebellar atrophy in neurodegeneration—a metaanalysis. *Neurol. Neurosurg. Psychiatry* 88, 780–788.

Irwin, D. J., McMillan, C. T., Brettschneider, J., Libon, D. J., Powers, J., Rascovsky, K., et al. (2013). Cognitive decline and reduced survival in C9orf72 expansion frontotemporal degeneration and amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* 84, 163–169. doi: 10.1136/jnnp-2012-303507

Machts, J., Loewe, K., Kaufmann, J., Jakubiczka, S., Abdulla, S., Petri, S., et al. (2015). Basal ganglia pathology in ALS is associated with neuropsychological deficits. *Neurology* 85, 1301–1309. doi: 10.1212/WNL.0000000000002017

Mackenzie, I. R., Arzberger, T., Kremmer, E., Troost, D., Lorenzl, S., Mori, K., et al. (2013). Dipeptide repeat protein pathology in C9ORF72 mutation cases: clinico-pathological correlations. *Acta Neuropathol.* 126, 859–879. doi: 10.1007/s00401-013-1181-y

Manjón, J. V., and Coupe, P. (2016). volBrain: an online MRI brain volumetry system. *Frontiers in Neuroinformatics* 10:30. doi: 10.3389/fninf.2016.00030

Omer, T., Finegan, E., Hutchinson, S., Doherty, M., Vajda, A., McLaughlin, R. L., et al. (2017). Neuroimaging patterns along the ALS-FTD spectrum: a multiparametric imaging study. *Amyotroph. Lateral Scler. Frontotemporal. Degener.* 18, 611–623. doi: 10.1080/21678421.2017.1332077

Phukan, J., Elamin, M., Bede, P., Jordan, N., Gallagher, L., Byrne, S., et al. (2012). The syndrome of cognitive impairment in amyotrophic lateral sclerosis: a population-based study. *J. Neurol. Neurosurg. Psychiatry* 83, 102–108. doi: 10.1136/jnnp-2011-300188

Prell, T., and Grosskreutz, J. (2013). The involvement of the cerebellum in amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Frontotemporal Dementia* 14, 507–515.

Rascovsky, K., Hodges, J. R., Knopman, D., Mendez, M. F., Kramer, J. H., Neuhaus, J., et al. (2011). Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain* 134, 2456–2477. doi: 10.1093/brain/awr179

Romero, J. E., Coupé, P., Giraud, R., Ta, V. T., Fonov, V., Park, M. T. M., et al. (2017). CERES: a new cerebellum lobule segmentation method. *Neuroimage* 147, 916–924. doi: 10.1016/j.neuroimage.2016.11.003

Schmahmann, J. D., and Sherman, J. C. (1998). The cerebellar cognitive affective syndrome. *Brain* 121, 561–579.

Schönecker, S., Neuhofer, C., Otto, M., Ludolph, A., Kassubek, J., Landwehrmeyer, B., et al. (2018). Atrophy in the thalamus but not cerebellum is specific for C9orf72 FTD and ALS patients - an atlas-based volumetric MRI study. *Front. Aging Neurosci.* 10:45. doi: 10.3389/fnagi.2018.00045

Schuster, C., Kasper, E., Dyrba, M., Machts, J., Bittner, D., Kaufmann, J., et al. (2014). Cortical thinning and its relation to cognition in amyotrophic lateral sclerosis. *Neurobiol. Aging* 35, 240–246. doi: 10.1016/j.neurobiolaging.2013.07.020

Stoodley, C. J., and Schmahmann, J. D. (2009). Functional topography in the human cerebellum: a meta-analysis of neuroimaging studies. *Neuroimage* 44, 489–501. doi: 10.1016/j.neuroimage.2008.08.039

Strong, M. J., Abrahams, S., Goldstein, L. H., Woolley, S., Mclaughlin, P., Snowden, J., et al. (2017). Amyotrophic lateral sclerosis - frontotemporal spectrum disorder (ALS-FTSD): revised diagnostic criteria. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 18, 153–174. doi: 10.1080/21678421.2016.1267768

Tan, R. H., Devenney, E., Dobson-Stone, C., Kwok, J. B., Hodges, J. R., Kiernan, M. C., et al. (2014). Cerebellar integrity in the amyotrophic lateral sclerosis-frontotemporal dementia continuum. *PLoS One* 9:e0105632. doi: 10.1371/journal.pone.0105632

Thivard, L., Pradat, P. F., Lehericy, S., Lacomblez, L., Dormont, D., Chiras, J., et al. (2007). Diffusion tensor imaging and voxel based morphometry

study in amyotrophic lateral sclerosis: relationships with motor disability. *J. Neurol. Neurosurg. Psychiatry* 78, 889–892. doi: 10.1136/jnnp.2006.101758

Tsujimoto, M., Senda, J., Ishihara, T., Niimi, Y., Kawai, Y., and Mizoguchi, K. (2011). Behavioral changes in early ALS correlate with voxel-based morphometry and diffusion tensor imaging. *J. Neurol. Sci.* 15, 136–140. doi: 10.1016/j.jns.2011.05.025

Westeneng, H. J., Walhout, R., Straathof, M., Schmidt, R., Hendrikse, J., Veldink, J. H., et al. (2016). Widespread structural brain involvement in ALS is not limited to the C9orf72 repeat expansion. *J. Neurol. Neurosurg. Psychiatry* 87, 1354–1360.

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.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 31 May 2019
doi: 10.3389/fnins.2019.00487

Metabolic and Stress Response Changes Precede Disease Onset in the Spinal Cord of Mutant SOD1 ALS Mice

*Gavin Pharaoh[1,2], Kavithalakshmi Sataranatarajan[1], Kaitlyn Street[1], Shauna Hill[1,3], Jake Gregston[1], Bumsoo Ahn[1], Caroline Kinter[1], Michael Kinter[1] and Holly Van Remmen[1,2,4]**

[1] Aging and Metabolism Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, United States, [2] Department of Physiology, University of Oklahoma Health Science Center, Oklahoma City, OK, United States, [3] Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States, [4] Oklahoma City VA Medical Center, Oklahoma City, OK, United States

OPEN ACCESS

Edited by:
Foteini Christidi,
National and Kapodistrian University of Athens Medical School, Greece

Reviewed by:
Chiara F. Valori,
German Center for Neurodegenerative Diseases (DZNE), Germany
Susanne Petri,
Hannover Medical School, Germany

***Correspondence:**
Holly Van Remmen
Holly-VanRemmen@omrf.org

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: 10 December 2018
Accepted: 29 April 2019
Published: 31 May 2019

Citation:
Pharaoh G, Sataranatarajan K, Street K, Hill S, Gregston J, Ahn B, Kinter C, Kinter M and Van Remmen H (2019) Metabolic and Stress Response Changes Precede Disease Onset in the Spinal Cord of Mutant SOD1 ALS Mice. Front. Neurosci. 13:487. doi: 10.3389/fnins.2019.00487

Many Amyotrophic Lateral Sclerosis (ALS) patients experience hypermetabolism, or an increase in measured vs. calculated metabolic rate. The cause of hypermetabolism and the effects on neuronal metabolism in ALS are currently unknown, but the efficacy of dietary interventions shows promise for metabolism as an ALS therapeutic target. The goal of this study is to measure changes in metabolic pathways as a function of disease progression in spinal cords of the $SOD1^{G93A}$ mouse model of ALS. We conducted a comprehensive assessment of protein expression for metabolic pathways, antioxidants, chaperones, and proteases in lumbar spinal cord from male $SOD1^{G93A}$ mice at pre-onset, onset, and end-stages of the disease using targeted proteomic analysis. These results reveal that protein content of metabolic proteins including proteins involved in glycolysis, β-oxidation, and mitochondrial metabolism is altered in $SOD1^{G93A}$ mouse spinal cord well before disease onset. The changes in mitochondrial metabolism proteins are associated with decreased maximal respiration and glycolytic flux in $SOD1^{G93A}$ dermal fibroblasts and increased hydrogen peroxide and lipid hydroperoxide production in mitochondria from sciatic nerve and gastrocnemius muscle fibers at end stage of disease. Consistent with redox dysregulation, expression of the glutathione antioxidant system is decreased, and peroxiredoxins and catalase expression are increased. In addition, stress response proteases and chaperones, including those involved in the mitochondrial unfolded protein response (UPR^{mt}), are induced before disease onset. In summary, we report that metabolic and stress response changes occur in $SOD1^{G93A}$ lumbar spinal cord before motor symptom onset, and are primarily caused by $SOD1^{G93A}$ expression and do not vary greatly as a function of disease course.

Keywords: amyotrophic lateral sclerosis (ALS), $SOD1^{G93A}$ ALS mouse model, metabolism, hypermetabolism, mitochondria, antioxidants, stress response, mitochondrial unfolded protein response

INTRODUCTION

Amyotrophic Lateral Sclerosis (ALS) is a cell non-autonomous disease affecting motor neurons as well as surrounding support cells including astrocytes, oligodendrocytes, and microglia (Ilieva et al., 2009; Rizzo et al., 2014) and skeletal muscle (Dobrowolny et al., 2008). Motor neuron cell death causes loss of neuromuscular innervation resulting in severe muscle atrophy and paralysis culminating in death by respiratory failure (Rowland and Shneider, 2001). While recent advances in sequencing technologies have led to the identification of genes underlying over 60% of familial cases (fALS) and over 10% of sporadic cases (sALS), the pathogenesis of ALS is still poorly understood delaying the development of effective treatments (Renton et al., 2014). More importantly, the only FDA approved drugs for the treatment of ALS, riluzole and edaravone, provide only minor improvements for ALS patients (Zoccolella et al., 2007; Abe et al., 2014).

The first mutated gene identified in fALS was superoxide dismutase 1 (*Sod1*), an antioxidant enzyme that detoxifies superoxide. Several transgenic mouse lines expressing mutant *Sod1*, including SOD1^{G93A}, have been generated that recapitulate fALS symptoms and have allowed exploration of biochemical mechanisms underlying fALS pathogenesis (Ilieva et al., 2009). One phenotype of SOD1 mutant mice that also recapitulates the human disease is a hypermetabolic phenotype that occurs before the onset of motor symptoms (Dupuis et al., 2004). Approximately 50% of ALS patients experience the phenomenon of hypermetabolism, wherein their measured resting energy expenditure is increased compared to their calculated resting energy expenditure (Bouteloup et al., 2009). Patients rarely switch metabolic groups during disease progression, and the hypermetabolism phenotype does not correlate with fat free mass, age, sex, BMI, or an ALS functional test (Bouteloup et al., 2009). However, hypermetabolic patients display a trend toward decreased survival length from symptom onset (Bouteloup et al., 2009), and a phase II clinical trial suggested that high calorie diets had beneficial survival effects in ALS patients (Wills et al., 2014). In support of this, a high calorie diet extends SOD1 mutant mouse lifespan (Dupuis et al., 2004), while caloric restriction reduced lifespan (Hamadeh et al., 2005). The hypermetabolism phenotype is a potential disease modifier that can be targeted to affect patient disease progression.

We hypothesized that increased energy demands (hypermetabolism) cause an energetic stress affecting metabolism in motor neurons that contributes to disease progression. To test our hypothesis, we collected lumbar spinal cord from SOD1^{G93A} mice and age-matched wildtype controls before and at onset of motor symptoms and at disease end-stage. We measured changes in protein abundance of major metabolic pathway enzymes at all three disease stages via a targeted proteomics approach. In addition, because mitochondrial damage occurs early in ALS pathogenesis, we measured sciatic nerve and skeletal muscle mitochondrial function and lipid hydroperoxide and hydrogen peroxide production. We also measured expression of spinal cord and skeletal muscle mitochondrial proteases and chaperones, including those involved in the mitochondrial unfolded protein response (UPR^{mt}), a stress response pathway responsible for maintaining mitochondrial protein quality control in response to accumulation of misfolded or unfolded proteins in the mitochondria (Hill and Van Remmen, 2014). Our results reported here represent the first comprehensive measurement of major metabolic and stress response pathways in spinal cord of ALS mutant mice as a function of disease stage.

RESULTS

Metabolic Pathways Are Differentially Regulated in the Spinal Cord of SOD1^{G93A} Mice, and Stress Response Proteins Are Induced

The SOD1^{G93A} ALS mouse model recapitulates the hypermetabolism phenotype (Dupuis et al., 2004). In addition to the well characterized decline in body and muscle mass, we also observed reduced fat mass in end-stage SOD1^{G93A} mice (**Supplemental Figure 1**). Because ALS is a neurodegenerative disease characterized by pathological changes in the spinal cord, we used targeted proteomic analysis to measure differential protein content of metabolic and stress response pathways in spinal cord homogenates from wild-type and SOD1^{G93A} mice over the time course of the disease. We interrogated changes in 113 proteins detected in at least one genotype (**Figure 1** and **Supplemental Table 1**). We categorized proteins into panels for carbohydrate metabolism, fatty acid metabolism, oxidative mitochondrial metabolism [the tricarboxylic acid cycle and electron transport chain (ETC)], and stress response (antioxidants, chaperones, heat shock proteins, and proteases). Metabolic and stress response protein expression differences in SOD1^{G93A} lumbar spinal cord are evident throughout disease course (**Figure 1A**). Principal component analysis (PCA) demonstrates that protein expression in lumbar spinal cord from SOD1^{G93A} mice clusters distinctly from spinal cord from control mice beginning before onset of symptoms, but no separation occurs in clustering between disease stages (**Figure 1B**). 49% of assayed proteins exhibit a significant difference due to genotype effect, while only 10% exhibited a significant difference based on disease stage using two-way ANOVA corrected for false discovery rate with the Benjamini-Hochberg procedure. These results show that metabolism and stress response protein content is already dramatically altered in spinal cords from SOD1^{G93A} mice before onset of motor symptoms.

Glycolysis and the Malate-Aspartate Shuttle

Two key proteins involved in glycolysis are elevated prior to disease onset, but we also observe a decline at end-stage in several glycolytic proteins along with a decline in some key proteins involved in the malate aspartate shuttle in spinal cord from SOD1^{G93A} mice Specifically, levels for the first two enzymes in glycolysis (HK1 and GPI1) are increased beginning at pre-onset or disease onset. However, ALDOA and GAPDH are reduced at end-stage and PGK1 exhibits a genotype specific reduction in spinal cords from SOD1^{G93A} mice compared to wild-type

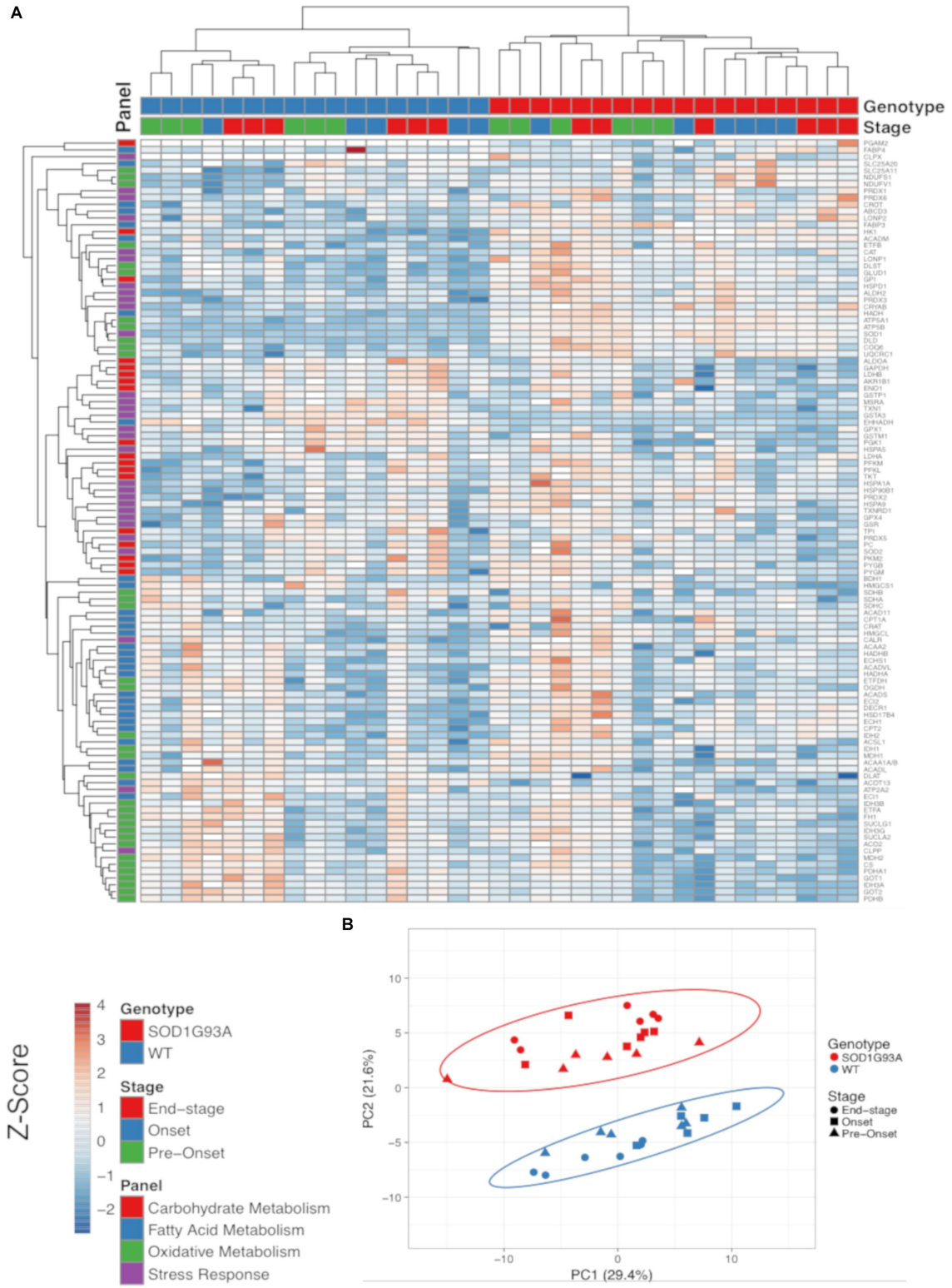

FIGURE 1 | Metabolic pathways are differentially regulated in the spinal cord of SOD1^{G93A} mice, and stress response proteins are induced. Protein content from spinal cords of pre-onset, onset, and end-stage control and SOD1^{G93A} mice (n = 5–6) measured by mass spectrometry. The assayed proteins were targeted for representative panels of carbohydrate metabolism, fatty acid metabolism, oxidative mitochondrial metabolism (tricarboxylic acid cycle and electron transport chain), and stress response (antioxidants, chaperones, heat shock proteins, and proteases). **(A)** Heatmap with clustering for each sample using a Z-score scale. **(B)** Principal component analysis (PCA) plot for principal components 1 and 2. Protein names, IDs, and absolute expression values are detailed in **Supplemental Table 1**.

controls (**Figure 2A**). PGAM2 only reached the threshold of detection in SOD1^{G93A} samples (**Figure 2A**). At end-stage, protein content of key components of the malate-aspartate shuttle declines (**Figure 2B**). While SLC25A11, the mitochondrial inner-membrane α-ketoglutarate/malate carrier, is increased at onset, at end-stage the cytoplasmic and mitochondrial aspartate aminotransferases (GOT1 and GOT2) and the mitochondrial malate dehydrogenase (MDH2) are decreased compared to wild-type controls (Maglott et al., 2011; **Figure 2B**). TKT, a key enzyme in the pentose-phosphate pathway is increased in spinal cords from SOD1^{G93A} mice (**Supplemental Table 1**). Combined these results suggest altered carbohydrate utilization in end-stage spinal cords from SOD1^{G93A} mice compared to wild-type control mice.

β-Oxidation

Several proteins involved in mitochondrial β-oxidation are increased in spinal cords from SOD1^{G93A} mice. CPT2, which is involved in import of fatty acids to mitochondria, and CROT, which is involved in export of β-oxidation products from peroxisomes, are both increased in SOD1^{G93A} samples (**Figure 3A**). The mitochondrial medium chain acyl-CoA dehydrogenase (ACADM) is increased at onset and end-stage in spinal cord from SOD1^{G93A} mice compared to wild-type mice, and the mitochondrial β-hydroxyl acyl CoA dehydrogenase (HADH) is increased throughout the disease course in SOD1^{G93A} mice (**Figure 3A**). Enoyl-CoA Hydratase 1 (ECH1), which is targeted to both mitochondria and peroxisomes, and DECR1, an auxiliary enzyme of mitochondrial β-oxidation are increased at disease onset in SOD1^{G93A} mice (**Figures 3A,B**). The fatty acid transporters ABCD3 and FABP3 are upregulated beginning at disease onset (**Figure 3B**). In the peroxisome, the peroxisomal bifunctional enzyme HSD17B4 is increased in spinal cord from SOD1^{G93A} while EHHADH is decreased throughout disease course (**Figure 3A**).

Several proteins involved in other aspects of fatty acid metabolism are differentially regulated. BDH1 is a mitochondrial dehydrogenase that interconverts two ketone bodies produced during fatty acid catabolism (Maglott et al., 2011) and is increased in onset and end-stage compared to pre-onset in spinal cords from SOD1^{G93A} mice (**Figure 3B**). ACOT13 is a mitochondrial medium- and long-chain acyl-CoA thioesterase and is decreased at end-stage compared to wild-type (**Figure 3B**). HMGCS1 catalyzes production of HMG-CoA, an intermediate in ketogenesis and isoprenoid biosynthesis, including synthesis of a wide variety of molecules such as cholesterol, ubiquinone, and steroid hormones (Holstein and Hohl, 2004; Maglott et al., 2011). HMGCS1 expression is decreased beginning at disease onset in SOD1^{G93A} mice (**Figure 3B**). HMGCL, a key enzyme in ketogenesis, is increased in SOD1^{G93A} spinal cords. Taken together, these data suggest an increase in mitochondrial fatty acid metabolism in ALS spinal cords.

Tricarboxylic Acid (TCA) Cycle

TCA proteins are generally decreased at end-stage in spinal cords from SOD1^{G93A} mice. Three subunits of pyruvate dehydrogenase (DLAT, PDHA1, PDHB), IDH3A, and MDH2 are decreased in end-stage in spinal cords from SOD1^{G93A} mice compared to wild-type control mice (**Figure 4A**). However DLD, the E3 component of α-ketoglutarate dehydrogenase is increased throughout disease course. Another subunit of α-ketoglutarate dehydrogenase, DLST, and glutamate dehydrogenase (GLUD1) are increased at onset suggesting increased entry of substrates into the TCA cycle from the glutamate pathway and decreased entry from carbohydrate metabolism (**Figure 4A**).

Mitochondrial Electron Transport Chain (ETC)

The content of several assayed proteins in the mitochondrial ETC is increased in spinal cord from the SOD1^{G93A} mice. Subunits of ETC Complex I (NDUFS1, NDUFV1), Complex III (UQCRC1), and Complex V (ATP5A1, ATP5B), as well as ETF (ETFB) are increased in SOD1^{G93A} spinal cord beginning before disease onset (**Figure 4B**). COQ6, which performs a biosynthetic step of ubiquinone synthesis, is also increased in SOD1^{G93A} spinal cord. In contrast, one subunit of Complex II (SDHB) is decreased (**Figure 4B**). Because we saw increased expression of ETC proteins in spinal cords from SOD1^{G93A} mice, we assessed gene expression for nuclear- and mitochondrial-encoded ETC subunits and PGC1α, a transcription factor involved in mitochondrial biogenesis (Scarpulla, 2011), in spinal cords and gastrocnemius muscles from SOD1^{G93A} mice. Expression of all assayed ETC complex subunits is increased and all exhibit a significant difference by genotype effect in spinal cords from SOD1^{G93A} mice compared to controls (**Supplemental Table 3** and **Supplemental Figure 2A**). However, no difference due to genotype is observed in PGC1α expression. In gastrocnemius muscle, no difference is identified between genotypes for ETC subunit expression, but *Ppargc1a* (PGC1α) expression is reduced at end-stage compared to wild-type controls (**Supplemental Table 4** and **Supplemental Figure 2B**).

Antioxidant Enzymes

Oxidative stress has been implicated as an important underlying factor in ALS pathogenesis (Barber and Shaw, 2010). We found many changes in the expression of hydrogen peroxide detoxification enzymes in spinal cord from SOD1^{G93A} mice compared to wild-type mice. The glutathione system, peroxiredoxins, and catalase are altered in spinal cords from SOD1^{G93A} mice (**Figure 5**). PRDX2, PRDX3, CAT, and PRDX6 are increased in SOD1^{G93A} compared to wild-type control (**Figure 5**). Simultaneously, GPX1 (glutathione peroxidase 1), GSTM1, and GSTA3 are decreased in spinal cords from SOD1^{G93A} mice (**Figure 5**). SOD1 is upregulated in SOD1^{G93A} compared to wild-type control, validating SOD1 overexpression in the spinal cord samples, while no change is observed in SOD2, the mitochondrial superoxide dismutase (**Figure 5**).

Stress Response Proteases and Chaperones

Misfolding and accumulation of ubiquitinated cytoplasmic mutant SOD1 inclusions has been observed early in disease progression and likely plays a role in pathogenesis (Ilieva et al., 2009). To understand the cellular response to accumulation of these aggregates, we measured the mRNA expression and protein content of a panel of proteases and chaperones. In spinal cord,

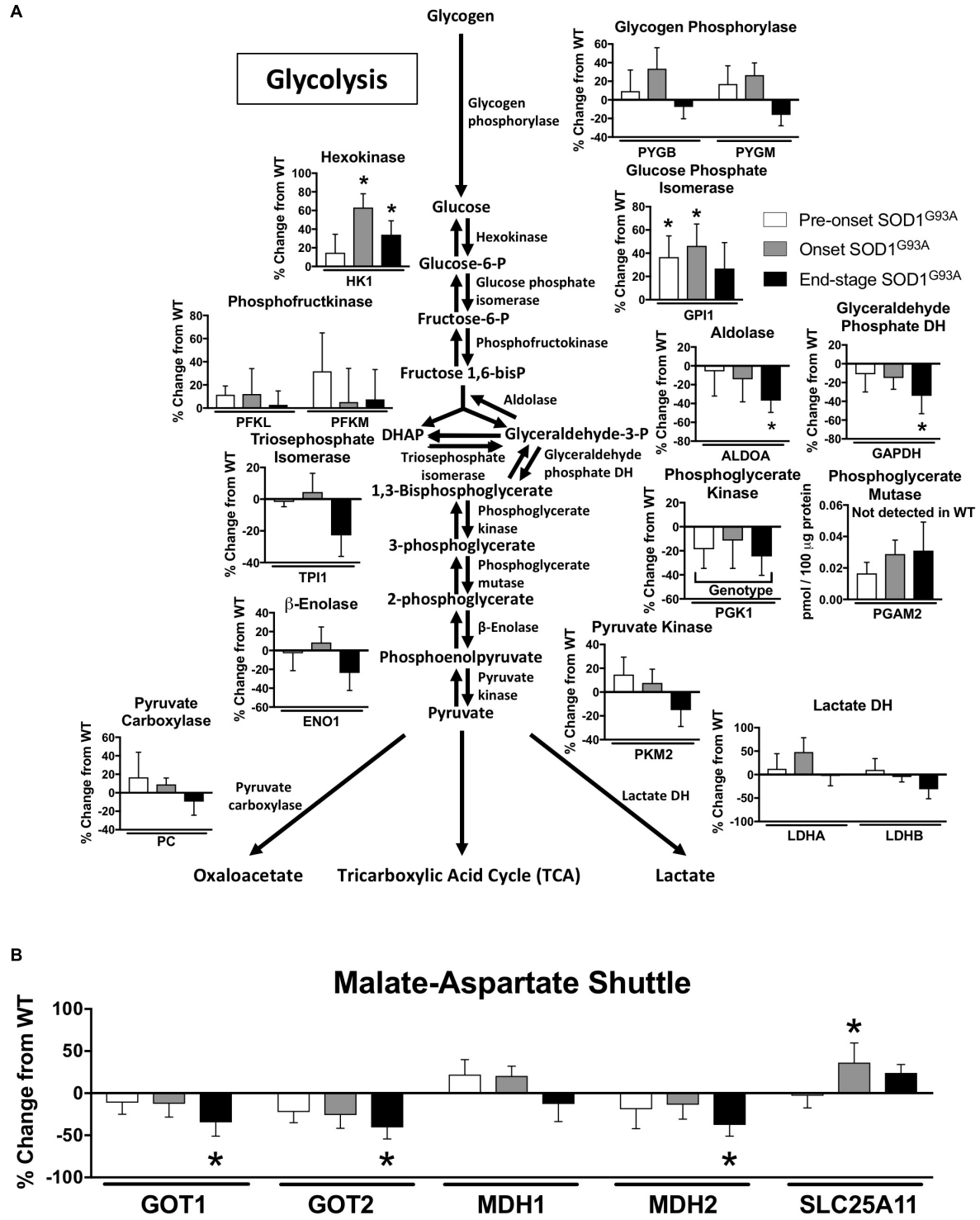

FIGURE 2 | Glycolysis and the malate-aspartate shuttle. Changes in protein content of carbohydrate metabolism in SOD1^{G93A} spinal cords compared to controls measured by mass spectrometry (n = 5–6). **(A)** Glycolysis. **(B)** Malate-aspartate shuttle. Data are represented as percent change relative to the same time point wild-type control. Bars represent means ± standard deviation. Phosphoglycerate mutase 2 (PGAM2) did not reach the level of detection in wild-type samples but did in SOD1^{G93A}. "Genotype" denotes a significant genotype effect in Benjamini-Hochberg corrected two-way ANOVA with $p < 0.05$ but no individual Multiple Comparison test was significant. Significance symbols demonstrate $p < 0.05$ for a genotype effect and additional significant results for the Tukey Multiple Comparison test. *$p < 0.05$ wild-type vs. SOD1^{G93A} at the designated time point. SOD1^{G93A} pre-onset (white), SOD1^{G93A} onset (gray), SOD1^{G93A} end-stage (black). DH, dehydrogenase; DHAP, Dihydroxyacetone phosphate. Protein names, IDs, and absolute expression values are detailed in **Supplemental Table 1**.

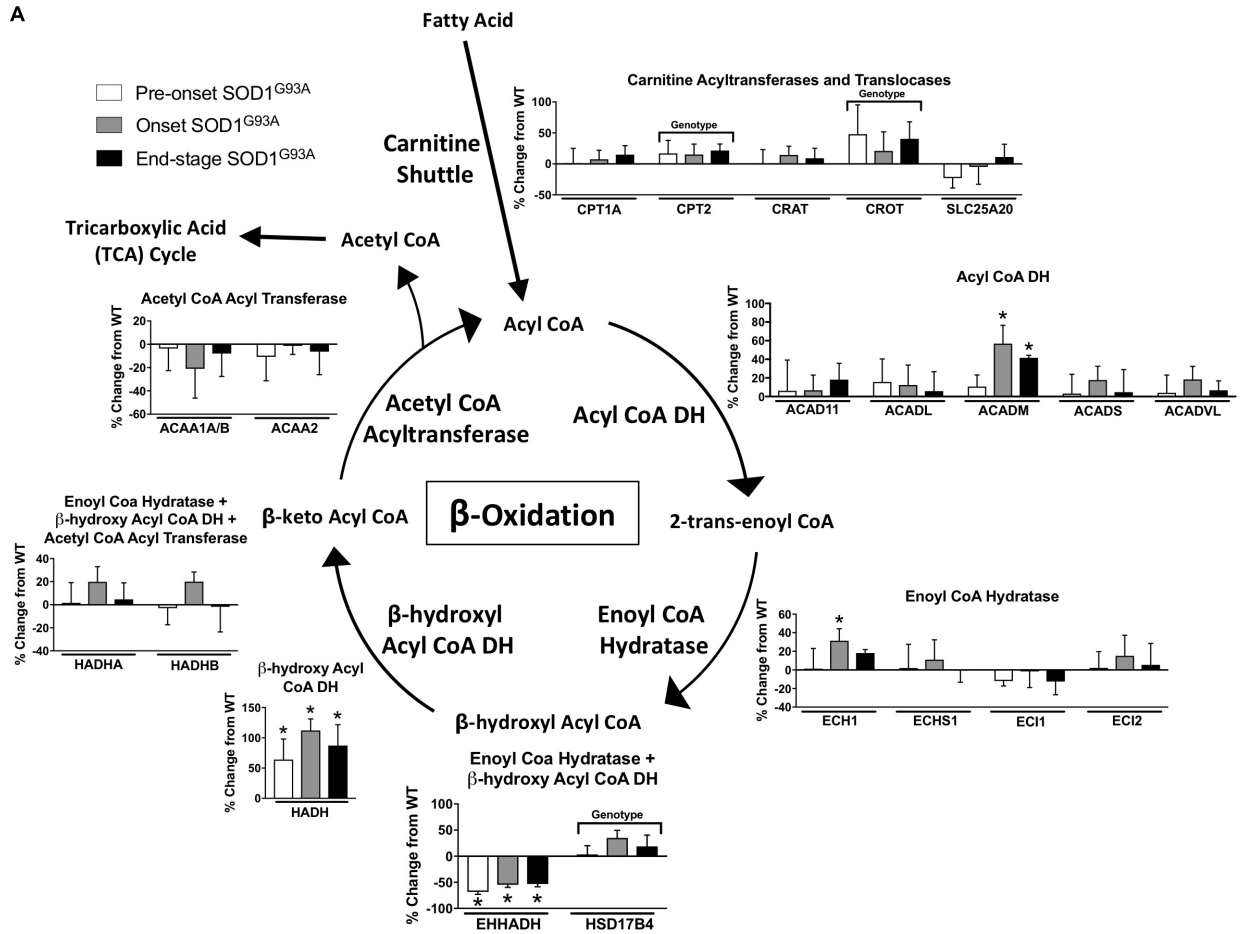

FIGURE 3 | Fatty acid metabolism. Changes in protein content of fatty acid metabolism in SOD1^{G93A} spinal cords compared to controls measured by mass spectrometry (n = 5–6). **(A)** β-oxidation. **(B)** Fatty acid metabolism related proteins. Data are represented as percent change relative to the same time point wild-type control. Bars represent means ± standard deviation. "Genotype" denotes a significant genotype effect in Benjamini-Hochberg corrected two-way ANOVA with $p < 0.05$ but no individual Multiple Comparison test was significant. Significance symbols demonstrate $p < 0.05$ for a genotype effect and additional significant results for the Tukey Multiple Comparison test. *$p < 0.05$ wild-type vs. SOD1^{G93A} at the designated time point; $^{\&}p < 0.05$ vs. SOD1^{G93A} pre-onset. SOD1^{G93A} pre-onset (white), SOD1^{G93A} onset (gray), SOD1^{G93A} end-stage (black). DH, dehydrogenase; CoA, coenzyme A. Protein names, IDs, and absolute expression values are detailed in **Supplemental Table 1**.

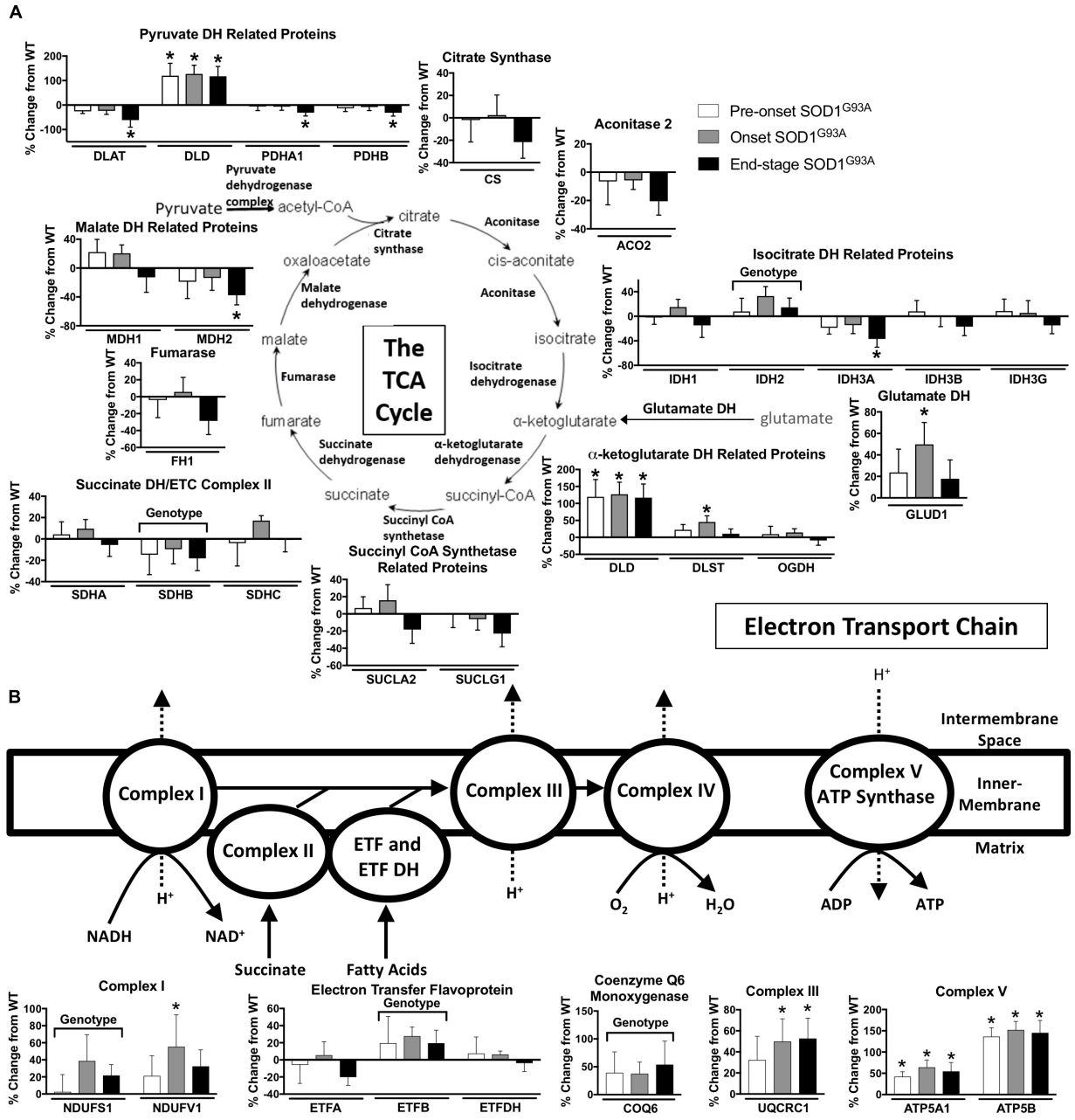

FIGURE 4 | Oxidative mitochondrial metabolism. Changes in protein content of oxidative mitochondrial metabolism in SOD1^{G93A} spinal cords compared to controls measured by mass spectrometry (n = 5–6). **(A)** The tricarboxylic acid (TCA) cycle. **(B)** The electron transport chain (ETC). Data are represented as percent change relative to the same time point wild-type control. Bars represent means ± standard deviation. "Genotype" denotes a significant genotype effect in Benjamini-Hochberg corrected two-way ANOVA with $p < 0.05$ but no individual Multiple Comparison test was significant. Significance symbols demonstrate $p < 0.05$ for a genotype effect and additional significant results for the Tukey Multiple Comparison test. *$p < 0.05$ wild-type vs. SOD1^{G93A} at the designated time point. SOD1^{G93A} pre-onset (white), SOD1^{G93A} onset (gray), SOD1^{G93A} end-stage (black). DH, dehydrogenase; CoA, coenzyme A; ETC, electron transport chain; ETF, electron transfer flavoprotein. Protein names, IDs, and absolute expression values are detailed in **Supplemental Table 1**.

mRNA expression of the mitochondrial proteases *Clpp*, *Lonp1*, *Afg3l2*, and *Oma1* is increased pre-onset, *Yme1l1* is decreased at end-stage, and the chaperone *Hspd1* is increased (**Figure 6B** and **Supplemental Table 3**). While CLPP is not increased at the protein level, CLPX, the chaperone subunit of the CLPX/CLPP oligomer, only reached the limit of detection in spinal cords from SOD1^{G93A} mice (**Supplemental Table 1**). In addition, protein expression of LONP1 and LONP2 as well as the heat shock proteins HSPD1 (Hsp60), CRYAB, and HSP90B1 is increased in spinal cords from SOD1^{G93A} mice compared to controls

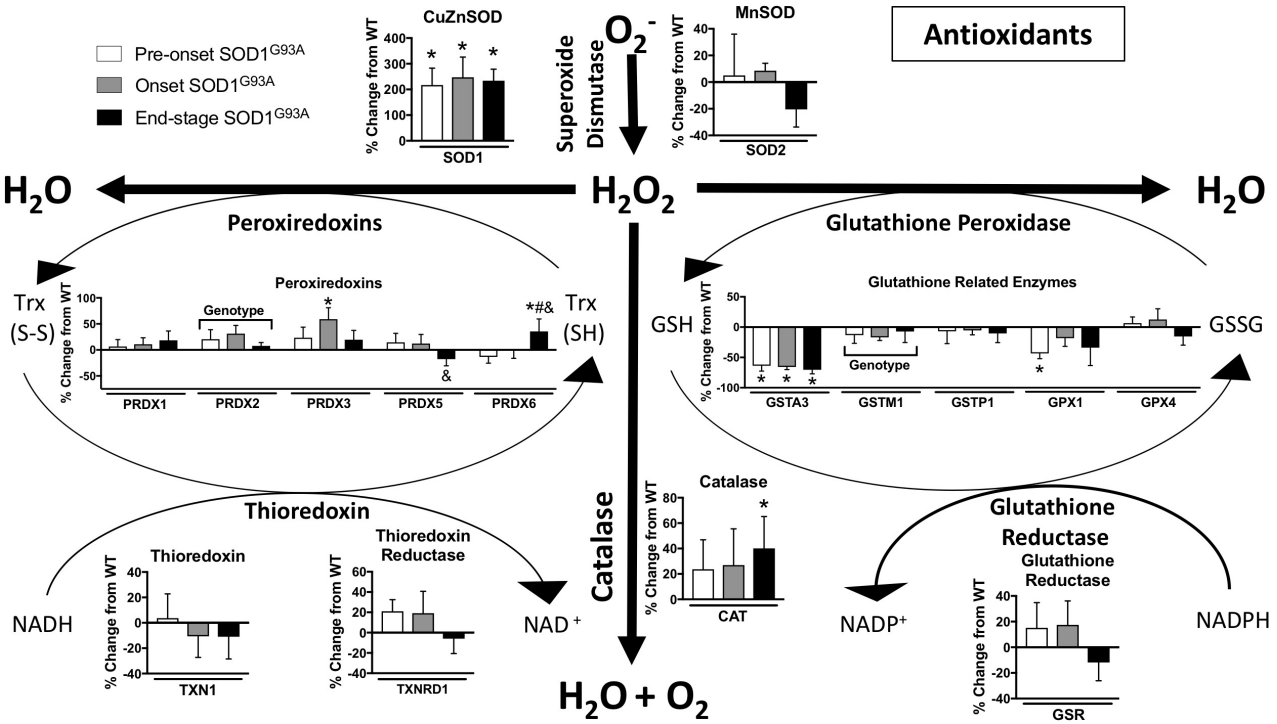

FIGURE 5 | Antioxidants. Changes in protein content of major antioxidants in SOD1^{G93A} spinal cords compared to controls measured by mass spectrometry (n = 5–6). Data are represented as percent change relative to the same time point wild-type control. Bars represent means ± standard deviation. "Genotype" denotes a significant genotype effect in Benjamini-Hochberg corrected two-way ANOVA with $p < 0.05$ but no individual Multiple Comparison test was significant. Significance symbols demonstrate $p < 0.05$ for a genotype effect and additional significant results for the Tukey Multiple Comparison test. $^{*}p < 0.05$ wild-type vs. SOD1^{G93A} at the designated time point; $^{\&}p < 0.05$ vs. SOD1^{G93A} pre-onset; $^{\#}p < 0.05$ vs. SOD1^{G93A} onset. SOD1^{G93A} pre-onset (white), SOD1^{G93A} onset (gray), SOD1^{G93A} end-stage (black). O_2^-, superoxide; H_2O_2, hydrogen peroxide; GSH, reduced glutathione; GSSG, oxidized glutathione disulfide; TRX (SH), reduced thioredoxin; TRX (S-S), oxidized thioredoxin. Protein names, IDs, and absolute expression values are detailed in **Supplemental Table 1**.

beginning before disease onset (**Figure 6C**). In contrast, mRNA expression of *Hspd1*, *Spg7*, *Yme1l1*, and *Oma1* is decreased in gastrocnemius at end-stage (**Figure 6A** and **Supplemental Table 4**). ALDH2, the mitochondrial aldehyde dehydrogenase, protects against oxidative stress (Ohta et al., 2004) and is upregulated in onset SOD1^{G93A} compared to wild-type control (**Supplemental Table 1**). ATP2A2, an endoplasmic reticulum calcium pump, is decreased at end-stage (**Supplemental Table 1**). Together, these data suggest that regulation of stress response to SOD1^{G93A} expression is tissue specific, and spinal cords from SOD1^{G93A} mice experience an increase beginning before onset of motor symptoms. mRNA expression or protein content of 4 proteins (HSPD1, CLPP, LONP1, and CLPX) involved in the UPR^{mt} was increased in spinal cords but not muscle from SOD1^{G93A} mice.

Mitochondrial Function in Cultured Fibroblasts, Sciatic Nerve Tissue, and Permeabilized Muscle Fibers of SOD1^{G93A} Mice

Mitochondrial dysfunction has been broadly implicated in the pathogenesis of neurodegenerative diseases including ALS (Schon and Manfredi, 2003), and impaired mitochondrial function is observed in human fALS SOD1 mutant cultured fibroblasts (Allen et al., 2014). We analyzed oxygen consumption rate (OCR) in pre-onset SOD1^{G93A} mouse dermal fibroblasts with the Seahorse XF24 using sequential additions of the Complex V/ATP synthase inhibitor oligomycin, protonophore FCCP, and Complex III inhibitor antimycin A to measure several parameters of mitochondrial function (**Figure 7A**). SOD1^{G93A} mouse fibroblasts have reduced maximal respiration and reserve capacity compared to wild-type controls, but no change in basal or ATP-linked respiration and no change in proton leak (**Figure 7B**). Extracellular acidification rate (ECAR) is a measurement of glycolysis using change in pH of the media (Wu et al., 2007). ECAR is non-significantly decreased at baseline but is significantly reduced when stressed with oligomycin and FCCP in SOD1^{G93A} mouse fibroblasts (**Figure 7D**). SOD1^{G93A} mouse fibroblasts have a decreased ability to respond to energetic demands in response to stress using glycolytic or oxidative mitochondrial metabolism (**Figure 7C**).

Because we found changes in the expression of ETC complex proteins in spinal cord, and previous work showed altered mitochondrial function in permeabilized SOD1^{G93A} mouse spinal cord (Cacabelos et al., 2016), we assessed mitochondrial function in end-stage nerve tissue and muscle. Due to technical limitations discussed later, we used the sciatic nerve to analyze

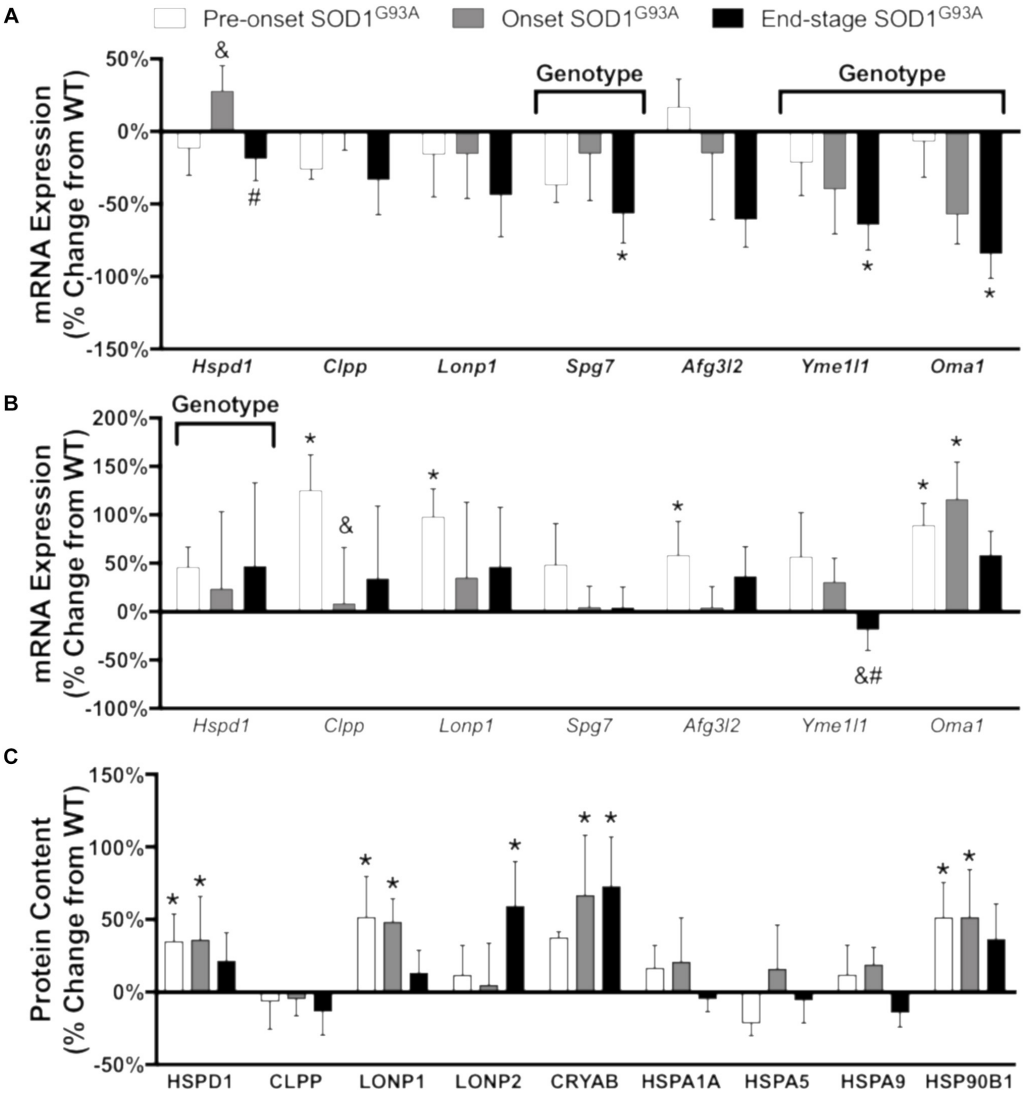

FIGURE 6 | Chaperones and proteases. Changes in mRNA expression and protein content of chaperones and proteases in SOD1^{G93A} gastrocnemius muscle and spinal cords compared to wild-type controls measured by RT-PCR and mass spectrometry (n = 5–6). **(A)** mRNA expression in the gastrocnemius muscle. **(B)** mRNA expression in the spinal cord. **(C)** Protein content in the spinal cord. Data are represented as percent change relative to the same time point wild-type control. Bars represent means ± standard deviation. "Genotype" denotes a significant genotype effect in Benjamini-Hochberg corrected two-way ANOVA with $p < 0.05$ but no individual Multiple Comparison test was significant. Significance symbols demonstrate $p < 0.05$ for a genotype effect and additional significant results for the Tukey Multiple Comparison test. $^{*}p < 0.05$ wild-type vs. SOD1^{G93A} at the designated time point; $^{\&}p < 0.05$ vs. SOD1^{G93A} pre-onset; $^{\#}p < 0.05$ vs. SOD1^{G93A} onset. SOD1^{G93A} pre-onset (white), SOD1^{G93A} onset (gray), SOD1^{G93A} end-stage (black). Gene IDs and relative expression (RQ) values are detailed in **Supplemental Tables 3** (spinal cord) and **4** (gastrocnemius). Protein names, IDs, and absolute expression values are detailed in **Supplemental Table 1**.

mitochondrial function in an affected nerve tissue. We measured simultaneous mitochondrial OCR and hydrogen peroxide and lipid hydroperoxide production rates in permeabilized sciatic nerves and muscle fibers using the OROBOROS Oxygraph-2k with Amplex UltraRed (AUR). Endogenous AUR reaction rate was measured directly after addition of the nerve tissue and muscle fiber bundle to the chamber. OCR and AUR reaction rate were analyzed for both tissues using a sequential addition of glutamate and malate in the absence of ADP to induce proton leak-linked respiration, ADP to stimulate Complex I-linked respiration, pyruvate as a further Complex I substrate, succinate to stimulate Complex I&II-linked respiration (OXPHOS capacity), rotenone to inhibit Complex I and measure Complex II-linked respiration, antimycin A to inhibit Complex III and measure non-mitochondrial OCR, and ascorbate and TMPD to activate CIV-linked respiration. We observe no significant difference in mitochondrial oxidative phosphorylation (OXPHOS) in either sciatic nerve or gastrocnemius muscle (**Figures 7E–H**). However, there is a significant increase in endogenous AUR reaction rate in gastrocnemius fibers from SOD1^{G93A} mice as well as a trend for an increase in rate in sciatic nerve (**Figures 7G,H**).

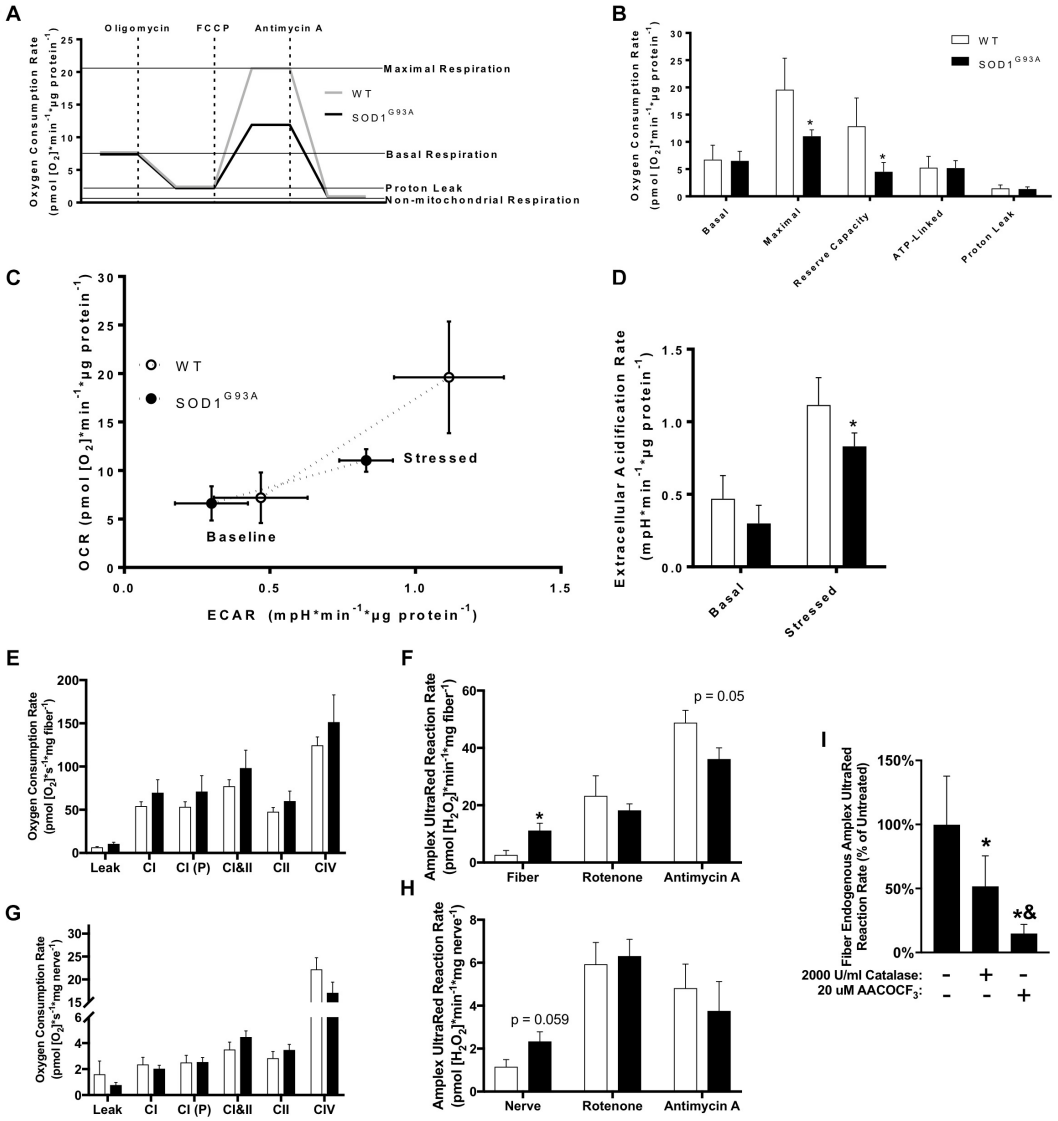

FIGURE 7 | Mitochondrial function in cultured fibroblasts, sciatic nerve tissue, and permeabilized muscle fibers of SOD1^{G93A} mice. **(A)** Schematic showing the output from Seahorse XF24 Flux Analyzer and calculation of measurements. **(B)** Oxygen consumption rate (OCR), **(C)** Extracellular acidification rate (ECAR), and **(D)** Comparison of OCR and ECAR of dermal fibroblasts isolated from wild-type (WT) and SOD1^{G93A} mice (n = 5) measured by Seahorse XF24 Flux Analyzer. Stressed ECAR was measured in the presence of oligomycin and FCCP. Simultaneous OCR and hydrogen peroxide and lipid hydroperoxide production rates (Amplex UltraRed) were assessed in permeabilized sciatic nerves (n = 8–11) and red gastrocnemius fiber biopsies (n = 7–9) from end-stage female mice using the OROBOROS Oxygraph-2k with fluorometer. **(E)** Gastrocnemius fiber OCR. **(F)** Gastrocnemius fiber hydrogen peroxide and lipid hydroperoxide production rates. **(G)** Sciatic nerve OCR. **(H)** Sciatic nerve hydrogen peroxide and lipid hydroperoxide production rates. $^{*}p < 0.05$ wild-type vs. SOD1^{G93A} determined by unpaired student's t-test. **(I)** Basal hydrogen peroxide and lipid hydroperoxide production rates for SOD1^{G93A} gastrocnemius fibers in the OROBOROS O2K fluorometer vehicle treated, or pre-treated with 2000 U/ml Catalase or 20 µM AACOCF$_3$ (n = 7). $^{*}p < 0.05$ treated (either catalase or AACOCF$_3$) vs. untreated control, $^{\&}p < 0.05$ catalase vs. AACOCF$_3$ treated. Substrate-inhibitor protocol and definitions of states are described in Materials and Methods. Bars represent means ± standard deviation. Wild-type (white) and SOD1^{G93A} (black). CI, mitochondrial Complex I (with glutamate, malate, and ADP); CI(P), mitochondrial Complex I (with glutamate, malate, pyruvate, and ADP); CII, mitochondrial Complex II; CIV, mitochondrial Complex IV; FCCP, p-trifluoromethoxy carbonyl cyanide phenyl hydrazone; AACOCF$_3$, Arachidonyl trifluoromethyl ketone.

While the probe AUR is generally used as a measurement for production rate of the reactive oxygen species (ROS) hydrogen peroxide, we have previously demonstrated that it also reacts with lipid hydroperoxides produced in muscle in response to loss of innervation (Bhattacharya et al., 2009). Muscles of SOD1^{G93A} mice experience denervation and atrophy and increase expression of genes associated with loss of innervation including acetylcholine receptor subunits (*Chrna1, Chrnd, Chrne*) and *Gadd45a* (**Supplemental Figure 2C**). *Sln*, which we previously demonstrated is dramatically increased in sarcopenia, is also increased in gastrocnemius muscle from SOD1^{G93A} mice along

with the chemokines *Cxcl2* and *Cxcl5* (Qaisar et al., 2018; **Supplemental Figure 2C**). To distinguish the source of the AUR reaction signal, we treated $SOD1^{G93A}$ gastrocnemius samples with either catalase or arachidonyl trifluoromethyl ketone ($AACOCF_3$). $AACOCF_3$ is an inhibitor of calcium-dependent phospholipase A2 ($cPLA_2$) that we have previously shown inhibits lipid hydroperoxide production in denervated muscle (Bhattacharya et al., 2009). Treatment with $AACOCF_3$ more effectively inhibits the endogenous AUR reaction rate than catalase, suggesting the signal is produced primarily by reaction with lipid hydroperoxides and not hydrogen peroxide or superoxide produced by the mitochondrial ETC (**Figure 7I**). Addition of substrates and inhibitors that increase mitochondrial ETC-derived superoxide and hydrogen peroxide production demonstrates that supraphysiological concentrations of catalase entirely inhibits the increased ETC-derived hydroperoxide production, while $AACOCF_3$ only significantly decreases endogenous hydroperoxide production (**Supplemental Figures 3A,B**).

DISCUSSION

The goal of this study is to determine the changes in metabolism and stress response in lumbar spinal cord of an ALS mouse model as a function of disease course. Understanding metabolic changes in ALS may help us develop specific dietary interventions that can delay disease progression. We find significant differences in protein content of metabolic pathway enzymes, antioxidant systems, heat shock proteins, and proteases that occur early in the lifespan of the mice and before onset of motor symptoms. Expression of the $SOD1^{G93A}$ mutant protein also leads to tissue specific regulation of chaperones and proteases, specifically, the expression of these proteins is induced in spinal cords but not skeletal muscle from $SOD1^{G93A}$ mice. The differences we observed in metabolism and stress response protein content are explained primarily by expression of the $SOD1^{G93A}$ transgene and not greatly modulated by disease stage.

Several previous studies have utilized proteomics approaches to analyze differences in protein content in the spinal cord of pre-onset ALS rodent models (Lukas et al., 2006; Massignan et al., 2007; Li et al., 2010). Massignan et al. (2007) used the two-dimensional gel electrophoresis proteomic method to analyze protein content changes in whole spinal cords from pre-onset $SOD1^{G93A}$ and control mice and also observed significant changes in content of glycolytic, mitochondrial, and stress response proteins. Li et al. (2010) used both two-dimensional gel electrophoresis as well as liquid chromatography followed by tandem mass spectrometry (MudPIT) to analyze changes in protein content of mitochondria isolated from spinal cords of pre-onset $SOD1^{G93A}$ rats and rats overexpressing wildtype SOD1 ($SOD1^{WT}$). Many proteins identified in their MudPIT analysis were not assessed in our targeted approach. However, Li et al. (2010) also observed many changes pre-onset in metabolic and stress response protein content and some were also observed in our analysis including a decrease in MDH2 and an increase or trend for increase in DECR1, IDH2, and PYGB. Lukas et al. (2006) analyzed protein content of spinal cords from end-stage wildtype, $SOD1^{WT}$, and $SOD1^{G93A}$ fractionated into mitochondrial, soluble, and particulate fractions with two-dimensional liquid chromatography coupled to tandem mass spectrometry. The authors found significant changes in metabolic and antioxidant proteins, among others (Lukas et al., 2006). Previous studies have not analyzed metabolic and stress response protein content as a function of disease course. We report here that modification of metabolic and stress response protein content is primarily due to $SOD1^{G93A}$ expression and not disease course.

Metabolism Is Altered by $SOD1^{G93A}$ Expression Before Disease Onset

In the early stages of disease development in this ALS mouse model, there is a significant change in the content of proteins related to glycolysis. Transfection of a neuronal cell line to express $SOD1^{G93A}$ protein has been found to increase glycolytic flux (Valbuena et al., 2016). However, at the end stages of the disease, we find that the content of several proteins involved in the malate-aspartate shuttle, pyruvate dehydrogenase, and TCA cycle is decreased, but the content of some proteins related to mitochondrial fatty acid oxidation and the mitochondrial ETC are increased in spinal cords from $SOD1^{G93A}$ mice. Upon activation, astrocytes undergo metabolic changes that optimize glucose utilization to produce lactate including the upregulation of proteins involved in glycolysis (Iglesias et al., 2017). Gliosis in spinal cords from $SOD1^{G93A}$ mice may partially explain the observed increase in some glycolytic proteins. Activated astrocytes also increase beta-oxidation to produce ketone bodies, which is consistent with our finding that HMGCL, a key enzyme in ketogenesis, is upregulated throughout disease course glycolysis (Iglesias et al., 2017). Compared to glial cells, neurons have a higher rate of oxidative metabolism and a slower glycolytic rate (Bélanger et al., 2011). Glucose is shunted through the pentose-phosphate pathway to regenerate NADPH, which is used to reduce glutathione among other reactions in the neuronal antioxidant system (Wamelink et al., 2008). Increased glycolytic rate comes at the expense of the pentose phosphate pathway and cellular antioxidant capacity (Bélanger et al., 2011). The decrease in ECAR also suggests decreased glycolytic potential in $SOD1^{G93A}$ fibroblasts. Altered fatty acid metabolism has been observed in ALS animal models and patients, where hyperlipidemia is associated with increased survival (Schmitt et al., 2014). Interestingly, ACADM catalyzes the first steps of β-oxidation for medium chain fatty acids, and feeding $SOD1^{G93A}$ mice with a medium chain triglyceride-supplemented diet delayed paralysis and motor neuron cell death (Zhao et al., 2012). In addition to fatty acid metabolism, changes in fat mass have also previously been linked to ALS. Decreased body mass index (BMI) is associated with increased risk of ALS (Gallo et al., 2013). Furthermore, high calorie diet appeared to be beneficial in a small phase 2 clinical trial (Wills et al., 2014). Metabolism plays a key role in

ALS pathogenesis before onset of symptoms, and therapies targeting metabolism and nutrition have potential to delay disease progression.

Metabolomics experiments have been conducted in ALS patients and mouse models. Metabolomic analysis of skeletal muscle, cerebral cortex, and plasma from wildtype and $SOD1^{G93A}$ mice reveals a distinct metabolite profile in $SOD1^{G93A}$ samples in each tissue (Patin et al., 2016). ALS patients also have significantly different plasma and cerebrospinal fluid (CSF) metabolites compared to controls. Metabolites associated with glucose metabolism are significantly different in the CSF of ALS patients compared to controls (Blasco et al., 2010, 2016; Gray et al., 2015). Significantly different plasma metabolites include amino acids, carbohydrates, lipids, peptides, nucleotides, and xenobiotics (Rozen et al., 2005; Lawton et al., 2012, 2014). The difference in plasma or CSF metabolite levels has been used to predict ALS in patients in preliminary studies, although no definitive metabolite panel has been developed for diagnosis of ALS (Blasco et al., 2014; Lawton et al., 2014). Treatment with Riluzole also significantly alters concentration of several metabolites (Rozen et al., 2005). Metabolite level has also been shown to correlate with disease progression, which suggests that metabolites could be used as a biomarker to diagnose ALS (Kumar et al., 2010; Blasco et al., 2016, 2018). However, there is a significant difference in CSF metabolite profile between sporadic, familial, and familial SOD1 mutant patents (Wuolikainen et al., 2011, 2012). Though metabolite profiles are heterogenous between patient subtypes, metabolite concentrations are clearly altered in ALS patients and future studies may provide evidence of common underlying metabolic pathophysiology.

Mitochondrial Dysfunction Is Tissue Specific

Mitochondrial dysfunction has been found to occur in numerous studies of ALS tissues, although observed differences are often not consistent between studies. A potential reason for this discrepancy in study outcomes is the diverse genetic causes and disease progressions in patients (Renton et al., 2014). Several studies have shown changes in neuronal ATP levels, ETC complex activities, mitochondrial calcium buffering capacity, and spinal cord astrocyte mitochondrial damage in ALS mouse models (Jung et al., 2002; Ilieva et al., 2009; Cacabelos et al., 2016). Fibroblasts from human patients are commonly studied as a model for mitochondrial function due to their ease of biopsy compared to the primary affected tissues in humans (spinal cord and skeletal muscle). Fibroblast studies in familial and sporadic ALS patient report differences compared to control samples as well as to each other. Familial *TARDBP(p.A328T)* mutant fibroblasts have decreased mitochondrial membrane potential but no change in OCR (Onesto et al., 2016). Familial *C9ORF72* mutant fibroblasts conversely exhibit mitochondrial hyperpolarization, increased OCR and ATP content, increased ROS and increased mitochondrial DNA content (Onesto et al., 2016). Familial SOD1I113T mutants exhibit decreased basal and coupled OCR and decreased reserved capacity (Allen et al., 2014). Sporadic ALS patient fibroblasts presented with decreased basal and coupled OCR (Raman et al., 2015). We observed reduced maximal respiration and reserve capacity in $SOD1^{G93A}$ mutant mouse fibroblasts compared to controls. Together, these findings suggest that ALS pathology affects mitochondrial function in fibroblasts regardless of underlying genetic cause, but mitochondrial phenotypes may be specific to the gene mutation.

ALS is primarily considered a motor neuron disease, and a previous study reported that basal and Complex I-linked respiration were reduced in onset $SOD1^{G93A}$ mouse spinal cords compared to pre-onset controls (Miquel et al., 2014; Cacabelos et al., 2016). However, a major issue with measuring OCR in tissues is that time of oxygen diffusion increases exponentially with distance and the lumbar spinal cord has much larger circumference than the sciatic nerve. Therefore, we chose permeabilized sciatic nerves for nerve tissue measurements, because they have a much smaller radius than spinal cords. Here we report no difference in sciatic nerve tissue OCR but a trend ($p = 0.059$) for an increase in AUR reaction rate. However, protein content data measured in spinal cord demonstrated an increased ETC subunit content from ALS compared to control mice. Sciatic nerve tissue may show similar compensatory changes.

Oxidative phosphorylation has been assayed in permeabilized fibers from ALS patient *M. vastus lateralis* (MVL) muscle biopsy permeabilized fibers finding a reduction in CI-linked OXPHOS (Wiedemann et al., 1998). However, another study of sALS patient MVL permeabilized fibers found no difference between patient and control muscles for CI-, CII-, or CIV-linked respiration (Echaniz-Laguna et al., 2002). The mean duration of disease in this study was low compared to others, and biopsies were taken before most of the patients exhibited MVL muscle atrophy (Echaniz-Laguna et al., 2002). Here we report no significant difference in OCR of permeabilized gastrocnemius fibers from end-stage $SOD1^{G9EA}$ mice compared to control. However, muscle-specific expression of $SOD1^{G93A}$ is sufficient to induce muscle dysmetabolism by inhibiting glucose uptake and shifting muscle toward using lipid metabolism, which suggests muscle metabolism also plays a significant role in ALS hypermetabolism (Dobrowolny et al., 2018).

Antioxidant and Stress Response Proteins Are Induced

We find that the levels of catalase and peroxiredoxins are induced, but the glutathione system expression is decreased. End-stage sciatic nerve tissue and gastrocnemius muscle fibers from $SOD1^{G93A}$ mice both exhibit increased reactivity *ex vivo* with AUR, which is primarily caused by lipid hydroperoxides in muscle. The role of lipid hydroperoxides in muscle atrophy and contractile dysfunction are still unclear, but these results are consistent with previous findings we reported in denervated muscle fibers (Bhattacharya et al., 2009). GPX4, which reduces lipid hydroperoxides, is not significantly different in spinal cords from $SOD1^{G93A}$ mice. In denervated muscle, we identified

cytosolic phospholipase A2 ($cPLA_2$) as the primary producer of lipid hydroperoxides (Bhattacharya et al., 2009). Previous studies have shown an upregulation of $cPLA_2$ in sporadic ALS patients and pre-onset $SOD1^{G93A}$ mouse spinal cords, and inhibition of $cPLA_2$ exhibits protective effects (Solomonov et al., 2016).

Changes in mitochondrial function may play a causal role in ALS pathogenesis for both motor neuron degeneration and skeletal muscle atrophy. Mutant SOD1 localizes to the mitochondrial outer membrane, intermembrane space, and matrix, where it forms protein aggregates possibly including other mitochondrial proteins such as SOD2 (Vijayvergiya et al., 2005). SOD1 mutant protein aggregates form in spinal cord mitochondria before the onset of motor symptoms (Liu et al., 2004), and motor neuron mitochondrial morphology is altered in SOD1 mutants prior to disease onset (Wong et al., 1995; Kong and Xu, 1998). Changes in muscle mitochondrial morphology occur pre-symptomatically, and muscle-specific $SOD1^{G93A}$ expression is sufficient to induce changes in mitochondrial morphology and cause mutant SOD1 aggregation inside mitochondria (Dobrowolny et al., 2008; Luo et al., 2013). We find a significant increase in heat shock proteins and chaperones in spinal cords from $SOD1^{G93A}$ mice throughout disease course. The mitochondrial UPR^{mt} recognizes a decline in mitochondrial protein quality and induces expression of mitochondrial stress response proteins (Hill and Van Remmen, 2014). A previous study found an upregulation of the mitochondrial intermembrane space UPR^{mt} in lumbar spinal cord of $SOD1^{G93A}$ mice (Riar et al., 2017). Here we report mRNA expression of three proteins (*Hspd1, Clpp, Lonp1*) and protein content of 3 proteins (HSPD1, LONP1, CLPX) involved in the mitochondrial matrix UPR^{mt} are increased throughout disease course in spinal cords of $SOD1^{G93A}$ mice (Wu et al., 2014). Despite increased mRNA expression, CLPP protein content is not altered in spinal cords from $SOD1^{G93A}$ mice. CLPP is required in *C. elegans* to initiate the mitochondrial UPR^{mt}, though recent evidence suggests it is not required in mammals (Bhaskaran et al., 2018). One hypothesis for the selective vulnerability of motor neurons in ALS is a reduced ability to upregulate stress response proteins in response to metabolic or oxidative stress compared to other neuronal populations (Duan and Mattson, 1999; Batulan et al., 2003; Sinclair, 2005; Mattson et al., 2007). The inability to increase CLPP protein content despite an increase in mRNA expression may provide evidence toward this hypothesis.

Several peroxisomal proteins are differentially regulated in ALS spinal cords. ABCD3 is a peroxisomal ATP-dependent fatty acid transporter with implicated roles in peroxisomal biogenesis (Gärtner and Valle, 1993) and uptake of fatty acid overflow from mitochondrial β-oxidation (van Roermund et al., 2014). Two other proteins involved in peroxisomal quality control are upregulated at end-stage: LONP2 and catalase (CAT). LONP2 is a peroxisomal isoform of the mitochondrial LON protease (LONP1), and both degrade proteins damaged by oxidation (Pomatto et al., 2017). Both LON proteases are increased in $SOD1^{G93A}$ suggesting a response to oxidative damage of proteins in both mitochondria and peroxisomes. SOD1 is expressed in peroxisomes, and peroxisomes are associated with the mitochondrial vacuoles occurring in spinal cords from $SOD1^{G93A}$ mice (Higgins et al., 2003). While wild-type SOD1 has been found in peroxisomes, it is unknown whether SOD1 aggregates form there (Islinger et al., 2009). These results suggest a potential role of peroxisomes in ALS that is poorly understood.

Expression of mitochondrial inner-membrane proteases is also affected (*Afg3l2, Yme1l1, Oma1*). *Yme1l1* and *Oma1* are both inner-membrane proteases involved in mitochondrial fission/fusion dynamics (Quirós et al., 2015). *Afg3l2 and Spg7* are subunits of the mAAA protease, which is responsible for maintaining quality of ETC subunits (Quirós et al., 2015). Alterations in mitochondrial dynamics and morphology have been widely reported in ALS patients and animal models (Muyderman and Chen, 2014). In spinal cords from $SOD1^{G93A}$ mice we observe an increase in protein content of some ETC complex proteins. OPA1 processing by the inner-membrane proteases YME1L1 and OMA1 affects mitochondrial fusion and cristae remodeling (Quirós et al., 2015). YME1L1 cleavage of OPA1 promotes mitochondrial fusion, while OPA1 cleavage by OMA1 is activated by mitochondrial oxidative stress, heat stress, or depolarization and inhibits mitochondrial fusion (Quirós et al., 2015). *Oma1* gene expression is induced in spinal cords from $SOD1^{G93A}$ mice. Increased processing of L-OPA1 by OMA1 is associated with swollen mitochondrial cristae, increased mitochondrial fragmentation, and increased sensitivity to apoptosis (Anand et al., 2014), phenotypes reported in ALS (Muyderman and Chen, 2014). OMA1 activation increases turnover of OMA1 as a feedback loop to limit stress response (Quirós et al., 2015). *Oma1* missense mutations were identified in sporadic ALS patients, however, its role in pathogenesis is unclear (Daoud et al., 2011).

$SOD1^{G93A}$ Expression Has Tissue Specific Effects

Metabolism and stress response proteins are differentially regulated in spinal cords from $SOD1^{G93A}$ mice and skeletal muscle over the disease course. While UPR^{mt} mRNA and protein content are increased in spinal cords from $SOD1^{G93A}$ mice, we do not observe a similar change in gastrocnemius fibers. Furthermore, mRNA expression of inner mitochondrial membrane proteases is decreased in gastrocnemius muscle. Several previous proteomics studies have been conducted in different tissues or cell lines of ALS models. Engelen-Lee et al. (2017) performed proteomics analysis in spinal cords from post-mortem sporadic ALS patients, and identified 3 differentially expressed proteins in the posterior horn and 18 in the anterior horn. While they observed changes in metabolism and protein folding pathways, no specific proteins were consistent with those reported here in the $SOD1^{G93A}$ familial ALS mouse model. Szelechowski et al. (2018) performed proteomic analysis in cultured primary motor neuron from pre-onset $SOD1^{G93A}$ mice as well as cultured patient fibroblasts. They found significant alterations in metabolic and misfolded protein response pathways in both cell lines. Interestingly, the

same malate-aspartate shuttle proteins (GOT1, GOT2, MDH2) we identified as decreased in end-stage spinal cords were all decreased in patient fibroblasts. Capitanio et al. (2012) examined some of the targeted proteins we identified in spinal cords in the gastrocnemius and triceps muscles of pre-onset and onset $SOD1^{G93A}$ mice. Only ALDH2 and CRYAB are consistently differentially regulated between spinal cord and muscle samples for stress response. αB-crystallin (CRYAB) has been extensively studied as a potential treatment for ALS treatment, because over-expression reduced SOD1 aggregates in cell culture models and knockout in SOD1 mutant mice accelerated disease onset (Xu et al., 2015). However, transgenic overexpression of αB-crystallin in SOD1 mutant mouse lines did not slow disease progression or reduce aggregate formation, making it a poor therapeutic target for ALS (Xu et al., 2015). The only significant differences in mitochondrial metabolism enzymes conserved between muscle and spinal cord are UQCRC1 and ATP5B, which are both increased in onset triceps and spinal cord (Capitanio et al., 2012). Elf et al. (2014) performed proteomics analysis in muscles of control and ALS patients. In ALS patients, ACADM, PGAM, and PRDX6 ($p < 0.06$) were also found to be differentially regulated. Few changes in ALS protein content compared to control appear to be conserved between tissues.

Caveats and Future Directions

This study provides a view of metabolism in the spinal cord of an ALS model throughout disease course. However, it is impossible to separate metabolism of different cell types in the spinal cord using this design. A recent proteomics study on cultured primary motor neurons from $SOD1^{G93A}$ mice found upregulation of oxidative metabolism including fatty acid β-oxidation and TCA cycle and downregulation of glycolysis (Szelechowski et al., 2018). In addition, survival of motor neurons from an ALS mouse model depended on fatty acid β-oxidation to a much greater extent than neurons from wild-type mice (Szelechowski et al., 2018). Combined, these data suggest these metabolic changes are occurring *in vivo* in motor neurons before onset of motor symptoms. Thus, metabolic reprogramming may play a role in early ALS pathogenesis and provides rationale for further development of dietary interventions as well as other interventions targeting metabolism in ALS patients.

This study also provides evidence for upregulation of components of the mitochondrial UPR^{mt} in spinal cords of $SOD1^{G93A}$ mice before onset of symptoms. Whether upregulation of this pathway delays onset of symptoms requires further study and could provide a new avenue for development of ALS treatments.

MATERIALS AND METHODS

Animals

All animal experiments were carried out in accordance with protocol approved by Institutional Animal Care and Use Committee at Oklahoma Medical Research Foundation. The $G93A^{SOD1}$ mice [B6-Tg(SOD1-G93A)1Gur/J(G93AGur1)] were purchased from Jackson Laboratories (stock number 004435, Jackson Laboratories, Bar Harbor, Maine) and were bred on a C57BL/6J background from our lab and a colony was established for use in this study. The colony is updated with mice purchased from Jackson laboratories every 10–12 months to maintain the stability of the colony. Mice were caged in a pathogen free environment with free access to standard chow and water and maintained on a 12 h light/dark cycle. Mice were weighed weekly and disease assessment was carried out as described earlier (Evans et al., 2015). Based on the severity of the disease, male and female mice were classified as pre-onset (63–69 days), onset (102–121 days), and end stage (135–152 days) and sacrificed accordingly. Wild-type littermates of same age served as controls. Mice were monitored and euthanized if symptoms became too severe. At the time of sacrifice, brain, spinal cord, sciatic nerve, epigonadal white adipose tissue (eWAT), quadriceps, soleus, and gastrocnemius were dissected, weighed and flash frozen in liquid nitrogen for biochemical analysis. One batch of mice was sacrificed separately and fresh sciatic nerve was used for the O2K measurements.

Quantification of Protein Content Using Targeted Proteomics

Protein was isolated from lumbar mouse spinal cords of male and female wild-type and $SOD1^{G93A}$ (pre-onset, onset, and end-stage, $n = 5–6$) in RIPA buffer: 10 mM Tris-Cl (pH 8.0), 1 mM EDTA, 1% Triton X-100 (v/v), 0.1% sodium deoxycholate (w/v), 0.1% SDS (w/v), 140 mM NaCL, and 1 mM PMSF, with protease inhibitor cocktail (Calbiochem Set III, EDTA-free; EMD Millipore; Billerica, MA, United States) as previously described (Ahn et al., 2018). An aliquot containing 100 μg of each samples was taken, 8 pmol BSA was added as an internal standard, and the samples were incubated at 80°C for 15 min in 1% SDS (w/v). Proteins were precipitated with 80% acetone (v/v) at −20°C overnight. The dried protein pellet was reconstituted in 100 μl Laemmli sample buffer and run into a short (1.5 cm) SDS-PAGE gel. The gels were fixed and stained. Each sample was cut from the gel as the entire lane and divided into smaller pieces. The gel pieces were washed to remove the Coomassie blue then reduced, alkylated, and digested overnight with trypsin. The mixture of peptides was extracted from the gel, evaporated to dryness in a SpeedVac and reconstituted in 150 μl 1% acetic acid (v/v) for analysis.

The analyses were carried out on a TSQ Vantage triple quadrupole mass spectrometry system (ThermoScientific TSQ Vantage, San Jose, CA, United States). The HPLC was an Eksigent splitless nanoflow system with a 10 cm × 75 μm i.d. C18 reversed phase capillary column (Eksigent, Dublin, CA, United States). 7 μl aliquots were injected and the peptide eluted with a 60 min gradient of acetonitrile in 0.1% formic acid (v/v). The mass spectrometer was operated in the selected reaction monitoring mode. For each protein, the method was developed to measure 2 ideal peptides. Assays for multiple proteins were bundled together in larger panels. Data were analyzed using the program SkyLine to determine the integrated peak area of the appropriate

chromatographic peaks. The response for each protein was calculated as the geometric mean of the two peptide area. These values were normalized to the response for the BSA standard and multiple housekeeping proteins were also monitored. Entrez Gene name and ID and UniprotKB entry, name, and gene names, and protein names are listed in **Supplemental Table 1** along with results for each protein. The Skyline files containing peak identification and analysis is available at: https://zenodo.org/record/2633276 (doi: 10.5281/zenodo.2633276).

Quantitative Real-Time Polymerase Chain Reaction (RT-PCR)

RT-PCR was performed as previously described (Sataranatarajan et al., 2015). Total RNA was extracted from gastrocnemius and lumbar spinal cord of male and female mice using TRIzol reagent (Invitrogen, Carlsbad, CA, United States). Equal amounts of extracted RNA (1 μg) were converted to first strand cDNA using a cDNA synthesis kit (Bio-Rad, Herculus, CA, United States). 5 ng of the cDNA samples was amplified using primers for *Afg3l2, Cxcl2, Cxcl5, Chrna1, Chrnd, Chrne, Clpp, Gadd45a, Hspd1, Lonp1, mt-Atp6, mt-Co2, mt-Nd1, Ndufs3, Oma1, Ppargc1a, Rfesd, Rn18s* (18S rRNA), *Sdha, Sdhb, Sln, Spg7,* and *Yme1l1* (details in **Supplemental Table 2**) and SYBER green (Invitrogen, Carlsbad, CA, United States). Real time PCR (RT-PCR) was done in Quant Studio 6 (Applied Biosystems, Foster City, CA, United States). The $\Delta\Delta C_t$ method was used to calculate relative mRNA expression.

Primary Fibroblast Cell Culture

Primary fibroblasts were isolated from tail snips of wild-type and $SOD1^{G93A}$ mice as previously described (Pharaoh et al., 2016). Briefly, snips were washed, minced, and incubated overnight with DMEM supplemented with Liberase DL (Roche). The next day the samples were suspended in complete DMEM (4.5 g/l glucose DMEM supplemented with 10% Fetal Bovine Serum and 1% Penicillin/Streptomycin), centrifuged at 200 $\times g$ for 5 min, and the cell pellet was resuspended in complete DMEM and transferred to a 25 mL cell culture flask.

Mitochondrial Function

Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of dermal fibroblasts (30,000 cells/well, $n = 5$, three independent experiments) was measured with a Seahorse XF24 Flux Analyzer under basal conditions and after sequential addition of 1 μM oligomycin (Complex V inhibitor), 1 μM FCCP (proton uncoupler), and 1 μM antimycin A (Complex III inhibitor) normalized to total protein (μg per well) measured by Bradford Protein Assay with BSA standard as previously described (Pharaoh et al., 2016). All OCR measurements were corrected for non-mitochondrial respiration (NMR) using the antimycin A values; basal is the third baseline value, maximal is the highest FCCP value corrected for NMR, reserve capacity is the difference between basal and maximal respiration, ATP-linked respiration is the difference between basal and the lowest oligomycin value, and proton leak is the lowest oligomycin value. For ECAR, basal is the third baseline value, and stressed is the highest value recorded after addition of oligomycin and FCCP.

Gastrocnemius fibers and sciatic nerves were collected from end-stage female mice and placed in ice-cold buffer X for mitochondrial function measurements. OCR and the rate of hydrogen peroxide and lipid hydroperoxide production were simultaneously measured using the Oxygraph-2k (O2k, OROBOROS Instruments, Innsbruck, Austria) in gastrocnemius fibers as previously reported with minor modifications (Ahn et al., 2018). We mechanically separated ∼3–5 mg of red gastrocnemius fibers with forceps in buffer X, permeabilized their plasma membrane with saponin (30 μg/ml) for 30 min, and washed them (3 × 5 min) in buffer Z at 4°C. We mechanically separated sciatic nerves nerve and minced them into 1–1.5 mm segments in buffer X, permeabilized their plasma membrane with saponin (50 μg/ml) for 30min, and washed them (3 × 5 min) in buffer Z at 4°C (Zheng et al., 2011).

OCR was measured using an oxygen probe, while AUR reaction rate was measured using the O2k-Fluo LED2-Module Fluorescence-Sensor Green. Measurements were performed on permeabilized fibers and sciatic nerve sections in buffer Z media at 37°C containing 10 μM Amplex® UltraRed (Molecular Probes, Eugene, OR), 1 U/ml horseradish peroxidase (HRP), 2.5 U/ml superoxide dismutase 1 (SOD1), and blebbistatin (25 μM). HRP catalyzes the reaction between peroxides, including hydrogen peroxide and lipid hydroperoxides, and AUR to produce the fluorescent resorufin (excitation: 565 nm; emission: 600 nM). The fluorescent signal was normalized between chambers via a hydrogen peroxide standard curve established on each day of experiments. Rates of respiration and hydrogen peroxide and lipid hydroperoxide production were measured during sequential additions of substrates and inhibitors as follows: baseline, glutamate (10 mM) and malate (2 mM) for Leak, ADP (5 mM) for Complex I-linked respiration, pyruvate (5 mM) for maximal Complex I-linked respiration, succinate (10 mM) for Complex I and II-linked respiration, rotenone (1μM) for Complex II-linked respiration, antimycin A (1 μM) to measure NMR, and TMPD (0.5 mM) immediately followed by ascorbate (5 mM) for Complex IV-linked respiration. All respiration measurements were normalized to antimycin A to account for non-mitochondrial oxygen consumption. Background resorufin production was subtracted from each experimental measurement of hydrogen peroxide and lipid hydroperoxide production. Graphs for AUR reaction rate show measurements from baseline, after addition of rotenone, and after addition of antimycin A. Data for both OCR and rates of hydrogen peroxide and lipid hydroperoxide generation were normalized by milligrams of muscle bundle wet weights. All substrates and inhibitors were prepared according to the protocols from OROBOROS instruments.

For the catalase vs. $AACOCF_3$ study, 2000 U/ml of catalase was added during permeabilization, washes, and assay, while 20 μM $AACOCF_3$ was added during permeabilization and washes. 2000 U/ml catalase was used as a final concentration for experimentation in order to have maximal inhibition. One unit of catalase will decompose 1.0 μmole of hydrogen peroxide per minute. Because our chambers are 2 ml, and we use 2000 U/ml,

our catalase concentration is capable of decomposing 4 mmoles hydrogen peroxide H_2O_2 per minute. Our measurements are in the pmole hydrogen peroxide per minute scale.

Statistical Analyses and Data Visualization

Statistical analysis and visualization were performed using GraphPad Prism version 7.0b for OS X (GraphPad Software, La Jolla, CA, United States[1]). For RT-PCR and targeted proteomics data sets, outliers were removed using the Grubbs' outlier test (Alpha = 0.05). Statistical significance for protein content was determined by ordinary two-way ANOVA with Tukey's Multiple Comparison test and Benjamini–Hochberg procedure. "Genotype" denotes a significant genotype effect in two-way ANOVA with $q < 0.05$ but no individual Tukey Multiple Comparison test was significant. Significance symbols demonstrate $q < 0.05$ for a genotype effect and additional significant results for the Tukey Multiple Comparison test. Values for CAT, GPI1, HSPD1, and MDH1 were the average from multiple experiments.

Statistical significance for RT-PCR and longitudinal tissue masses is determined by ordinary two-way ANOVA with Tukey's Multiple Comparison test. "Genotype" denotes a significant genotype effect in Benjamini-Hochberg corrected two-way ANOVA with $p < 0.05$ but no individual Multiple Comparison test was significant. Significance symbols demonstrate $p < 0.05$ for a genotype effect and additional significant results for the Tukey Multiple Comparison test. For eWAT end-stage mass, statistical significance is determined by unpaired two-tailed student's t-test ($^*p < 0.05$). Unpaired two-tailed student's t-test was used for comparisons between two groups in the mitochondrial function experiments, and ordinary one-way ANOVA with Tukey's Multiple Comparison Test was used for the OROBOROS O2K experiment of gastrocnemius fibers from $SOD1^{G93A}$ mice treated with catalase or $AACOCF_3$.

Heatmap and the PCA plot were generated using ClustVis with default settings (Row scaling = unit variance scaling, PCA method = SVD with imputation, clustering distance for rows = correlation, clustering method for rows = average, tree ordering for rows = tightest cluster first) (Metsalu and Vilo, 2015).

[1] www.graphpad.com

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of USDA guidelines by the Institutional Animal Care and Use Committee at Oklahoma Medical Research Foundation. The protocol was approved by the Institutional Animal Care and Use Committee at Oklahoma Medical Research Foundation.

AUTHOR CONTRIBUTIONS

Study was conceived and designed by HVR. Experiments were performed and data collected by GP, KSa, KSt, SH, JG, CK, and MK. BA developed the sciatic nerve mitochondrial experimental procedure used here. Data analysis and visualization was performed by GP. The manuscript was written by GP with input from HVR. All authors edited and approved the final manuscript.

FUNDING

This work was supported by a grant from the Pape Adams Foundation to HVR, the Oklahoma Medical Research Foundation (OMRF) Fleming Scholar Program, and the OMRF John and Mildred Carson Ph.D. Scholarship Fund and The National Institute on Aging T32 AG052363 training fellowships awarded to GP.

ACKNOWLEDGMENTS

We would like to thank the Oklahoma Nathan Shock Center for Excellence in the Biology of Aging Multiplexing Protein Quantification Core.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2019.00487/full#supplementary-material

REFERENCES

Abe, K., Itoyama, Y., Sobue, G., Tsuji, S., Aoki, M., Doyu, M., et al. (2014). Confirmatory double-blind, parallel-group, placebo-controlled study of efficacy and safety of edaravone (MCI-186) in amyotrophic lateral sclerosis patients. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 15, 610–617. doi: 10.3109/21678421.2014.959024

Ahn, B., Pharaoh, G., Premkumar, P., Huseman, K., Ranjit, R., Kinter, M., et al. (2018). Nrf2 deficiency exacerbates age-related contractile dysfunction and loss of skeletal muscle mass. *Redox Biol.* 17, 47–58. doi: 10.1016/j.redox.2018.04.004

Allen, S. P., Rajan, S., Duffy, L., Mortiboys, H., Higginbottom, A., Grierson, A. J., et al. (2014). Superoxide dismutase 1 mutation in a cellular model of amyotrophic lateral sclerosis shifts energy generation from oxidative phosphorylation to glycolysis. *Neurobiol. Aging* 35, 1499–1509. doi: 10.1016/j.neurobiolaging.2013.11.025

Anand, R., Wai, T., Baker, M. J., Kladt, N., Schauss, A. C., Rugarli, E., et al. (2014). Thei-AAA protease YME1L and OMA1 cleave OPA1 to balance mitochondrial fusion and fission. *J. Cell Biol.* 204, 919–929. doi: 10.1083/jcb.201308006

Barber, S. C., and Shaw, P. J. (2010). Oxidative stress in ALS: key role in motor neuron injury and therapeutic target. *Free Radic. Biol. Med.* 48, 629–641. doi: 10.1016/j.freeradbiomed.2009.11.018

Batulan, Z., Shinder, G. A., Minotti, S., He, B. P., Doroudchi, M. M., Nalbantoglu, J., et al. (2003). High threshold for induction of the stress response in motor neurons is associated with failure to activate HSF1. *J. Neurosci.* 23, 5789–5798. doi: 10.1523/jneurosci.23-13-05789.2003

Bélanger, M., Allaman, I., and Magistretti, P. J. (2011). Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. *Cell Metab.* 14, 724–738. doi: 10.1016/j.cmet.2011.08.016

Bhaskaran, S., Pharaoh, G., Ranjit, R., Murphy, A., Matsuzaki, S., Nair, B. C., et al. (2018). Loss of mitochondrial protease ClpP protects mice from diet-induced obesity and insulin resistance. *EMBO Rep* 19:e45009. doi: 10.15252/embr.201745009

Bhattacharya, A., Muller, F. L., Liu, Y., Sabia, M., Liang, H., Song, W., et al. (2009). Denervation induces cytosolic phospholipase A2-mediated fatty acid hydroperoxide generation by muscle mitochondria. *J. Biol. Chem.* 284, 46–55. doi: 10.1074/jbc.M806311200

Blasco, H., Corcia, P., Moreau, C., Veau, S., Fournier, C., Vourc'h, P., et al. (2010). 1H-NMR-based metabolomic profiling of CSF in early amyotrophic lateral sclerosis. *PLoS One* 5:e13223. doi: 10.1371/journal.pone.0013223

Blasco, H., Nadal-Desbarats, L., Pradat, P.-F., Gordon, P. H., Antar, C., Veyrat-Durebex, C., et al. (2014). Untargeted 1H-NMR metabolomics in CSF: toward a diagnostic biomarker for motor neuron disease. *Neurology* 82, 1167–1174. doi: 10.1212/WNL.0000000000000274

Blasco, H., Nadal-Desbarats, L., Pradat, P.-F., Gordon, P. H., Madji Hounoum, B., Patin, F., et al. (2016). Biomarkers in amyotrophic lateral sclerosis: combining metabolomic and clinical parameters to define disease progression. *Eur. J. Neurol.* 23, 346–353. doi: 10.1111/ene.12851

Blasco, H., Patin, F., Descat, A., Garçon, G., Corcia, P., Gelé, P., et al. (2018). A pharmaco-metabolomics approach in a clinical trial of ALS: identification of predictive markers of progression. *PLoS One* 13:e0198116. doi: 10.1371/journal.pone.0198116

Bouteloup, C., Desport, J.-C., Clavelou, P., Guy, N., Derumeaux-Burel, H., Ferrier, A., et al. (2009). Hypermetabolism in ALS patients: an early and persistent phenomenon. *J. Neurol.* 256, 1236–1242. doi: 10.1007/s00415-009-5100-z

Cacabelos, D., Ramírez-Núñez, O., Granado-Serrano, A. B., Torres, P., Ayala, V., Moiseeva, V., et al. (2016). Early and gender-specific differences in spinal cord mitochondrial function and oxidative stress markers in a mouse model of ALS. *Acta Neuropathol. Commun.* 4:3. doi: 10.1186/s40478-015-0271-6

Capitanio, D., Vasso, M., Ratti, A., Grignaschi, G., Volta, M., Moriggi, M., et al. (2012). Molecular signatures of amyotrophic lateral sclerosis disease progression in hind and forelimb muscles of an SOD1G93A mouse Model. *Antioxid. Redox Signal.* 17, 1333–1350. doi: 10.1089/ars.2012.4524

Daoud, H., Valdmanis, P. N., Gros-Louis, F., Belzil, V., Spiegelman, D., Henrion, E., et al. (2011). Resequencing of 29 candidate genes in patients with familial and sporadic amyotrophic lateral sclerosis. *Arch. Neurol.* 68, 587–593. doi: 10.1001/archneurol.2010.351

Dobrowolny, G., Aucello, M., Rizzuto, E., Beccafico, S., Mammucari, C., Boncompagni, S., et al. (2008). Skeletal muscle is a primary target of SOD1G93A-mediated toxicity. *Cell Metab.* 8, 425–436. doi: 10.1016/j.cmet.2008.09.002

Dobrowolny, G., Lepore, E., Martini, M., Barberi, L., Nunn, A., Scicchitano, B. M., et al. (2018). Metabolic changes associated with muscle expression of SOD1. *Front. Physiol.* 9:831. doi: 10.3389/fphys.2018.00831

Duan, W., and Mattson, M. P. (1999). Dietary restriction and 2-deoxyglucose administration improve behavioral outcome and reduce degeneration of dopaminergic neurons in models of Parkinson's disease. *J. Neurosci. Res.* 57, 195–206. doi: 10.1002/(sici)1097-4547(19990715)57:2<195::aid-jnr5>3.0.co;2-p

Dupuis, L., Oudart, H., Rene, F., de Aguilar, J.-L. G., and Loeffler, J.-P. (2004). Evidence for defective energy homeostasis in amyotrophic lateral sclerosis: benefit of a high-energy diet in a transgenic mouse model. *Proc. Natl. Acad. Sci. U.S.A.* 101, 11159–11164. doi: 10.1073/pnas.0402026101

Echaniz-Laguna, A., Zoll, J., Ribera, F., Tranchant, C., Warter, J.-M., Lonsdorfer, J., et al. (2002). Mitochondrial respiratory chain function in skeletal muscle of ALS patients. *Ann. Neurol.* 52, 623–627. doi: 10.1002/ana.10357

Elf, K., Shevchenko, G., Nygren, I., Larsson, L., Bergquist, J., Askmark, H., et al. (2014). Alterations in muscle proteome of patients diagnosed with amyotrophic lateral sclerosis. *J. Proteom.* 108, 55–64. doi: 10.1016/j.jprot.2014.05.004

Engelen-Lee, J., Blokhuis, A. M., Spliet, W. G. M., Pasterkamp, R. J., Aronica, E., Demmers, J. A. A., et al. (2017). Proteomic profiling of the spinal cord in ALS: decreased ATP5D levels suggest synaptic dysfunction in ALS pathogenesis. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 18, 210–220. doi: 10.1080/21678421.2016.1245757

Evans, T. M., Jaramillo, C. A., Sataranatarajan, K., Watts, L., Sabia, M., Qi, W., et al. (2015). The effect of mild traumatic brain injury on peripheral nervous system pathology in wild-type mice and the G93A mutant mouse model of motor neuron disease. *Neuroscience* 298, 410–423. doi: 10.1016/j.neuroscience.2015.04.041

Gallo, V., Wark, P. A., Jenab, M., Pearce, N., Brayne, C., Vermeulen, R., et al. (2013). Prediagnostic body fat and risk of death from amyotrophic lateral sclerosis: the EPIC cohort. *Neurology* 80, 829–838. doi: 10.1212/WNL.0b013e3182840689

Gärtner, J., and Valle, D. (1993). The 70 kDa peroxisomal membrane protein: an ATP-binding cassette transporter protein involved in peroxisome biogenesis. *Semin. Cell Biol.* 4, 45–52. doi: 10.1006/scel.1993.1006

Gray, E., Larkin, J. R., Claridge, T. D. W., Talbot, K., Sibson, N. R., and Turner, M. R. (2015). The longitudinal cerebrospinal fluid metabolomic profile of amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 16, 456–463. doi: 10.3109/21678421.2015.1053490

Hamadeh, M. J., Rodriguez, M. C., Kaczor, J. J., and Tarnopolsky, M. A. (2005). Caloric restriction transiently improves motor performance but hastens clinical onset of disease in the Cu/Zn-superoxide dismutase mutant G93A mouse. *Muscle Nerve* 31, 214–220. doi: 10.1002/mus.20255

Higgins, C. M. J., Jung, C., and Xu, Z. (2003). ALS-associated mutant SOD1G93A causes mitochondrial vacuolation by expansion of the intermembrane space and by involvement of SOD1 aggregation and peroxisomes. *BMC Neurosci.* 4:16. doi: 10.1186/1471-2202-4-16

Hill, S., and Van Remmen, H. (2014). Mitochondrial stress signaling in longevity: a new role for mitochondrial function in aging. *Redox Biol.* 2, 936–944. doi: 10.1016/j.redox.2014.07.005

Holstein, S. A., and Hohl, R. J. (2004). Isoprenoids: remarkable diversity of form and function. *Lipids* 39, 293–309. doi: 10.1007/s11745-004-1233-3

Iglesias, J., Morales, L., and Barreto, G. E. (2017). Metabolic and inflammatory adaptation of reactive astrocytes: role of PPARs. *Mol. Neurobiol.* 54, 2518–2538. doi: 10.1007/s12035-016-9833-2

Ilieva, H., Polymenidou, M., and Cleveland, D. W. (2009). Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. *J. Cell Biol.* 187, 761–772. doi: 10.1083/jcb.200908164

Islinger, M., Li, K. W., Seitz, J., Völkl, A., and Lüers, G. H. (2009). Hitchhiking of Cu/Zn superoxide dismutase to peroxisomes–evidence for a natural piggyback import mechanism in mammals. *Traffic* 10, 1711–1721. doi: 10.1111/j.1600-0854.2009.00966.x

Jung, C., Higgins, C. M. J., and Xu, Z. (2002). Mitochondrial electron transport chain complex dysfunction in a transgenic mouse model for amyotrophic lateral sclerosis. *J. Neurochem.* 83, 535–545. doi: 10.1046/j.1471-4159.2002.01112.x

Kong, J., and Xu, Z. (1998). Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral sclerosis in mice expressing a mutant SOD1. *J. Neurosci.* 18, 3241–3250. doi: 10.1523/jneurosci.18-09-03241.1998

Kumar, A., Bala, L., Kalita, J., Misra, U. K., Singh, R. L., Khetrapal, C. L., et al. (2010). Metabolomic analysis of serum by (1) H NMR spectroscopy in amyotrophic lateral sclerosis. *Clin. Chim. Acta* 411, 563–567. doi: 10.1016/j.cca.2010.01.016

Lawton, K. A., Brown, M. V., Alexander, D., Li, Z., Wulff, J. E., Lawson, R., et al. (2014). Plasma metabolomic biomarker panel to distinguish patients with amyotrophic lateral sclerosis from disease mimics. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 15, 362–370. doi: 10.3109/21678421.2014.908311

Lawton, K. A., Cudkowicz, M. E., Brown, M. V., Alexander, D., Caffrey, R., Wulff, J. E., et al. (2012). Biochemical alterations associated with ALS. *Amyotroph. Lateral Scler.* 13, 110–118. doi: 10.3109/17482968.2011.619197

Li, Q., Vande Velde, C., Israelson, A., Xie, J., Bailey, A. O., Dong, M.-Q., et al. (2010). ALS-linked mutant superoxide dismutase 1 (SOD1) alters mitochondrial protein composition and decreases protein import. *Proc. Natl. Acad. Sci. U.S.A.* 107, 21146–21151. doi: 10.1073/pnas.1014862107

Liu, J., Lillo, C., Jonsson, P. A., Vande Velde, C., Ward, C. M., Miller, T. M., et al. (2004). Toxicity of familial ALS-linked SOD1 mutants from selective recruitment to spinal mitochondria. *Neuron* 43, 5–17. doi: 10.1016/j.neuron.2004.06.016

Lukas, T. J., Luo, W. W., Mao, H., Cole, N., and Siddique, T. (2006). Informatics-assisted protein profiling in a transgenic mouse model of amyotrophic lateral sclerosis. *Mol. Cell. Proteom.* 5, 1233–1244. doi: 10.1074/mcp.m500431-mcp200

Luo, G., Yi, J., Ma, C., Xiao, Y., Yi, F., Yu, T., et al. (2013). Defective mitochondrial dynamics is an early event in skeletal muscle of an amyotrophic lateral sclerosis mouse model. *PLoS One* 8:e82112. doi: 10.1371/journal.pone.0082112

Maglott, D., Ostell, J., Pruitt, K. D., and Tatusova, T. (2011). Entrez gene: gene-centered information at NCBI. *Nucleic Acids Res.* 39, D52–D57. doi: 10.1093/nar/gkq1237

Massignan, T., Casoni, F., Basso, M., Stefanazzi, P., Biasini, E., Tortarolo, M., et al. (2007). Proteomic analysis of spinal cord of presymptomatic amyotrophic lateral sclerosis G93A SOD1 mouse. *Biochem. Biophys. Res. Commun.* 353, 719–725. doi: 10.1016/j.bbrc.2006.12.075

Mattson, M. P., Cutler, R. G., and Camandola, S. (2007). Energy intake and amyotrophic lateral sclerosis. *Neuromol. Med.* 9, 17–20.

Metsalu, T., and Vilo, J. (2015). ClustVis: a web tool for visualizing clustering of multivariate data using principal component analysis and heatmap. *Nucleic Acids Res.* 43, W566–W570. doi: 10.1093/nar/gkv468

Miquel, E., Cassina, A., Martínez-Palma, L., Souza, J. M., Bolatto, C., Rodríguez-Bottero, S., et al. (2014). Neuroprotective effects of the mitochondria-targeted antioxidant MitoQ in a model of inherited amyotrophic lateral sclerosis. *Free Radic. Biol. Med.* 70, 204–213. doi: 10.1016/j.freeradbiomed.2014.02.019

Muyderman, H., and Chen, T. (2014). Mitochondrial dysfunction in amyotrophic lateral sclerosis – a valid pharmacological target? *Br. J. Pharmacol.* 171, 2191–2205. doi: 10.1111/bph.12476

Ohta, S., Ohsawa, I., Kamino, K., Ando, F., and Shimokata, H. (2004). Mitochondrial ALDH2 Deficiency as an Oxidative Stress. *Ann. N. Y. Acad. Sci.* 1011, 36–44. doi: 10.1007/978-3-662-41088-2_4

Onesto, E., Colombrita, C., Gumina, V., Borghi, M. O., Dusi, S., Doretti, A., et al. (2016). Gene-specific mitochondria dysfunctions in human TARDBP and C9ORF72 fibroblasts. *Acta Neuropathol. Commun.* 4:47. doi: 10.1186/s40478-016-0316-5

Patin, F., Baranek, T., Vourc'h, P., Nadal-Desbarats, L., Goossens, J.-F., Marouillat, S., 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. doi: 10.1007/s13311-016-0461-3

Pharaoh, G., Pulliam, D., Hill, S., Sataranatarajan, K., and Van Remmen, H. (2016). Ablation of the mitochondrial complex IV assembly protein Surf1 leads to increased expression of the UPR(MT) and increased resistance to oxidative stress in primary cultures of fibroblasts. *Redox Biol.* 8, 430–438. doi: 10.1016/j.redox.2016.05.001

Pomatto, L. C. D., Raynes, R., and Davies, K. J. A. (2017). The peroxisomal Lon protease LonP2 in aging and disease: functions and comparisons with mitochondrial Lon protease LonP1. *Biol. Rev. Camb. Philos. Soc.* 92, 739–753. doi: 10.1111/brv.12253

Qaisar, R., Bhaskaran, S., Premkumar, P., Ranjit, R., Natarajan, K. S., Ahn, B., et al. (2018). Oxidative stress-induced dysregulation of excitation-contraction coupling contributes to muscle weakness. *J. Cachexia Sarcopenia Muscle.* 9, 1003–1017. doi: 10.1002/jcsm.12339

Quirós, P. M., Langer, T., and López-Otín, C. (2015). New roles for mitochondrial proteases in health, ageing and disease. *Nat. Rev. Mol. Cell Biol.* 16, 345–359. doi: 10.1038/nrm3984

Raman, R., Allen, S. P., Goodall, E. F., Kramer, S., Ponger, L.-L., Heath, P. R., et al. (2015). Gene expression signatures in motor neurone disease fibroblasts reveal dysregulation of metabolism, hypoxia-response and RNA processing functions. *Neuropathol. Appl. Neurobiol.* 41, 201–226. doi: 10.1111/nan.12147

Renton, A. E., Chiò, A., and Traynor, B. J. (2014). State of play in amyotrophic lateral sclerosis genetics. *Nat. Neurosci.* 17, 17–23. doi: 10.1038/nn.3584

Riar, A. K., Burstein, S. R., Palomo, G. M., Arreguin, A., Manfredi, G., and Germain, D. (2017). Sex specific activation of the ERα axis of the mitochondrial UPR (UPRmt) in the G93A-SOD1 mouse model of familial ALS. *Hum. Mol. Genet.* 26, 1318–1327. doi: 10.1093/hmg/ddx049

Rizzo, F., Riboldi, G., Salani, S., Nizzardo, M., Simone, C., Corti, S., et al. (2014). Cellular therapy to target neuroinflammation in amyotrophic lateral sclerosis. *Cell. Mol. Life Sci.* 71, 999–1015. doi: 10.1007/s00018-013-1480-4

Rowland, L. P., and Shneider, N. A. (2001). Amyotrophic lateral sclerosis. *N. Engl. J. Med.* 344, 1688–1700.

Rozen, S., Cudkowicz, M. E., Bogdanov, M., Matson, W. R., Kristal, B. S., Beecher, C., et al. (2005). Metabolomic analysis and signatures in motor neuron disease. *Metabolomics* 1, 101–108. doi: 10.1007/s11306-005-4810-1

Sataranatarajan, K., Qaisar, R., Davis, C., Sakellariou, G. K., Vasilaki, A., Zhang, Y., et al. (2015). Neuron specific reduction in CuZnSOD is not sufficient to initiate a full sarcopenia phenotype. *Redox Biol.* 5, 140–148. doi: 10.1016/j.redox.2015.04.005

Scarpulla, R. C. (2011). Metabolic control of mitochondrial biogenesis through the PGC-1 family regulatory network. *Biochim. Biophys. Acta* 1813, 1269–1278. doi: 10.1016/j.bbamcr.2010.09.019

Schmitt, F., Hussain, G., Dupuis, L., Loeffler, J.-P., and Henriques, A. (2014). A plural role for lipids in motor neuron diseases: energy, signaling and structure. *Front. Cell. Neurosci.* 8:25. doi: 10.3389/fncel.2014.00025

Schon, E. A., and Manfredi, G. (2003). Neuronal degeneration and mitochondrial dysfunction. *J. Clin. Invest.* 111, 303–312. doi: 10.1172/jci17741

Sinclair, D. A. (2005). Toward a unified theory of caloric restriction and longevity regulation. *Mech. Ageing Dev.* 126, 987–1002. doi: 10.1016/j.mad.2005.03.019

Solomonov, Y., Hadad, N., and Levy, R. (2016). Reduction of cytosolic phospholipase A2α upregulation delays the onset of symptoms in SOD1G93A mouse model of amyotrophic lateral sclerosis. *J. Neuroinflamm.* 13:134. doi: 10.1186/s12974-016-0602-y

Szelechowski, M., Amoedo, N., Obre, E., Léger, C., Allard, L., Bonneu, M., et al. (2018). Metabolic reprogramming in amyotrophic lateral sclerosis. *Sci. Rep.* 8:3953. doi: 10.1038/s41598-018-22318-5

Valbuena, G. N., Rizzardini, M., Cimini, S., Siskos, A. P., Bendotti, C., Cantoni, L., et al. (2016). Metabolomic analysis reveals increased aerobic glycolysis and amino acid deficit in a cellular model of amyotrophic lateral sclerosis. *Mol. Neurobiol.* 53, 2222–2240. doi: 10.1007/s12035-015-9165-7

van Roermund, C. W. T., Ijlst, L., Wagemans, T., Wanders, R. J. A., and Waterham, H. R. (2014). A role for the human peroxisomal half-transporter ABCD3 in the oxidation of dicarboxylic acids. *Biochim. Biophys. Acta* 1841, 563–568. doi: 10.1016/j.bbalip.2013.12.001

Vijayvergiya, C., Beal, M. F., Buck, J., and Manfredi, G. (2005). Mutant superoxide dismutase 1 forms aggregates in the brain mitochondrial matrix of amyotrophic lateral sclerosis mice. *J. Neurosci.* 25, 2463–2470. doi: 10.1523/jneurosci.4385-04.2005

Wamelink, M. M. C., Struys, E. A., and Jakobs, C. (2008). The biochemistry, metabolism and inherited defects of the pentose phosphate pathway: a review. *J. Inherit. Metab. Dis.* 31, 703–717. doi: 10.1007/s10545-008-1015-6

Wiedemann, F. R., Winkler, K., Kuznetsov, A. V., Bartels, C., Vielhaber, S., Feistner, H., et al. (1998). Impairment of mitochondrial function in skeletal muscle of patients with amyotrophic lateral sclerosis. *J. Neurol. Sci.* 156, 65–72. doi: 10.1016/s0022-510x(98)00008-2

Wills, A.-M., Hubbard, J., Macklin, E. A., Glass, J., Tandan, R., Simpson, E. P., et al. (2014). Hypercaloric enteral nutrition in patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet* 383, 2065–2072. doi: 10.1016/S0140-6736(14)60222-1

Wong, P. C., Pardo, C. A., Borchelt, D. R., Lee, M. K., Copeland, N. G., Jenkins, N. A., et al. (1995). An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. *Neuron* 14, 1105–1116.

Wu, M., Neilson, A., Swift, A. L., Moran, R., Tamagnine, J., Parslow, D., et al. (2007). Multiparameter metabolic analysis reveals a close link between attenuated mitochondrial bioenergetic function and enhanced glycolysis dependency in human tumor cells. *Am. J. Physiol. Cell Physiol.* 292, C125–C136.

Wu, Y., Williams, E. G., Dubuis, S., Mottis, A., Jovaisaite, V., Houten, S. M., et al. (2014). Multilayered genetic and omics dissection of mitochondrial activity in a mouse reference population. *Cell* 158, 1415–1430. doi: 10.1016/j.cell.2014.07.039

Wuolikainen, A., Andersen, P. M., Moritz, T., Marklund, S. L., and Antti, H. (2012). ALS patients with mutations in the SOD1 gene have an unique metabolomic profile in the cerebrospinal fluid compared with ALS patients without mutations. *Mol. Genet. Metab.* 105, 472–478. doi: 10.1016/j.ymgme.2011.11.201

Wuolikainen, A., Moritz, T., Marklund, S. L., Antti, H., and Andersen, P. M. (2011). Disease-related changes in the cerebrospinal fluid metabolome in amyotrophic

lateral sclerosis detected by GC/TOFMS. *PLoS One* 6:e17947. doi: 10.1371/journal.pone.0017947

Xu, G., Fromholt, S., Ayers, J. I., Brown, H., Siemienski, Z., Crosby, K. W., et al. (2015). Substantially elevating the levels of αB-crystallin in spinal motor neurons of mutant SOD1 mice does not significantly delay paralysis or attenuate mutant protein aggregation. *J. Neurochem.* 133, 452–464. doi: 10.1111/jnc.13022

Zhao, W., Varghese, M., Vempati, P., Dzhun, A., Cheng, A., Wang, J., et al. (2012). Caprylic triglyceride as a novel therapeutic approach to effectively improve the performance and attenuate the symptoms due to the motor neuron loss in ALS disease. *PLoS One* 7:e49191. doi: 10.1371/journal.pone.0049191

Zheng, H., Xiao, W. H., and Bennett, G. J. (2011). Functional deficits in peripheral nerve mitochondria in rats with paclitaxel- and oxaliplatin-evoked painful peripheral neuropathy. *Exp. Neurol.* 232, 154–161. doi: 10.1016/j.expneurol.2011.08.016

Zoccolella, S., Beghi, E., Palagano, G., Fraddosio, A., Guerra, V., Samarelli, V., et al. (2007). Riluzole and amyotrophic lateral sclerosis survival: a population-based study in southern Italy. *Eur. J. Neurol.* 14, 262–268.

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.

frontiers in Neurology

MINI REVIEW
published: 04 June 2019
doi: 10.3389/fneur.2019.00574

The Gut and Parkinson's Disease—A Bidirectional Pathway

Susanne Fonseca Santos[1], *Hadassa Loth de Oliveira*[2], *Elizabeth Sumi Yamada*[1], *Bianca Cruz Neves*[2] *and Antonio Pereira Jr.*[1,3]*

[1] Graduate Program in Neuroscience and Cell Biology, Institute of Biology, Federal University of Pará, Belém, Brazil, [2] Department of Biochemistry, Institute of Chemistry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [3] Department of Electrical and Biomedical Engineering, Institute of Technology, Federal University of Pará, Belém, Brazil

Humans evolved a symbiotic relationship with their gut microbiome, a complex microbial community composed of bacteria, archaea, protists, and viruses, including bacteriophages. The enteric nervous system (ENS) is a gateway for the bidirectional communication between the brain and the gut, mostly through the vagus nerve (VN). Environmental exposure plays a pivotal role in both the composition and functionality of the gut microbiome and may contribute to susceptibility to neurodegenerative disorders, such as Parkinson's disease (PD). The neuropathological hallmark of PD is the widespread appearance of alpha-synuclein aggregates in both the central and peripheral nervous systems, including the ENS. Many studies suggest that gut toxins can induce the formation of α-syn aggregates in the ENS, which may then be transmitted in a prion-like manner to the CNS through the VN. PD is strongly associated with aging and its negative effects on homeostatic mechanisms protecting from inflammation, oxidative stress, and protein malfunction. In this mini-review, we revisit some landmark discoveries in the field of Parkinson's research and focus on the gut-brain axis. In the process, we highlight evidence showing gut-associated dysbiosis and related microbial-derived components as important players and risk factors for PD. Therefore, the gut microbiome emerges as a potential target for protective measures aiming to prevent PD onset.

Keywords: Parkinson's disease, enteric nervous system, microbiome, neurotoxicants, probiotics

OPEN ACCESS

Edited by:
Foteini Christidi, National and Kapodistrian University of Athens, Greece

Reviewed by:
Kun Lu, University of North Carolina at Chapel Hill, United States
Denis Soulet, Laval University, Canada

***Correspondence:**
Antonio Pereira Jr.
apereira@ufpa.br

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neurology

Received: *25 January 2019*
Accepted: *15 May 2019*
Published: *04 June 2019*

Citation:
Santos SF, de Oliveira HL, Yamada ES, Neves BC and Pereira A Jr (2019) The Gut and Parkinson's Disease—A Bidirectional Pathway. Front. Neurol. 10:574. doi: 10.3389/fneur.2019.00574

INTRODUCTION

Parkinson's Disease (PD) is a common neurodegenerative disorder typically associated with the progressive loss of dopaminergic neurons located in the midbrain nucleus substantia nigra pars compacta (SNpc) (1). Although the cardinal symptoms of PD are motor impairments attributed to the depletion of the neurotransmitter dopamine in the striatum, a major target of the SNpc (2), it has been long recognized [for review, see (3)] that other non-motor symptoms, including olfactory (4–6) and gastrointestinal (GI) dysfunction (4), appear during the so-called premotor phase of the disease.

The neuropathological hallmark of PD is the presence of cytoplasmic inclusions, called Lewy bodies (LB) or Lewy neurites (7–9), in SNpc neurons (10). LBs are composed mostly of α-synuclein (α-syn) aggregates (11–13), whose aberrant soluble oligomeric conformations are thought to mediate its toxic effects (14). Alpha-syn is an intrinsically disordered protein (IDP), which lacks a stable 3D structure under physiological conditions and is characterized by exacerbated structural plasticity and conformational adaptability (15). As other IDPs possessing amyloidogenic

regions (16), α-syn can turn into a promiscuous binder leading to abnormal interactions and the development of PD (17). Tuttle et al. (18) provided a detailed 3D structure of functional α-syn fibrils (see **Figure 1**), using solid-state NMR spectroscopy. The study may serve as the basis for a better understanding of molecular mechanisms involved in α-syn fibril nucleation and propagation. In addition, such structural information may provide useful insights on possible interactions of α-synuclein with other proteins and small molecules and allow the emergence of new tools with potential to facilitate both the diagnosis and treatment of PD (e.g., imaging agents and therapeutic drugs).

Aggregates of α-syn fibrils are also found in neural tissue located outside the central nervous system (CNS) of PD patients, in both the autonomic and enteric nervous system (ENS), an outcome which may be associated with the non-motor symptoms of the disease [for review, see (3)]. These findings led Braak et al. (4) to propose a staging system for the progression of the disease following a specific pattern of α-syn aggregates spreading from peripheral toward more centralized locations in the brain. The triggering event would be the invasion of vulnerable neural structures such as the olfactory epithelium and the ENS, which interface directly with the external environment (5, 22), by a neurotoxicant ("neurotropic virus") (23). While both structures (24, 25) possess immunological and physical barriers protecting them against environmental insults, these barriers steadily deteriorate with aging [for review, see (26, 27)], which is the biggest risk factor for idiopathic PD (28).

Animal studies have supported the claim that α-syn aggregates propagate in a prion-like manner [(29); for review, see (30)] via microtubule-associated transport along axons (31). In summary, the prion hypothesis of PD proposes that amyloidogenic α-syn would induce a conformational change in the endogenous protein through permissive templating, convert it into a likeness of itself (32, 33) and propagate retrogradely through the vagus nerve or the olfactory tract from the ENS or the olfactory bulb, respectively. Even though definitive proof for this prion hypothesis is still missing (30) and there is the controversial possibility that intestinal α-syn aggregates have a brain origin (34, 35), it has been shown that vagotomy is associated with a decreased risk for PD in humans (36, 37). Also, grafted neurons in PD patients develop α-syn aggregate pathology (38–40) and α-syn from PD patients can cause nigrostriatal degeneration in mice and non-human primates (41). Remarkably, exogenous α-syn fibrils, either PD patient-derived or produced in *E. coli*, were able to seed the formation of LB-like inclusions which spread from the GI tract to the brain through the vagus nerve in rats (31).

Prior to Braak's hypothesis, however, there was already strong evidence pointing to the role played by exogenous toxins in the etiology of sporadic PD. For instance, postencephalitic parkinsonism (von Economo's disease), which has an autoimmune basis caused by a viral illness (42), is associated with degeneration of the basal ganglia (43). Additionally, the discovery of parkinsonism induced by 1-methyl-4-phenyl-1,2,4,5-tetrahydropyridine (MPTP) through self-administration, in 1982 (44) brought to light a new class of xenobiotic substances that may cause PD-like symptoms by environmental contact. MPTP is a lipophilic compound which readily passes into the brain where it is converted by monoamine oxidase B (MAO-B) to 1-methyl-4-phenylpyridinium (MPP+) (45) which is taken up by dopaminergic cells and impairs mitochondria respiration by poisoning complex 1 (46). There are many heterocyclic molecules that structurally resemble MPTP and are found in the brain from both endogenous and exogenous sources, such as tetrahydroisoquinolines (TIQ) and β-carbolines (β-C). For instance, a TIQ derivative, salsolinol, which is produced by enterobacteria (47) and has been found in the urine of PD patients, may have a double-faced, dose-dependent effect on the nigrostriatal pathway as either a harmful or protective agent (48).

The evidence for the role played by toxins in inducing parkinsonism and the relative scarcity of familial cases (about 10%) (49) underscore the importance of environmental and lifestyle factors over genetic ones in the etiology of the disease (50–52). Some chronic diseases have been associated with a phenomenon called evolutionary mismatch when ancestral traits are no longer adaptive in modern contexts (53, 54). For instance, α-syn is involved with normal synaptic function by regulating, among other things, the size of presynaptic vesicles (55) and the assembly of SNARE proteins involved with the docking of synaptic vesicles to presynaptic membranes (56). However, as old age became common in humans after the early upper Paleolithic (57), the steady increase in longevity seen in modern times may have had a collateral effect on the protein homeostasis (proteostasis) network, which coordinates protein synthesis, folding, trafficking, disaggregation, and degradation (58, 59). The breakdown of proteostasis, which is a common feature of many neurodegenerative diseases (60), means that misfolded proteins may accumulate due to lack of clearance or failure to refold into their native structures (61). In the case of prion-like proteins, this may cause further protein misfolding (template effect) leading to protein aggregation and ultimately cell death (62).

THE GUT-BRAIN AXIS AND PARKINSON'S DISEASE

The gut-brain axis is mediated by intense bidirectional communication between the CNS and the ENS (63). Through the ENS, the gut microbiota influences the development and function of all divisions of the nervous system (64) and this association was established very early during the evolution of multicellular organisms. The first nervous system appeared more than 500 million years ago before the divergence of cnidarians and bilaterians, the two metazoan sister groups (65). That primitive brain had a simple structure, organized as a diffuse nerve net which controlled a restricted set of basic behaviors and was the template for the subsequent evolution of the mammalian ENS (66–68), which retained many of its basic structural characteristics, such as a network of nervous ganglia distributed in the myenteric and submucous plexuses (69). Higher vertebrates went to evolve an additional set of neural structures in the central nervous system (CNS), tasked with the control of more sophisticated behaviors (70). However, the ENS and the CNS maintain intense crosstalk through reciprocal connections mediated by the VN (**Figure 1**) and pelvic nerve in mammals

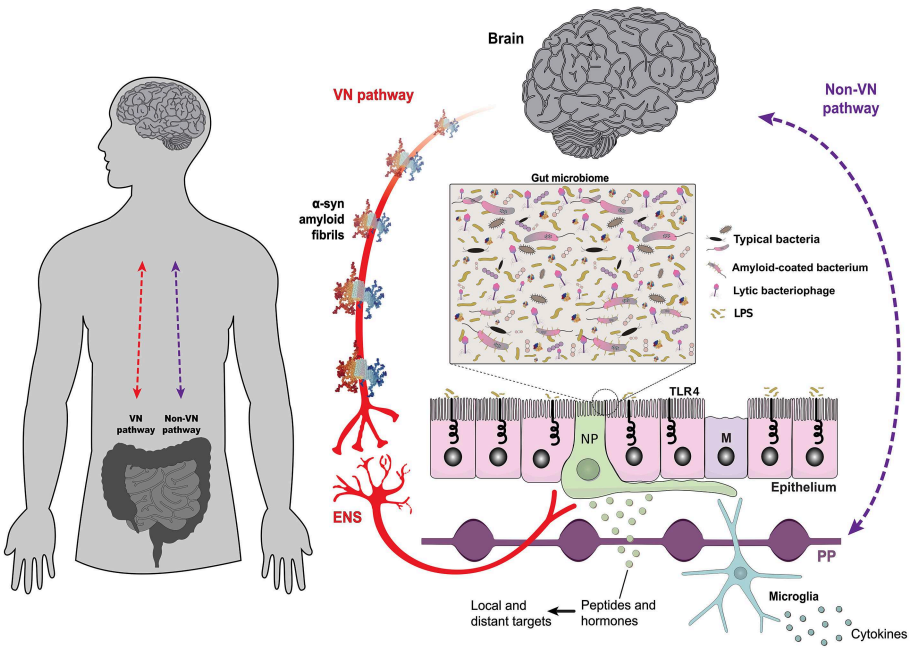

FIGURE 1 | The gut epithelium is a multifunctional interface. The bidirectional interplay between the brain and the gut is mediated by neural, such as the vagus nerve (VN-gateway), and humoral pathways, such as the lymphatic tissue and the bloodstream (Non-VN gateways). A monolayer of epithelial cells separates the intestinal lumen and the complex gut microbiome from the underlying lymphoid and enteric nervous tissues. The structure of alpha-synuclein amyloid fibrils (PDB 2N0A) is based on atomic-resolution molecular data from NGL Viewer (19). Members of the gut microbiome and their extracellular compounds may trigger responses in the VN through enteroendocrine cells, which are contacted by vagus nerve terminals through specialized structures called neuropods (NP) (20). Microbial antigens can cross the gut epithelium through microfold cells, playing a central role in localized inflammatory responses [adapted from Bohórquez et al. (21)]. Toll-like receptors are microbe-sensing proteins, present in intestinal epithelial cells, mediating recognition of commensal bacteria from the harmful/inflammatory ones. ENS, enteric nervous system; M, microfold cells; NP, neuropods; PP, Peyer's patches; TLR4, Toll-like receptor 4; VN, vagus nerve.

(71, 72). As the main substrate for this information exchange, the vagus nerve is an attractive target of neurostimulation therapies for the treatment of psychiatric and gastrointestinal disorders (73, 74).

The GI tract harbors a complex microbial ecosystem (**Figure 1**), consisting of bacteria, archaea, protists, and eukaryotic and prokaryotic viruses, also known as bacteriophages (75–77). The human microbiome has coevolved with its host (78), which keeps a tight leash on the intrinsic competitive nature of the microorganisms that comprise the microbiome, through both the nervous (71, 79, 80) and the immune systems (81, 82). This arrangement maximizes the benefits the host gains from the symbiotic relationship, including protection against pathogens, improved nutrition, and mental health (81). A sub-type of intestinal epithelial cells called enteroendocrine cells, provide a signaling pathway through which the microbiome interacts with the CNS via the vagus nerve (20, 83). Enteroendocrine cells have diverse phenotypes and express a variety of peptides/hormones that can act as signaling molecules on distinct targets, both local and distant, and some are chemoreceptors responding to a variety of luminal stimuli (84, 85). As other intestinal epithelial cells, enteroendocrine cells express toll-like receptors (86), allowing them to detect bacterial products, and activate vagal afferents through basal processes called neuropods (see **Figure 1**) (20, 87).

THE GUT MICROBIOME AND BRAIN FUNCTION

There is increasing evidence of the association between microbiome dysfunction and CNS-related co-morbidities, such as anxiety, depression, autism spectrum disorders, Alzheimer's disease and PD (88–92). This association probably arose as a by-product of natural selection forces acting on microorganisms to adapt to the host and vice-versa (93). The effect of the microbiota on the CNS can lead to behavior modifications (93–95) and even to host manipulation (96) associated with increasing fitness of its bacterial populations. For instance, the microbiome can influence social interactions by acting on the nutritional behavior of individual animals, particularly those from social species where individuals share microbes and interact around foods (97). The proximate neuro-endocrinological and inflammatory mechanisms underlying this type of host manipulation are largely shared by the microbiome and the host (98, 99). For instance, levels of many neurotransmitters that are important for the expression of social behavior, such as serotonin (5-HT), dopamine, norepinephrine (NE), γ-aminobutyric acid (GABA), and glutamate are either expressed or regulated by bacteria (100–102). Particularly, most of the body's serotonin (5-HT) (5-hydroxytryptamine) is produced in the gut by enterochromaffin cells (EC) under the influence

of the microbiome (103). The activation of 5-HT_4 receptors induces the maturation of the ENS and regulates its adult function (104). In the gut, there are three major metabolic pathways leading from the essential amino acid tryptophan (Trp) to 5-HT, kynurenine (Kyn), and indole derivatives, which are under the direct or indirect control of the microbiota (105). During inflammatory states, most tryptophan is diverted to the production of Kyn and its metabolites kynurenine acid (KYNA) and quinolinic acid (QUIN) (106). While KYNA is considered neuroprotective, QUIN can cause excitotoxicity as an agonist of N-methyl-d-aspartate (NMDA) receptor and contribute to the neuropathogenesis of PD [for review, see (107)].

Although α-syn aggregates are also seen in the ENS of normally aging subjects (108), especially in the appendix (109), it is more prevalent in PD patients (110). Recent *in vivo* models showed that accumulation of α-syn aggregates in the ENS can be induced by alterations in the gut microbiome (111). Interestingly, Sampson et al. (112) demonstrated in mice, genetically modified to overexpress α-syn, that the presence of gut microbiota is necessary to promote pathological alterations and motor deficits similar to PD. They also demonstrated that fecal transplants from PD patients impair motor function in the same mouse strain, strongly suggesting that gut microbes may play a pivotal role in the onset of synucleinopathies such as PD (112). Underlying these findings is the fact that microbial amyloids produced by some members of the gut microbiota can be released in the extracellular space, where they can be internalized by neighboring cells, including neurons, and seed the formation of pathological aggregates of endogenous α-syn through permissive templating (113, 114). The failure of normal clearance mechanisms such as the ubiquitin-proteasome system, characteristic of both familial and idiopathic PD (115), to degrade the misfolded protein, may facilitate the seeding process.

The concept of microbial dysbiosis also comprises the bacteriophage components of the microbiome (116). Bacteriophages (phages) are viral parasites of bacteria and are important regulators of host-microbiome interactions through horizontal gene transfer and antagonistic coevolution (117, 118). Besides targeting bacteria, phages can impact human health by playing a direct role on intestinal inflammatory processes (119) and possibly causing α-syn misfolding (120). A recent study showed significant differences in the gut phagobiota of PD patients and healthy individuals and a depletion of *Lactococcus* bacteria (121) in the former, which is associated with the regulation of gut permeability (122) and dopamine production (102), two factors linked with the early signs of PD in the gut (123). Phage therapy has recently returned to the spotlight as an alternative antimicrobial strategy (124, 125). Eventually, it may also contribute to fighting PD through targeted approaches to manipulate the microbiome (121).

Probiotic bacteria have been linked to improved GI symptoms associated with PD (126). Probiotics affect the functionality of the CNS through beneficial interactions with the commensal gut microbiota and modulation of gut-derived inflammation (127). The microbiota of PD patients exhibits a pro-inflammatory profile (128, 129) due to increased intestinal permeability to endotoxins (lipopolysaccharide) (130). Bacterial amyloids may also favor a pro-inflammatory environment in the gut (131). A common bacterial component, the Curli fimbriae, share structural and biophysical properties with amyloids and are produced by *E. coli* through coordinated biosynthetic processes (132). Other components of the gut microbiome are also known to produce functional extracellular amyloids [e.g., *Salmonella*, *Klebsiella*, *Citrobacter*, and *Bacillus* species; (133)]. Since probiotic treatment induces an anti-inflammatory peripheral immune response in multiple sclerosis patients (134) there is a possibility they may also be beneficial for PD patients, although there are no reports corroborating this hypothesis. One option is to take advantage of Lactobacilli's ability to inhibit the formation of biofilms by pathogenic bacteria (135, 136). One caveat, however, is that the effects of probiotics are highly variable, being person-specific, as shown in a recent study (137). This limitation may be counteracted with the use of genetically-modified probiotics able to deliver novel therapeutics efficiently and with site specificity (138). Despite the increasing number of probiotic products available to consumers and the aggressive marketing proclaiming their efficacy, there have been few studies addressing concerns about efficacy and, more importantly, the safety of these products (139). There is an urgent need for more studies about the therapeutic potential of specific bacterial strains to help maintain oxidative and protein homeostasis in the ENS.

CONCLUDING REMARKS

Aging is the main risk factor for the development of PD (140) and delaying the aging process is neuroprotective to PD in animal models (141). Aging is also associated with the accumulation of neuroinflammatory sequelae and the breakdown of homeostatic mechanisms that protect against protein misfolding, oxidative stress, decreased mitochondrial function, etc. The gut, as one of the main gateways to environmental exposure to the brain, may contribute to increasing the susceptibility to these factors. The microbiome has a protective effect mediating this exposure, and dysbiosis seems to be a pivotal risk factor for PD and other neurological disorders. Thus, the adoption of preventive measures to ensure a healthy microbiome throughout the lifetime can potentially decrease the risk of developing PD and other neurodegenerative diseases. The widespread use of antibiotics, for instance, which can kill gut bacteria indiscriminately, can cause a shift of the microbiome to an alternative stable state with unknown consequences in the long term (142).

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. *Neuron.* (2003) 39:889–909. doi: 10.1016/S0896-6273(03)00568-3
2. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry.* (1992) 55:181–4.
3. Garcia-Ruiz PJ, Chaudhuri KR, Martinez-Martin P. Non-motor symptoms of Parkinson's disease A review…from the past. *J Neurol Sci.* (2014) 338:30–3. doi: 10.1016/j.jns.2014.01.002
4. Braak H, Rüb U, Gai WP, Del Tredici K. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. *J Neural Transm.* (2003) 110:517–36. doi: 10.1007/s00702-002-0808-2
5. Doty RL, Deems DA, Stellar S. Olfactory dysfunction in parkinsonism A general deficit unrelated to neurologic signs, disease stage, or disease duration. *Neurology.* (1988) 38:1237–1237.
6. Pearce RK, Hawkes CH, Daniel SE. The anterior olfactory nucleus in Parkinson's disease. *Mov Disord.* (1995) 10:283–7. doi: 10.1002/mds.870100309
7. Lewy F. Paralysis agitans pathologische anatomie. In: Lewandowski M, editor. *Handbuch der Neurologie*. Berlin: Springer (1912). p. 920–33.
8. Okazaki H, Lipkin LE, Aronson SM. Diffuse intracytoplasmic ganglionic inclusions (Lewy type) associated with progressive dementia and quadriparesis in flexion. *J Neuropathol Exp Neurol.* (1961) 20:237–44.
9. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the α-synuclein gene identified in families with Parkinson's disease. *Science.* (1997) 276:2045–7. doi: 10.1126/science.276.5321.2045
10. Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. *J Neurol Neurosurg Psychiatry.* (1988) 51:745–52. doi: 10.1136/jnnp.51.6.745
11. Goedert M. Alpha-synuclein and neurodegenerative diseases. *Nat Rev Neurosci.* (2001) 2:492–501. doi: 10.1038/35081564
12. Gründemann J, Schlaudraff F, Haeckel O, Liss B. Elevated α-synuclein mRNA levels in individual UV-laser-microdissected dopaminergic substantia nigra neurons in idiopathic Parkinson's disease. *Nucleic Acids Res.* (2008) 36:e38. doi: 10.1093/nar/gkn084
13. Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M. α-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies. *Proc Natl Acad Sci USA.* (1998) 95:6469–73. doi: 10.1073/pnas.95.11.6469
14. Stefanis L. α-Synuclein in Parkinson's disease. *Cold Spring Harb Perspect Med.* (2012) 2:a009399. doi: 10.1101/cshperspect.a009399
15. Uversky VN, Dunker AK. Understanding protein non-folding. *Biochim Biophys Acta BBA Proteins Proteomics.* (2010) 1804:1231–64. doi: 10.1016/j.bbapap.2010.01.017
16. Esteras-Chopo A, Serrano L, López de la Paz M. The amyloid stretch hypothesis: recruiting proteins toward the dark side. *Proc Natl Acad Sci USA.* (2005) 102:16672–7. doi: 10.1073/pnas.0505905102
17. Uversky VN. Wrecked regulation of intrinsically disordered proteins in diseases: pathogenicity of deregulated regulators. *Front Mol Biosci.* (2014) 1:6. doi: 10.3389/fmolb.2014.00006
18. Tuttle MD, Comellas G, Nieuwkoop AJ, Covell DJ, Berthold DA, Kloepper KD, et al. Solid-state NMR structure of a pathogenic fibril of full-length human α-synuclein. *Nat Struct Mol Biol.* (2016) 23:409–15. doi: 10.1038/nsmb.3194
19. Rose AS, Bradley AR, Valasatava Y, Duarte JM, Prlic A, Rose PW. NGL viewer: web-based molecular graphics for large complexes. *Bioinforma.* (2018) 34:3755–8. doi: 10.1093/bioinformatics/bty419
20. Kaelberer MM, Buchanan KL, Klein ME, Barth BB, Montoya MM, Shen X, et al. A gut-brain neural circuit for nutrient sensory transduction. *Science.* (2018) 361:eaat5236. doi: 10.1126/science.aat5236
21. Bohórquez DV, Shahid RA, Erdmann A, Kreger AM, Wang Y, Calakos N, et al. Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. *J Clin Invest.* (2015) 125:782–6. doi: 10.1172/JCI78361
22. Anselmi L, Bove C, Coleman FH, Le K, Subramanian MP, Venkiteswaran K, et al. Ingestion of subthreshold doses of environmental toxins induces ascending Parkinsonism in the rat. *NPJ Parkinson Dis.* (2018) 4:30. doi: 10.1038/s41531-018-0066-0
23. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: a dual-hit hypothesis. *Neuropathol Appl Neurobiol.* (2007) 33:599–614. doi: 10.1111/j.1365-2990.2007.00874.x
24. Gershon MD, Bursztajn S. Properties of the enteric nervous system: limitation of access of intravascular macromolecules to the myenteric plexus and muscularis externa. *J Comp Neurol.* (1978) 180:467–88. doi: 10.1002/cne.901800305
25. Mellert TK, Getchell ML, Sparks L, Getchell TV. Characterization of the immune barrier in human olfactory mucosa. *Otolaryngol Head Neck Surg.* (1992) 106:181–8.
26. Attems J, Walker L, Jellinger KA. Olfaction and aging: a mini-review. *Gerontology.* (2015) 61:485–90. doi: 10.1159/000381619
27. Bowman GL, Dayon L, Kirkland R, Wojcik J, Peyratout G, Severin IC, et al. Blood-brain barrier breakdown, neuroinflammation, and cognitive decline in older adults. *Alzheimer Dement.* (2018) 14:1640–50. doi: 10.1016/j.jalz.2018.06.2857
28. Reeve A, Simcox E, Turnbull D. Ageing and Parkinson's disease: why is advancing age the biggest risk factor? *Ageing Res Rev.* (2014) 14:19–30. doi: 10.1016/j.arr.2014.01.004
29. Okuzumi A, Kurosawa M, Hatano T, Takanashi M, Nojiri S, Fukuhara T, et al. Rapid dissemination of alpha-synuclein seeds through neural circuits in an in-vivo prion-like seeding experiment. *Acta Neuropathol Commun.* (2018) 6:96. doi: 10.1186/s40478-018-0587-0
30. Steiner JA, Quansah E, Brundin P. The concept of alpha-synuclein as a prion-like protein: ten years after. *Cell Tissue Res.* (2018) 373:161–73. doi: 10.1007/s00441-018-2814-1
31. Holmqvist S, Chutna O, Bousset L, Aldrin-Kirk P, Li W, Björklund T, et al. Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. *Acta Neuropathol.* (2014) 128:805–20. doi: 10.1007/s00401-014-1343-6
32. Eisenberg D, Jucker M. The amyloid state of proteins in human diseases. *Cell.* (2012) 148:1188–203. doi: 10.1016/j.cell.2012.02.022
33. Stopschinski BE, Diamond MI. The prion model for progression and diversity of neurodegenerative diseases. *Lancet Neurol.* (2017) 16:323–32. doi: 10.1016/S1474-4422(17)30037-6
34. Lawson VA, Furness JB, Klemm HM, Pontell L, Chan E, Hill AF, et al. The brain to gut pathway: a possible route of prion transmission. *Gut.* (2010) 59:1643–51. doi: 10.1136/gut.2010.222620
35. Ulusoy A, Phillips RJ, Helwig M, Klinkenberg M, Powley TL, Di Monte DA. Brain-to-stomach transfer of α-synuclein via vagal preganglionic projections. *Acta Neuropathol.* (2017) 133:381–93. doi: 10.1007/s00401-016-1661-y
36. Liu B, Fang F, Pedersen NL, Tillander A, Ludvigsson JF, Ekbom A, et al. Vagotomy and Parkinson disease: a Swedish register-based matched-cohort study. *Neurology.* (2017a) 88:1996–2002. doi: 10.1212/WNL.0000000000003961
37. Svensson E, Horváth-Puhó E, Thomsen RW, Djurhuus JC, Pedersen L, Borghammer P, et al. Vagotomy and subsequent risk of Parkinson's disease. *Ann Neurol.* (2015) 78:522–9. doi: 10.1002/ana.24448
38. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat Med.* (2008a) 14:504–6. doi: 10.1038/nm1747
39. Kordower JH, Chu Y, Hauser RA, Olanow CW, Freeman TB. Transplanted dopaminergic neurons develop PD pathologic changes: a second case report. *Mov Disord.* (2008b) 23:2303–6. doi: 10.1002/mds.22369
40. Li J-Y, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, et al. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat Med.* (2008) 14:501–3. doi: 10.1038/nm1746
41. Recasens A, Dehay B, Bové J, Carballo-Carbajal I, Dovero S, Pérez-Villalba A, et al. Lewy body extracts from Parkinson disease brains trigger α-synuclein pathology and neurodegeneration in mice and monkeys. *Ann Neurol.* (2014) 75:351–62. doi: 10.1002/ana.24066
42. Jang H, Boltz DA, Webster RG, Smeyne RJ. Viral parkinsonism. *Biochim Biophys Acta.* (2009) 1792:714–21. doi: 10.1016/j.bbadis.2008.08.001
43. von Economo C. Encephalitis lethargica: its sequelae and treatment. *JAMA.* (1931) 98:255. doi: 10.1001/jama.1932.02730290071039

44. Langston JW. The MPTP story. *J Parkinson Dis.* (2017) 7:S11–S19. doi: 10.3233/JPD-179006
45. Chiba K, Trevor A, Castagnoli N. Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. *Biochem Biophys Res Commun.* (1984) 120:574–8.
46. Javitch JA, D'Amato RJ, Strittmatter SM, Snyder SH. Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6 -tetrahydropyridine: uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proc Natl Acad Sci USA.* (1985) 82:2173–7.
47. Villageliú DN, Borts DJ, Lyte M. Production of the neurotoxin salsolinol by a gut-associated bacterium and its modulation by alcohol. *Front Microbiol.* (2018) 9:3092. doi: 10.3389/fmicb.2018.03092
48. Kurnik-Łucka M, Panula P, Bugajski A, Gil K. Salsolinol: an unintelligible and double-faced molecule—lessons learned from *in vivo* and *in vitro* experiments. *Neurotox Res.* (2018) 33:485–514. doi: 10.1007/s12640-017-9818-6
49. Thomas B, Beal MF. Parkinson's disease. *Hum Mol Genet.* (2007) 16:R183–94. doi: 10.1093/hmg/ddm159
50. Garcia-Ruiz PJ, Espay AJ. Parkinson disease: an evolutionary perspective. *Front Neurol.* (2017) 8:157. doi: 10.3389/fneur.2017.00157
51. Jomova K, Vondrakova D, Lawson M, Valko M. Metals, oxidative stress and neurodegenerative disorders. *Mol Cell Biochem.* (2010) 345:91–104. doi: 10.1007/s11010-010-0563-x
52. Kalinderi K, Bostantjopoulou S, Fidani L. The genetic background of Parkinson's disease: current progress and future prospects. *Acta Neurol Scand.* (2016) 134:314–26. doi: 10.1111/ane.12563
53. Gluckman PD, Hanson MA. Living with the past: evolution, development, and patterns of disease. *Science.* (2004) 305:1733–6. doi: 10.1126/science.1095292
54. Tooby J, Cosmides L. The past explains the present: emotional adaptations and the structure of ancestral environments. *Ethol Sociobiol.* (1990) 11:375–424.
55. Murphy DD, Rueter SM, Trojanowski JQ, Lee VM. Synucleins are developmentally expressed, and alpha-synuclein regulates the size of the presynaptic vesicular pool in primary hippocampal neurons. *J Neurosci.* (2000) 20:3214–20. doi: 10.1523/jneurosci.20-09-03214.2000
56. Burré J, Sharma M, Tsetsenis T, Buchman V, Etherton MR, Südhof TC. Alpha-synuclein promotes SNARE-complex assembly *in vivo* and *in vitro*. *Science.* (2010) 329:1663–7. doi: 10.1126/science.1195227
57. Caspari R, Lee S-H. Older age becomes common late in human evolution. *Proc Natl Acad Sci USA.* (2004) 101:10895–900. doi: 10.1073/pnas.0402857101
58. Kaushik S, Cuervo AM. Proteostasis and aging. *Nat Med.* (2015) 21:1406–15. doi: 10.1038/nm.4001
59. Powers ET, Morimoto RI, Dillin A, Kelly JW, Balch WE. Biological and chemical approaches to diseases of proteostasis deficiency. *Annu Rev Biochem.* (2009) 78:959–91. doi: 10.1146/annurev.biochem.052308.114844
60. Balch WE, Morimoto RI, Dillin A, Kelly JW. Adapting proteostasis for disease intervention. *Science.* (2008) 319:916–9. doi: 10.1126/science.1141448
61. Kikis EA, Gidalevitz T, Morimoto RI. Protein homeostasis in models of aging and age-related conformational disease. In: N. Tavernarakis, editor. *Protein Metabolism and Homeostasis in Aging Advances in Experimental Medicine and Biology*. Boston, MA: Springer (2010). p. 138–59.
62. Bobela W, Aebischer P, Schneider BL. Alpha-synuclein as a mediator in the interplay between aging and Parkinson's disease. *Biomolecules.* (2015) 5:2675–700. doi: 10.3390/biom5042675
63. Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol Q Publ Hell Soc Gastroenterol.* (2015) 28:203–9.
64. Diaz Heijtz R, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci USA.* (2011) 108:3047–52. doi: 10.1073/pnas.1010529108
65. Kelava I, Rentzsch F, Technau U. Evolution of eumetazoan nervous systems: insights from cnidarians. *Philos Trans R Soc B Biol Sci.* (2015) 370:20150065. doi: 10.1098/rstb.2015.0065
66. Furness JB, Stebbing MJ. The first brain: species comparisons and evolutionary implications for the enteric and central nervous systems. *Neurogastroenterol Motil.* (2018) 30:13234. doi: 10.1111/nmo.13234
67. Koizumi O. Origin and evolution of the nervous system considered from the diffuse nervous system of cnidarians. In: Goffredo S, Dubinsky, Z, editors. *The Cnidaria, Past, Present and Future: The World of Medusa and Her Sisters*. Cham: Springer International Publishing (2016). p. 73–91.
68. Shimizu H, Koizumi O, Fujisawa T. Three digestive movements in Hydra regulated by the diffuse nerve net in the body column. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol.* (2004) 190:623–30. doi: 10.1007/s00359-004-0518-3
69. Costa M, Brookes SJH, Hennig GW. Anatomy and physiology of the enteric nervous system. *Gut.* (2000) 47:iv15–9. doi: 10.1136/gut.47.suppl_4.iv15
70. Kaas J. *Evolution of Nervous Systems. 2nd ed*. Academic Press (2017). Available online at: https://www.sciencedirect.com/referencework/9780128040966/evolution-of-nervous-systems (accessed April 6, 2019).
71. Powell N, Walker MM, Talley NJ. The mucosal immune system: master regulator of bidirectional gut-brain communications. *Nat Rev Gastroenterol Hepatol.* (2017) 14:143–59. doi: 10.1038/nrgastro.2016.191
72. Rao M, Gershon MD. The bowel and beyond: the enteric nervous system in neurological disorders. *Nat Rev Gastroenterol Hepatol.* (2016) 13:517–28. doi: 10.1038/nrgastro.2016.107
73. Breit S, Kupferberg A, Rogler G, Hasler G. Vagus nerve as modulator of the brain–gut axis in psychiatric and inflammatory disorders. *Front Psychiatry.* (2018) 9:44. doi: 10.3389/fpsyt.2018.00044
74. Johnson RL, Wilson CG. A review of vagus nerve stimulation as a therapeutic intervention. *J Inflamm Res.* (2018) 11:203–13. doi: 10.2147/JIR.S163248
75. Gerritsen J, Smidt H, Rijkers GT, de Vos WM. Intestinal microbiota in human health and disease: the impact of probiotics. *Genes Nutr.* (2011) 6:209–40. doi: 10.1007/s12263-011-0229-7
76. Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JI. Worlds within worlds: evolution of the vertebrate gut microbiota. *Nat Rev Microbiol.* (2008) 6:776–88. doi: 10.1038/nrmicro1978
77. Selber-Hnatiw S, Rukundo B, Ahmadi M, Akoubi H, Al-Bizri H, Aliu AF, et al. Human gut microbiota: toward an ecology of disease. *Front Microbiol.* (2017) 8:1265. doi: 10.3389/fmicb.2017.01265
78. Moeller AH, Caro-Quintero A, Mjungu D, Georgiev AV, Lonsdorf EV, Muller MN, et al. Cospeciation of gut microbiota with hominids. *Science.* (2016) 353:380–2. doi: 10.1126/science.aaf3951
79. Bailey MT, Dowd SE, Galley JD, Hufnagle AR, Allen RG, Lyte M. Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. *Brain Behav Immun.* (2011) 25:397–407. doi: 10.1016/j.bbi.2010.10.023
80. Galley JD, Nelson MC, Yu Z, Dowd SE, Walter J, Kumar PS, et al. Exposure to a social stressor disrupts the community structure of the colonic mucosa-associated microbiota. *BMC Microbiol.* (2014) 14:189. doi: 10.1186/1471-2180-14-189
81. Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S. The evolution of the host microbiome as an ecosystem on a leash. *Nature.* (2017) 548:43–51. doi: 10.1038/nature23292
82. Zilber-Rosenberg I, Rosenberg E. Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiol Rev*. (2008) 32:723–35. doi: 10.1111/j.1574-6976.2008.00123.x
83. Hoffman BU, Lumpkin EA. A gut feeling. *Science.* (2018) 361:1203–4. doi: 10.1126/science.aau9973
84. Feher J. 8.3—Intestinal and colonic chemoreception and motility. In: Feher J, editor. *Quantitative Human Physiology, 2nd Edn*. Boston: Academic Press (2017). p. 796–809.
85. Latorre R, Sternini C, De Giorgio R, Greenwood-Van Meerveld B. Enteroendocrine cells: a review of their role in brain-gut communication. *Neurogastroenterol Motil.* (2016) 28:620–30. doi: 10.1111/nmo.12754
86. Bogunovic M, Davé SH, Tilstra JS, Chang DTW, Harpaz N, Xiong H, et al. Enteroendocrine cells express functional Toll-like receptors. *Am J Physiol Gastrointest Liver Physiol.* (2007) 292:G1770–1783. doi: 10.1152/ajpgi.00249.2006
87. Liddle RA. Neuropods. *Cell Mol Gastroenterol Hepatol.* (2019) 7:739–47. doi: 10.1016/j.jcmgh.2019.01.006
88. Dinan TG, Cryan JF. Gut feelings on ParkinsonŠs and depression. *Cerebrum.* (2017) 2017:cer-04-17.

89. Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell.* (2013) 155:1451–63. doi: 10.1016/j.cell.2013.11.024
90. Kelly JR, Minuto C, Cryan JF, Clarke G, Dinan TG. Cross talk: the microbiota and neurodevelopmental disorders. *Front Neurosci.* (2017) 11:490. doi: 10.3389/fnins.2017.00490
91. Pellegrini C, Antonioli L, Colucci R, Blandizzi C, Fornai M. Interplay among gut microbiota, intestinal mucosal barrier and enteric neuro-immune system: a common path to neurodegenerative diseases? *Acta Neuropathol.* (2018) 136:345–61. doi: 10.1007/s00401-018-1856-5
92. Unger MM, Spiegel J, Dillmann K-U, Grundmann D, Philippeit H, Bürmann J, et al. Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls. *Parkinson Relat Disord.* (2016) 32:66–72. doi: 10.1016/j.parkreldis.2016.08.019
93. Johnson KV-A, Foster KR. Why does the microbiome affect behaviour? *Nat Rev Microbiol.* (2018) 16:647–55. doi: 10.1038/s41579-018-0014-3
94. Akami M, Andongma AA, Zhengzhong C, Nan J, Khaeso K, Jurkevitch E, et al. Intestinal bacteria modulate the foraging behavior of the oriental fruit fly Bactrocera dorsalis (Diptera: Tephritidae). *PLoS ONE.* (2019) 14:e0210109. doi: 10.1371/journal.pone.0210109
95. Schretter CE, Vielmetter J, Bartos I, Marka Z, Marka S, Argade S, et al. A gut microbial factor modulates locomotor behaviour in *Drosophila*. *Nature.* (2018) 563:402. doi: 10.1038/s41586-018-0634-9
96. Lewin-Epstein O, Aharonov R, Hadany L. Microbes can help explain the evolution of host altruism. *Nat Commun.* (2017) 8:14040. doi: 10.1038/ncomms14040
97. Pasquaretta C, Gómez-Moracho T, Heeb P, Lihoreau M. Exploring interactions between the gut microbiota and social behavior through nutrition. *Genes.* (2018) 9:E534. doi: 10.3390/genes9110534
98. Freestone P. Communication between bacteria and their hosts. *Scientifica.* (2013) 2013:361073. doi: 10.1155/2013/361073
99. Mazzoli R, Pessione E. The Neuro-endocrinological Role of Microbial Glutamate and GABA Signaling. *Front Microbiol.* (2016) 7:1934. doi: 10.3389/fmicb.2016.01934
100. Liu R, Hong J, Xu X, Feng Q, Zhang D, Gu Y, et al. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nat Med.* (2017b) 23:859–68. doi: 10.1038/nm.4358
101. Strandwitz P, Kim KH, Terekhova D, Liu JK, Sharma A, Levering J, et al. GABA-modulating bacteria of the human gut microbiota. *Nat Microbiol.* (2019) 4:396. doi: 10.1038/s41564-018-0307-3
102. Vodolazov IR, Dbar SD, Oleskin AV, Stoyanova LG. Exogenous and endogenous neuroactive biogenic amines: studies with *Lactococcus lactis* subsp. *lactis Appl Biochem Microbiol.* (2018) 54:603–10. doi: 10.1134/S0003683818060157
103. Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell.* (2015) 161:264–76. doi: 10.1016/j.cell.2015.02.047
104. Vadder FD, Grasset E, Holm LM, Karsenty G, Macpherson AJ, Olofsson LE, et al. Gut microbiota regulates maturation of the adult enteric nervous system via enteric serotonin networks. *Proc Natl Acad Sci USA.* (2018) 115:6458–63. doi: 10.1073/pnas.1720017115
105. Agus A, Planchais J, Sokol H. Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host Microbe.* (2018) 23:716–24. doi: 10.1016/j.chom.2018.05.003
106. Keszthelyi D, Troost FJ, Jonkers DM, van Donkelaar EL, Dekker J, Buurman WA, et al. Does acute tryptophan depletion affect peripheral serotonin metabolism in the intestine? *Am J Clin Nutr.* (2012) 95:603–8. doi: 10.3945/ajcn.111.028589
107. Lim CK, Fernández-Gomez FJ, Braidy N, Estrada C, Costa C, Costa S, et al. Involvement of the kynurenine pathway in the pathogenesis of Parkinson's disease. *Prog Neurobiol.* (2017) 155:76–95. doi: 10.1016/j.pneurobio.2015.12.009
108. Böttner M, Zorenkov D, Hellwig I, Barrenschee M, Harde J, Fricke T, et al. Expression pattern and localization of alpha-synuclein in the human enteric nervous system. *Neurobiol Dis.* (2012) 48:474–80. doi: 10.1016/j.nbd.2012.07.018
109. Gray MT, Munoz DG, Gray DA, Schlossmacher MG, Woulfe JM. Alpha-synuclein in the appendiceal mucosa of neurologically intact subjects. *Mov Disord.* (2014) 29:991–8. doi: 10.1002/mds.25779
110. Barrenschee M, Zorenkov D, Böttner M, Lange C, Cossais F, Scharf AB, et al. Distinct pattern of enteric phospho-alpha-synuclein aggregates and gene expression profiles in patients with Parkinson's disease. *Acta Neuropathol Commun.* (2017) 5:1. doi: 10.1186/s40478-016-0408-2
111. Chen SG, Stribinskis V, Rane MJ, Demuth DR, Gozal E, Roberts AM, et al. Exposure to the functional bacterial amyloid protein curli enhances alpha-synuclein aggregation in aged fischer 344 rats and *Caenorhabditis elegans*. *Sci Rep.* (2016) 6:34477. doi: 10.1038/srep34477
112. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell.* (2016) 167:1469–80.e12. doi: 10.1016/j.cell.2016.11.018
113. Friedland RP, Chapman MR. The role of microbial amyloid in neurodegeneration. *PLoS Pathog.* (2017) 13:e1006654. doi: 10.1371/journal.ppat.1006654
114. Soto C, Pritzkow S. Protein misfolding, aggregation, and conformational strains in neurodegenerative diseases. *Nat Neurosci.* (2018) 21:1332–40. doi: 10.1038/s41593-018-0235-9
115. McNaught KSP, Olanow CW, Halliwell B, Isacson O, Jenner P. Failure of the ubiquitin–proteasome system in Parkinson's disease. *Nat Rev Neurosci.* (2001) 2:589–94. doi: 10.1038/35086067
116. Manrique P, Dills M, Young MJ. The human gut phage community and its implications for health and disease. *Viruses.* (2017) 9:E141. doi: 10.3390/v9060141
117. Duerkop BA. Bacteriophages shift the focus of the mammalian microbiota. *PLoS Pathog.* (2018) 14:e1007310. doi: 10.1371/journal.ppat.1007310
118. Scanlan PD. Bacteria–bacteriophage coevolution in the human gut: implications for microbial diversity and functionality. *Trends Microbiol.* (2017) 25:614–23. doi: 10.1016/j.tim.2017.02.012
119. Gogokhia L, Buhrke K, Bell R, Hoffman B, Brown DG, Hanke-Gogokhia C, et al. Expansion of bacteriophages is linked to aggravated intestinal inflammation and colitis. *Cell Host Microbe.* (2019) 25:285–99.e8. doi: 10.1016/j.chom.2019.01.008
120. Tetz G, Tetz V, Tetz G, Tetz V. Bacteriophages as new human viral pathogens. *Microorganisms.* (2018b) 6:54. doi: 10.3390/microorganisms6020054
121. Tetz G, Brown SM, Hao Y, Tetz V. Parkinson's disease and bacteriophages as its overlooked contributors. *Sci Rep.* (2018a) 8:10812. doi: 10.1038/s41598-018-29173-4
122. Darby TM, Owens JA, Saeedi BJ, Luo L, Matthews JD, Robinson BS, et al. *Lactococcus lactis* subsp. *cremoris is an efficacious beneficial bacterium that limits tissue injury in the intestine iScience.* (2019) 12:356–67. doi: 10.1016/j.isci.2019.01.030
123. Houser MC, Tansey MG. The gut-brain axis: is intestinal inflammation a silent driver of Parkinson's disease pathogenesis? *NPJ Parkinson Dis.* (2017) 3:3. doi: 10.1038/s41531-016-0002-0
124. Chan BK, Abedon ST, Loc-Carrillo C. Phage cocktails and the future of phage therapy. *Future Microbiol.* (2013) 8:769–83. doi: 10.2217/fmb.13.47
125. Górski A, Miedzybrodzki R, Weber-Dabrowska B, Fortuna W, Letkiewicz S, Rogóz P, et al. Phage therapy: combating infections with potential for evolving from merely a treatment for complications to targeting diseases. *Front Microbiol.* (2016) 7:1515. doi: 10.3389/fmicb.2016.01515
126. Barichella M, Pacchetti C, Bolliri C, Cassani E, Iorio L, Pusani C, et al. Probiotics and prebiotic fiber for constipation associated with Parkinson disease: an RCT. *Neurology.* (2016) 87:1274–80. doi: 10.1212/WNL.0000000000003127
127. Wang H, Lee I-S, Braun C, Enck P. Effect of probiotics on central nervous system functions in animals and humans: a systematic review. *J Neurogastroenterol Motil.* (2016) 22:589–605. doi: 10.5056/jnm16018
128. Bedarf JR, Hildebrand F, Coelho LP, Sunagawa S, Bahram M, Goeser F, et al. Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naïve Parkinson's disease patients. *Genome Med.* (2017) 9:39. doi: 10.1186/s13073-017-0428-y
129. Petrov VA, Saltykova IV, Zhukova IA, Alifirova VM, Zhukova NG, Dorofeeva YB, et al. Analysis of gut microbiota in patients with Parkinson's

disease. *Bull Exp Biol Med.* (2017) 162:734–7. doi: 10.1007/s10517-017-3700-7
130. Forsyth CB, Shannon KM, Kordower JH, Voigt RM, Shaikh M, Jaglin JA, et al. Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. *PLoS ONE.* (2011) 6:e28032. doi: 10.1371/journal.pone.0028032
131. Miraglia F, Colla E. Microbiome, Parkinson's disease and molecular mimicry. *Cells.* (2019) 8:222. doi: 10.3390/cells8030222
132. Taglialegna A, Lasa I, Valle J. Amyloid Structures as Biofilm Matrix Scaffolds. *J Bacteriol.* (2016) 198:2579–88. doi: 10.1128/JB.00122-16
133. Van Gerven N, Van der Verren SE, Reiter DM, Remaut H. The role of functional amyloids in bacterial virulence. *J Mol Biol.* (2018) 430:3657–84. doi: 10.1016/j.jmb.2018.07.010
134. Tankou SK, Regev K, Healy BC, Tjon E, Laghi L, Cox LM, et al. A probiotic modulates the microbiome and immunity in multiple sclerosis. *Ann Neurol.* (2018) 83:1147–61. doi: 10.1002/ana.25244
135. Söderling EM, Marttinen AM, Haukioja AL. Probiotic lactobacilli interfere with Streptococcus mutans biofilm formation *in vitro*. *Curr Microbiol.* (2011) 62:618–22. doi: 10.1007/s00284-010-9752-9
136. Vuotto C, Longo F, Donelli G. Probiotics to counteract biofilm-associated infections: promising and conflicting data. *Int J Oral Sci.* (2014) 6:189–94. doi: 10.1038/ijos.2014.52
137. Zmora N, Zilberman-Schapira G, Suez J, Mor U, Dori-Bachash M, Bashiardes S, et al. Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. *Cell.* (2018) 174:1388–405. doi: 10.1016/j.cell.2018.08.041
138. Singh B, Mal G, Marotta F. Designer probiotics: paving the way to living therapeutics. *Trends Biotechnol.* (2017) 35:679–82. doi: 10.1016/j.tibtech.2017.04.001
139. Cohen PA. Probiotic safety—no guarantees. *JAMA Intern Med.* (2018) 178:1577–8. doi: 10.1001/jamainternmed.2018.5403
140. Driver JA, Logroscino G, Gaziano JM, Kurth T. Incidence and remaining lifetime risk of Parkinson disease in advanced age. *Neurology.* (2009) 72:432–8. doi: 10.1212/01.wnl.0000341769.50075.bb
141. Cooper JF, Dues DJ, Spielbauer KK, Machiela E, Senchuk MM, Raamsdonk JMV. Delaying aging is neuroprotective in Parkinson's disease: a genetic analysis in *C. elegans models NPJ Parkinson Dis.* (2015) 1:15022. doi: 10.1038/npjparkd.2015.22
142. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc Natl Acad Sci USA.* (2011) 108 (Suppl. 1):4554–61. doi: 10.1073/pnas.1000087107

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.

frontiers in Neuroscience

REVIEW
published: 25 June 2019
doi: 10.3389/fnins.2019.00601

The Peripheral Nervous System in Amyotrophic Lateral Sclerosis: Opportunities for Translational Research

Francesco Gentile[1], Stefania Scarlino[1], Yuri Matteo Falzone[1,2], Christian Lunetta[3], Lucio Tremolizzo[4], Angelo Quattrini[1†] and Nilo Riva[1,2†]*

[1] Experimental Neuropathology Unit, Division of Neuroscience, Institute of Experimental Neurology – San Raffaele Scientific Institute, Milan, Italy, [2] Department of Neurology, San Raffaele Scientific Institute, Milan, Italy, [3] NeuroMuscular Omnicentre, Serena Onlus Foundation, Milan, Italy, [4] Neurology Unit, ALS Clinic, San Gerardo Hospital, University of Milano-Bicocca, Monza, Italy

OPEN ACCESS

Edited by:
Foteini Christidi, National and Kapodistrian University of Athens, Greece

Reviewed by:
Chiara F. Valori, German Center for Neurodegenerative Diseases (DZNE), Germany
Yasushi Kitaoka, St. Marianna University School of Medicine, Japan

***Correspondence:**
Nilo Riva
riva.nilo@hsr.it

† These authors share last senior authorship

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: *15 February 2019*
Accepted: *27 May 2019*
Published: *25 June 2019*

Citation:
Gentile F, Scarlino S, Falzone YM, Lunetta C, Tremolizzo L, Quattrini A and Riva N (2019) The Peripheral Nervous System in Amyotrophic Lateral Sclerosis: Opportunities for Translational Research. Front. Neurosci. 13:601. doi: 10.3389/fnins.2019.00601

Although amyotrophic lateral sclerosis (ALS) has been considered as a disorder of the motor neuron (MN) cell body, recent evidences show the non-cell-autonomous pathogenic nature of the disease. Axonal degeneration, loss of peripheral axons and destruction of nerve terminals are early events in the disease pathogenic cascade, anticipating MN degeneration, and the onset of clinical symptoms. Therefore, although ALS and peripheral axonal neuropathies should be differentiated in clinical practice, they also share damage to common molecular pathways, including axonal transport, RNA metabolism and proteostasis. Thus, an extensive evaluation of the molecular events occurring in the peripheral nervous system (PNS) could be fundamental to understand the pathogenic mechanisms of ALS, favoring the discovery of potential disease biomarkers, and new therapeutic targets.

Keywords: motor neuron disease, lower motor neuron syndrome, nerve, neuropathy, CMT, distal SMA, hereditary neuropathy, genetics

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is the most common and severe form of motor neuron disease (MND), an heterogeneous group of disorders defined by prominent motor neuron (MN) degeneration (Saberi et al., 2015; Riva et al., 2016). Recent evidence has challenged such traditional view of selective neuronal loss demonstrating widespread extra-motor involvement in ALS and implying that neuronal populations other than MNs may also be affected (Geser et al., 2008; Brettschneider et al., 2013; McCluskey et al., 2014). Furthermore, an expanded clinical spectrum has now been recognized, as overlapping phenotypes with other neuromuscular disorders (Sabatelli et al., 2016), including peripheral neuropathies, have been described (Rajabally and Jacob, 2008; Bhatt et al., 2009; Sawa et al., 2012). Although in clinical practice MNDs should be differentiated from other peripheral nervous system (PNS) disorders, sensory and autonomic neurons in the dorsal root ganglia (DRG) and lower MNs in the ventral horns share important challenges, as proper stability and functioning of their long projections throughout the body requires a protective environment and efficient communication between the central nervous system (CNS) and the outermost areas of these cells. Nonetheless, differences in

morphology and function may confer different patterns of resistance or vulnerability to specific stressors.

The aim of this review is to dissect PNS involvement in ALS, analyzing the evidence from clinical and pathological data to genetics in order to provide novel pathophysiological insights and potential implication for therapeutic strategies.

DIAGNOSTIC CHALLENGES IN ALS AND ALS-MIMICS

In the absence of pathognomonic diagnostic biomarkers, ALS diagnosis relies on clinical findings suggestive of selective upper, and lower MN (LMN) lesion and the exclusion of alternative causes (Ludolph et al., 2015). The wide clinical heterogeneity of the disease led to the development of several phenotypic classifications, mainly dependent on the distribution of UMN and LMN lesions. In patients presenting with isolated signs of LMN involvement (LMN Syndrome – LMNS), the differential diagnosis may be more challenging since the primary disease target may be the cell soma, the axon and its myelin, the neuromuscular junction (NMJ) or the muscle (Riva et al., 2011b; Garg et al., 2017; Muller et al., 2018). Such disorders not only share the motor unit as a common target of damage, but may be potentially underpinned by common pathogenic mechanisms. Within this context, ALS, with special reference to its restricted LMN phenotypes, such as flair legs and flail arm syndromes, shows high overlap in terms of clinical presentation, targets and mechanisms of damage with hereditary axonal neuropathies, such as axonal Charcot-Marie-Tooth neuropathy (CMT2) and distal hereditary motor neuropathy (dHMN) (Fischer and Glass, 2007). Besides the different rate of progression and spreading, distinction between these disorders could be difficult especially in the early phase, as clinical and neurophysiologic studies may be strikingly similar and may not able to discern the primary lesion site. Nonetheless, a recent study supported the value of the electroneurographic split hand index has recently been suggested for distinguishing ALS from mimic disorders (Menon et al., 2014). Moreover, pes cavus, traditionally considered a distinguishing clinical sign of CMT, has also been anecdotally reported in ALS patients (Ceroni et al., 2001), and is estimated to be as high as 2% our case series (data not published). Notably, the percentage of misdiagnosis in degenerative LMNS has been reported to be as high as 19%; moreover, up to 10% of patients initially diagnosed as ALS are ultimately re-diagnosed as having another disease, including peripheral neuropathy (Davenport et al., 1996; Traynor et al., 2000; Visser et al., 2002). Therefore, in some cases, MND diagnosis remains uncertain, and only follow up can lead to a satisfying level of diagnostic confidence. Of note, we previously showed that biopsy of obturator nerve may be useful in the distinction between ALS and motor neuropathies (Corbo et al., 1997; Riva et al., 2011b). Signs of acute axonal damage, focal/multifocal fiber loss and absent or scarce axonal regeneration are consistently observed in motor nerve biopsies from patients with ALS (**Figure 1A**), while neuropathies show a higher regeneration capacity and may present signs of myelin damage, inflammation, or pathologic deposits (Benedetti et al., 2010).

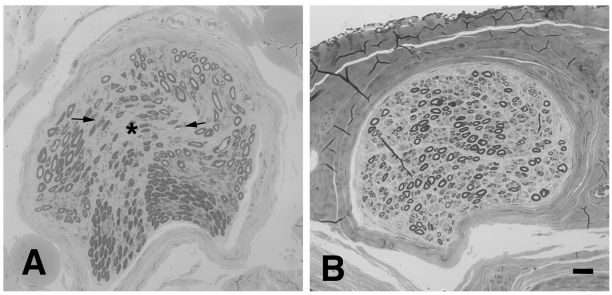

FIGURE 1 | Representative neuropathological cases. **(A)** Transverse semi-thin sections of motor nerve biopsy (obturator nerve), and sural sensory nerve biopsy **(B)** from a patients with MND. **(A)** Focal loss of myelinated nerve fibers is evident (asterisk) in the endoneurium together with signs of acute axonal degeneration (myelin ovoids, arrows); no signs of regeneration are observed. **(B)** In sural sensory nerve biopsies a diffuse mild reduction of large nerve fibers is observed. Bar: 20 μm.

THE PNS AS A CONVERGING POINT IN ALS

Evidence of Peripheral Nervous System Involvement in Human ALS

Although the circumstances leading to MN damage in ALS are still unknown, different mechanisms have been proposed to explain disease onset and spread. The dying-forward hypothesis states that the primary site of damage resides in the cell soma and subsequently spreads to the peripheral compartments. In the recent years, the dying-back pattern of degeneration has obtained a large attention in the context of ALS pathophysiology (Fischer et al., 2004), suggesting that ALS is a distal axonopathy, a pattern typically seen in peripheral neuropathies with a distal to proximal gradient of damage (England and Asbury, 2004). Therefore, considering that both motor and sensory nerves share mechanisms of axonal degeneration, it may be plausible that in ALS signs of PNS dysfunction may also extend to the sensory system. Notably, other neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease show evidence of distal axonopathy (Arendt, 2009; Chung et al., 2009).

While diagnostic assessment in ALS relays on the assumption that non-motor components of the PNS are usually spared, increasing amount of evidence suggests the presence of sensory and autonomic dysfunction in ALS. Sensory symptoms are reported in about 2–30% of cases (Gubbay et al., 1985; Hammad et al., 2007). Numbness, tingling and pain are the most common symptoms reported. Decreased vibration sense and abnormal pain and temperature thresholds are rarely observed during standard examination, however quantitative sensory testing shows abnormal findings in about 10% of patients, rising to almost half of the cases in advanced stages of the disease (Truini et al., 2015; Isak et al., 2017).

Autonomic dysfunction has been reported in 5–30% of cases, while quantitative autonomic testing detected abnormal sudomotor and cardiovagal response in up to 50% of patients (McCluskey et al., 2014; Piccione et al., 2015). Abnormalities in either sensory nerve amplitudes or nerve conduction velocity may be apparent in about 20% of cases (Mondelli et al., 1993; Pugdahl et al., 2008), with higher frequencies reported in patients with longer disease duration (Pugdahl et al., 2007; Isak et al., 2016).

Pathologic studies of the sural nerve in ALS have demonstrated a mild reduction of the number of large nerve fibers (**Figure 1B**) in most of the examined cases (Bradley et al., 1983; Hammad et al., 2007; Devigili et al., 2011; Luigetti et al., 2012). Clustering of mitochondria, dilation of small vesicles and accumulation of neurofilaments (NF) have also been reported (Dyck et al., 1975; Di Trapani et al., 1987; Heads et al., 1991). Skin biopsy shows in about 80% of ALS patients a reduction of intraepidermal nerve fiber density (Weis et al., 2011; Truini et al., 2015; Dalla Bella et al., 2016; Nolano et al., 2017) and focal axonal swellings, suggesting axonal degeneration (Isak et al., 2017). Interestingly, small nerve fibers in the cornea are also reduced in ALS patients, correlating with bulbar disability scores (Ferrari et al., 2014). Finally, a significant neuronal loss has been observed in DRG of ALS patients, with preferential involvement of large-sized cells (Kawamura et al., 1981). Notably, evidence of pTDP-43 pathology has been shown in Clarke's column, intermediolateral nucleus and dorsal root ganglia in a subset of cases, proving that these structures are also affected (Nishihira et al., 2008; Brettschneider et al., 2014).

The Role of Genetics in ALS: At the Borders of the Disease Spectrum

The knowledge about ALS genetics has had a relevant acceleration in the last decade, with more than 30 genes identified as pathogenic and more than 100 as ALS-related (Riva et al., 2016). The first gene identified as a cause of ALS was *SOD1* in 1993 (Rosen et al., 1993) and together with *TARDBP* (Sreedharan et al., 2008), *FUS* (Kwiatkowski et al., 2009), and chromosome 9 open reading frame 72 (*C9orf72*) (DeJesus-Hernandez et al., 2011; Renton et al., 2011), represent the most common ALS-related genes, covering up to 70% of familial and 10% of sporadic cases (Riva et al., 2016; Chia et al., 2018). ALS-key genes have been associated with almost all clinical ALS phenotypes, although patients with SOD1, and FUS mutations may show preferential LMN involvement (Waibel et al., 2013; Picher-Martel et al., 2016).

The frequency of gene variants in cases with extra-motor involvement is significantly higher compared to the pure ALS phenotype, and has been associated with poorer survival (McCluskey et al., 2014). Prominent sensory involvement had been described in rare mutated cases, with *SOD1* and *TARDBP* genes being reported most frequently (Camdessanche et al., 2011).

Many of the genes involved in ALS are associated also with other neurological disorders sharing common targets of neurodegeneration, such as hereditary spastic paraplegia (HSP), axonal CMT neuropathy and dHMN. Among the genes involved in dHMN and axonal CMT there are Berardinelli-Seip Congenital Lipodystrophy 2 (*BSCL2*), neurofilament light (*NEFL*), transient receptor potential cation channel subfamily V member 4 (*TRPV4*), heat shock protein family B1, B3, and B8 (*HSPB1*, *HSPB3*, and *HSPB8)* (Martini et al., 2000; Rossor et al., 2012). Despite the discrepancy between the major genes mutated in hereditary neuropathies and ALS, each of them exerts pleiotropic functions in neuronal homeostasis, including RNA metabolism, protein quality control, axonal transport, stress response. Therefore, generation of a spectrum of clinical phenotypes from alteration in master genes, broadly involved in key neuronal metabolic pathways, could be expected (**Table 1**).

Neurofilaments (NF) are composed of three subunits defined by their molecular weight and encoded by the NF light (*NEFL*), medium (*NEFM*), and heavy (*NEFH*) genes (Lieberburg et al., 1989). NFs are specifically expressed in neurons and are the most abundant cytoskeletal components of myelinated axons, regulating their caliber, growth, and conduction rate (Hoffman et al., 1987). Mutations in *NEFL* are known to cause both axonal and demyelinating forms of CMT with different phenotypes, including pyramidal signs (Mersiyanova et al., 2000; Jordanova et al., 2003; Rebelo et al., 2016; Jacquier et al., 2017). Mutations in NEFH gene are involved in the pathogenesis of sporadic ALS (Figlewicz et al., 1994; Al-Chalabi et al., 1999) but also in CMT (Jacquier et al., 2017; Nam et al., 2017).

The cellular abundance of PI(3,5)P2, a phosphoinositide involved in the control of vesicles trafficking, is regulated by a phosphoinositide 5-phosphatase encoded by the *FIG4* gene. CMT4J cases, clinically characterized by early onset and aggressive disease progression, have been associated with by an autosomal dominant pattern of transmission and by biallelic *FIG4* mutations (Nicholson et al., 2011). Notably, heterozygous autosomal dominant *FIG4* variants have been more recently associated with ALS and identified as ALS11 (Osmanovic et al., 2017).

The valosin containing protein (VCP) is member of the AAA ATPase family of proteins. This protein is ubiquitously expressed, and it is implicated in multiple cellular processes, such as cell survival (Vandermoere et al., 2006; Braun and Zischka, 2008), stress response and DNA and protein quality control (DeLaBarre et al., 2006; Ju et al., 2009; Weihl et al., 2009). Mutations in *VCP* have been described in patients with autosomal dominant inclusion body myopathy (IBM) associated with Paget disease and fronto-temporal dementia (FTD) (IBMPFD) (Watts et al., 2004), pure ALS patients (Johnson et al., 2010; Miller et al., 2012), and recently also CMT (Gonzalez et al., 2014; Jerath et al., 2015).

The exact function of senataxin (SETX) is unknown but it may be involved in RNA metabolism. Studies have shown a role in DNA transcription and repair (Suraweera et al., 2009; Skourti-Stathaki et al., 2011). Mutations in *STX* have been described in ataxia-ocular apraxia 2 (AOA2) (Moreira et al., 2004; Duquette et al., 2005; Fogel and Perlman, 2006; Arning et al., 2008; Airoldi et al., 2010; Fogel et al., 2014), autosomal dominant juvenile ALS (Chen et al., 2004; Zhao et al., 2009; Avemaria et al., 2011; Arning et al., 2013; Tripolszki et al., 2017), and in dHMN with pyramidal features (De Jonghe et al., 2002).

TABLE 1 | Key genes associated with ALS, hereditary neuropathy, and overlapping phenotypes.

Gene	Gene name	Chromosome	Disease*	Inheritance	References
ALS genes					
C9orf72	Chromosome 9 open reading frame 72	9p21	FTD and/or ALS 1	AD	DeJesus-Hernandez et al., 2011; Renton et al., 2011
FUS	FUS RNA Binding Protein	16p11	ALS6 with or without FTD	AD AR	Vance et al., 2009
OPTN	Optineurin	10p13	Open angle glaucoma – ALS12	AD AR	Rezaie et al., 2002 (Glaucoma); Maruyama et al., 2010 (ALS)
PFN1	Profilin 1	17p13	ALS18	AD	Wu et al., 2012
SOD1	Superoxide dismutase 1	21q22	ALS1	AD AR	Rosen et al., 1993
SQSTM1	Sequestosome 1	5q35	Paget disease of bone 3 – FTD and/or ALS3	AD	Laurin et al., 2002 (Paget Disease); Fecto et al., 2011 (ALS)
TARDBP	TAR DNA binding protein	1p36	ALS 10 with or without FTD	AD	Gitcho et al., 2008; Kabashi et al., 2008
UBQLN2	Ubiquilin 2	Xp11.21	ALS15 with or without FTD	X-linked; AD	Deng et al., 2011
Overlap genes					
DCTN1	Dynactin subunit 1	2p13	dHMNVIIB – Perry syndrome – ALS	AD/AR Risk factor (ALS)	Puls et al., 2003 (dHMN); Munch et al., 2005 (ALS); Farrer et al., 2009 (Perry syndrome)
DYNC1H1	Dynein cytoplasmic 1 heavy chain 1	14q32	CMT2O – dSMA1 – ALS	AD	Munch et al., 2004 (ALS); Weedon et al., 2011 (CMT); Harms et al., 2012 (dSMA)
FIG4	FIG4 phosphoinositide 5-phosphatase	6q21	CMT4J – ALS11 – Yunis-Varon syndrome	AR – AD	Chow et al., 2007, 2009 (CMT, ALS); Campeau et al., 2013 (Yunis-Varon)
GARS	Glycyl-tRNA synthetase	7p15	CMT2D – dHMNVA – ALS	AD	Antonellis et al., 2003 (CMT – dSMAV); Kruger et al., 2016 (ALS)
KIF5A	Kinesin family member 5A	12q13	CMT2 – SPG10 – ALS25	AD	Reid et al., 2002; Crimella et al., 2012 (HSP – CMT2); Nicolas et al., 2018 (ALS)
MFN2	Mitofusin 2	1p36	CMT2A2A – CMT2A2B – dHMNVIA – ALS like	AD AR	Zuchner et al., 2004a (CMT2A2A); MMarchesi et al., 2011 (ALS); Polke et al., 2011 (CMT2A2B)
NEFH	Neurofilament heavy	22q12	ALS – CMT2CC	AD – AR	Figlewicz et al., 1994 (ALS); Rebelo et al., 2016 (CMT)
PLEKHG5	Pleckstrin homology and RhoGEF domain containing G5	1p36	CMTC; SMA – SMA distal4 – ALS	AR	Maystadt et al., 2007 (SMA); Azzedine et al., 2013; Kim et al., 2013 (CMT); Ozoguz et al., 2015 (ALS)
SETX	Senataxin	9q34	dHMN with pyramidal signs – ALS4 juvenile – spinocerebellar ataxia 1	AD – AR	De Jonghe et al., 2002 (dHMN); Chen et al., 2004 (ALS); Moreira et al., 2004 (spinocerebellar ataxia)
SIGMAR1	Sigma non-opioid intracellular receptor 1	9p13	ALS16 juvenile – SMA distal 2	AR	Al-Saif et al., 2011 (ALS); Li X. et al., 2015 (dSMA2)
SPAST	Spastin	2p22	SPG4 – ALS juvenile	AD	Fonknechten et al., 2000 (HSP); Meyer et al., 2005 (ALS)
SPG11	Spatacsin vesicle trafficking associated	15q21	SPG11 – ALS5 juvenile – CMT2X	AR	Stevanin et al., 2007 (SPG); Orlacchio et al., 2010 (ALS); Montecchiani et al., 2016 (CMT)
VCP	Valosin containing protein	9p13	IBMPFD- ALS14 with or without FTD – CMT2Y	AD	Watts et al., 2004 (IBMPFD); Johnson et al., 2010 (ALS); Gonzalez et al., 2014 (CMT)

(Continued)

TABLE 1 | Continued

Gene	Gene name	Chromosome	Disease*	Inheritance	References
CMT/dHMN genes					
BSCL2	BSCL2, seipin lipid droplet biogenesis associated	11q13	Lipodystrophy, congenital generalized, type 2 – dHMNVA – silver spastic paraplegia syndrome	AR – AD	Magré et al., 2001 (Lypodistrophy); Windpassinger et al., 2004 (dHMNVA, Silver syndrome)
HSPB1	Heat shock protein family B (small) member 1	7q11	CMT2F – dHMN IIB	AD	Evgrafov et al., 2004 (CMT dHMN)
HSPB3	Heat shock protein family B (small) member 3	5q11	dHMN IIC	AD	Kolb et al., 2010
HSPB8	Heat shock protein family B (small) member 8	12q24	dHMN IIA – CMT2L	AD	Irobi et al., 2004 (dHMN); Tang et al., 2005 (CMT)
NEFL	Neurofilament light	8q21	CMT2E – CMT1F – CMTG	AD AR	Mersiyanova et al., 2000 (CMT2E); Jordanova et al., 2003 (CMT1F); Zuchner et al., 2004b (CMT2G)
TRPV4	Transient receptor potential cation channel subfamily V member 4	2q24	HMSN IIC – SMA – scapuloperoneal SMA	AD	Auer-Grumbach et al., 2010

**OMIM and report from literature. ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia; CMT, charcot marie tooth; SMA, spinal muscular atrophy; SPG, spastic paraplegia; HMSN, hereditary motor and sensory neuropathy; Dhmn, distal hereditary motor neuropathy; UPS, ubiquitin proteasome system; ERAD, endoplasmic reticulum-associated protein degradation; IBMPFD, inclusion body myopathy paget disease frontotemporal dementia.*

The Spastic Paraplegia 11 gene (*SPG11*) encodes the spatacsin protein, selectively expressed in neuron with a role in axonal growth, transport, and cytoskeletal stability (Perez-Branguli et al., 2014). *SPG11* variants were first described in patients with autosomal recessive spastic paraplegia 11 with thin corpus callosum (Hehr et al., 2007). Then, descriptions in juvenile ALS (ALS5) and in CMT2X were also reported (Orlacchio et al., 2010; Daoud et al., 2012; Iskender et al., 2015; Montecchiani et al., 2016).

The dynactin subunit 1 (*DCTN1*) and kinesin family member 5A (*KIF5A*) genes encode for dynactin and kinesin subunits, involved in retrograde and anterograde axonal transport, respectively (Hirokawa et al., 2009; Kwinter et al., 2009). *DCTN1* and *KIF5A* variants have been variably described in ALS (Munch et al., 2004, 2005; Liu et al., 2014, 2017; Brenner et al., 2018; Nicolas et al., 2018), CMT, and dHMN (Crimella et al., 2012; Lopez et al., 2015).

Finally, pleckstrin homology and RhoGEF domain containing G5 (*PLEKHG5*) and mitofusin 2 (*MFN2*) genes, whose mutations are known to cause CMT (Muglia et al., 2001; Zuchner et al., 2004a, 2006; Engelfried et al., 2006; Del Bo et al., 2008; Nicholson et al., 2008; Azzedine et al., 2013; Kim et al., 2013; Iapadre et al., 2018), have been also reported in patients with ALS or an "ALS-like" phenotype (Marchesi et al., 2011; Ozoguz et al., 2015).

PATHOGENIC MECHANISMS OF PNS DAMAGE IN ALS

Peripheral Motor and Sensory Dysfunction in ALS Models

The strongest evidence supporting the dying-back pattern of degeneration comes from transgenic mice overexpressing the mutated form of human SOD1 G93A (hSOD-1^{G93A}), the most studied ALS model. Axonal pathology precedes spinal MN death and symptom onset, with the NMJ being the first site of morphological alteration (Fischer et al., 2004). Along with disease progression, the burden of axonal pathology overcomes the moderate MN loss in spinal cord, and suggesting that the motor phenotype in these mice models is mainly driven by the PNS damage (Gould et al., 2006; Hegedus et al., 2007). Indeed, interventions leading to complete rescue of MNs in the spinal cord of mutant SOD1 (mSOD1) ALS mice were not sufficient to halt axonal loss and motor phenotype, suggesting that mechanisms of central, and peripheral degeneration may be at least partially independent (Gould et al., 2006; Rouaux et al., 2007; Suzuki et al., 2007).

Despite the wealth amount of data generated from this model about MN degeneration, important concerns remain about the reproducibility of these findings in human ALS. The preferential involvement of spinal compared to cortical MNs, the anatomical differences in CNS organization between mice and humans and the confounding effects of artificial manipulation, such as transgenic overexpression, limit the translation of ALS preclinical research on patients (Kato, 2008; Philips and Rothstein, 2015). Nonetheless, some studies reported evidence of early NMJ alterations and axonal injury preceding MN loss also in ALS patients (Bradley et al., 1983; Dengler et al., 1990; Fischer et al., 2004; Bruneteau et al., 2015). Moreover, progressive NMJ and motor axon loss is a consistent finding also in other transgenic ALS mice with mutations in *TARDBP*, *FUS*, and *C9orf72* repeat expansion (Picher-Martel et al., 2016). Overexpression of hTDP-43^{A315T} and hTDP-43^{WT} leads to NMJ denervation and loss of corticospinal axons, which in some cases predominate over MN al loss (Wegorzewska et al., 2009; Arnold et al., 2013; Herdewyn et al., 2014). In the hFUS^{P525L} mouse lines, where the mutation is

conditionally expressed in MNs, the progressive degeneration is preceded by early pre-symptomatic retraction of motor axons (Sharma et al., 2016).

Reflecting evidence of sensory dysfunction in human ALS, transgenic hSOD-1^{G93A} mice also display neurodegeneration in sensory axon, DRG and proprioceptive sensory fibers of muscle spindles. Signs of axonal damage are detected since the pre-symptomatic stage, progressing with a distal-to-proximal gradient (Guo et al., 2009; Vaughan et al., 2015). Furthermore, loss of dorsal root axons has been detected in rodent models overexpressing mutant TDP-43 and FUS (Huang et al., 2011; Arnold et al., 2013), suggesting that ALS-specific proteins may also affect sensory neurons. However, it is still unclear whether the damage comes from nearby disease spreading or arise independently from MNs. Interestingly, transfection of mutant TDP-43 and SOD1 in cultured sensory neurons exert a negative impact on neurites length and arborization after prolonged culture, suggesting a direct effect (Vaughan et al., 2018).

Therefore, the reported evidence demonstrates that all components of the PNS are affected in ALS, although the differing kinetics of damage and progression still point to differences in vulnerability between the sensory and motor axons and neurons.

Unraveling Mechanisms of Vulnerability and Resistance of Peripheral Motor and Sensory Neurons in ALS

Studies addressing the mechanisms of PNS involvement in ALS may provide a unique opportunity for unraveling the determinants of the different patterns of vulnerability of motor and sensory neurons. This would allow the identification of specific protective or deleterious factors amenable of therapeutic intervention. Hereafter, we discuss key factors at the PNS level potentially implied in such differences.

Intrinsic Axonal Vulnerability

Hyperexcitability of the axolemma, due to altered Na^+ and K^+ conductance properties, has been demonstrated in motor but not sensory axons of ALS patients (Shibuya et al., 2011; Park et al., 2017; Matamala et al., 2018). The consequent increase in Ca^{2+} influx may be selectively harmful in the motor compartment, since motor axons show a low Ca^{2+}-buffering capacity (Jaiswal, 2013; Leal and Gomes, 2015). In turn, Ca^{2+} overload leads to the activation of effector proteins such as calpain, a calcium-dependent protease, involved in TDP-43 fragmentation, which predisposes to aggregation, Wallerian degeneration and NMJ disassembly (Yamashita et al., 2012; Conforti et al., 2014; Campanari et al., 2016). Specific demise of MNs may also be driven by the involvement of ALS-key proteins, such as TDP-43 and FUS. They are mRNA-binding proteins, mainly localized in the nucleus, which participate in the metabolism of broad pools of mRNAs, by affecting splicing, stability, transport. Mutations in these proteins affect neurons by both loss and gain of function, leading to altered RNA processing, increasing cytoplasmic translocation and propensity to aggregation (Ratti and Buratti, 2016). They are essential during embryonic development, but only MNs in the cortex and anterior horns display sustained expression of both proteins during lifetime (Huang et al., 2010). Although most of the transcriptome is shared between motor and DRG axons, more than one third is unique (Rotem et al., 2017). It has been estimated that TDP43 binds roughly 30% of the whole transcriptome (Ling et al., 2013), including coding and non-coding RNAs, such as miRNA (Kawahara and Mieda-Sato, 2012). TDP-43 is actively recruited in RNA granules along motor axons for active transport (Fallini et al., 2012). Examples of TDP43-bound mRNAs include human neurofilaments, components of the endosomal trafficking and mitochondrial proteins (Stalekar et al., 2015; Schwenk et al., 2016; Wang et al., 2016). Over-expression of wild type and mutant TDP-43 alters the dynamics of its axonal transport (Alami et al., 2014), leading to mitochondrial toxicity and NMJ loss (Lin et al., 2011; Tripathi et al., 2014; Wang et al., 2016). Furthermore, a transient increase in TDP-43 localization along axonal routes is observed following axotomy, suggesting a role in peripheral nerve regeneration (Moisse et al., 2009). However, TDP-43^{G348C} mice show abnormal persistence of cytoplasmic levels after peripheral injury, impairing axonal regeneration (Swarup et al., 2012). Such finding may provide a potential additional explanation to the paucity of regenerative clusters observed in ventral roots and motor nerves of human and animal ALS (Riva et al., 2011b, 2014; Luigetti et al., 2012). In contrast, sensory nerves still show some level of regeneration capacity in ALS patients, suggesting that efficient repair mechanisms may dampen progressive neurodegeneration (Hammad et al., 2007; Isaacs et al., 2007).

Neuromuscular Junction

The early susceptibility of the NMJ in ALS relies on peculiar features that distinguish it from other peripheral ending structures, including the sensory receptors. It has high energy demands and requires efficient mechanisms of plasticity and repair to cope with the challenges faced by muscle contraction. Oxidative stress, which contributes to ALS pathogenesis (D'Amico et al., 2013), has a great influence on NMJ function, as the early production of reactive oxygen species (ROS) in distal muscles inhibits transmitter release (Kraft et al., 2007; Naumenko et al., 2011) and leads to synaptic loss (Fischer et al., 2011, Fischer et al., 2012). High levels of oxidative stress have also been observed in muscles and spinal cord of ALS patients (D'Amico et al., 2013; Lanfranconi et al., 2017). Mitochondrial dysfunction further participates to NMJ dismantling, as its role in energy production and Ca^{2+} buffering is essential for proper function and maintenance of the synapse (Fischer-Hayes et al., 2013). Non-neuronal factors are also involved, as terminal schwann cells (TSCs) exert important functions in NMJ stability and repair, adapting their state according to local synaptic environment. Such decoding ability depends on the muscarinic acetylcholine receptor (mAchR), a G-protein-coupled receptor (GPCR) present on the membrane of TSCs, which detects the level of Ach released in the synaptic cleft. SOD1 mutants have been shown to alter the mAchR sensitivity of these cells to local damage, leading to deficient repair of denervated NMJ in ALS mice models (Arbour et al., 2015). Still, such findings need confirmation in humans and other models of ALS, although a

relationship between TDP-43 function and TSC-related receptors has been suggested (Arbour et al., 2017).

PNS-Related Non-cell Autonomous Toxicity

Besides TSCs, other non-cell autonomous mechanisms in the PNS may further influence MNs vulnerability. The role of Schwann cells in the peripheral nerve has also been recently re-evaluated in ALS. Early deficits in axonal transport may be initially compensated by the transfer of polyribosomes from Schwann cells to the axonal compartment, boosting local protein synthesis as an adaptive response to mSOD1-induced injury (Verheijen et al., 2014). Moreover, Schwann cells may exert partial protection against the increased production of ROS related to axonal degeneration, as the loss of dismutase activity in these cells accelerated disease progression (Lobsiger et al., 2009). However, pathogenic gain-of-functions SOD1 mutants may affect Schwann cells functioning and protective effects as well (Wang et al., 2012). Neuroinflammatory response within the PNS and alterations of the blood-nerve barrier might offer additional insights in the pathogenesis of ALS (Kang and Rivest, 2007; Beers et al., 2008), as suggested by observations raised in mice models (Pupillo et al., 2014; Nardo et al., 2016, 2018) and in human ALS (Devigili et al., 2011; Riva et al., 2011a; Gerevini et al., 2016). Pathological studies of sural nerve biopsies of ALS patients have demonstrated, in a subset of cases, the presence of vasculitic-like inflammatory infiltrates associated with normal nerve morphology, suggesting a potential neuroprotective effect of neuroinflammation within the PNS (Devigili et al., 2011). Indeed, the recruitment of macrophages and stimulation of their phagocytic function in peripheral nerves is important to create a favorable milieu for axonal regeneration, as shown in mSOD1 ALS mice (Nardo et al., 2016).

Common Grounds for ALS and Hereditary Neuropathies

Despite the distinctive prognosis and disease course, ALS, related MNDs and hereditary neuropathies show a non-negligible degree of overlap in terms of both clinical presentations and genetics. Therefore, a better understanding of the key cellular pathways involved in both diseases may allow a broader comprehension of the molecular events leading to axonal and MN degeneration in ALS.

Neuronal Cytoskeleton

Microtubules, NFs and actin are the main components of axonal cytoskeleton, and impairment in either structure or functioning of any of these components may lead to axonal atrophy, retraction and degeneration or transport defects (Gentil et al., 2015; Kevenaar and Hoogenraad, 2015). Elevated levels of light NFs (NFL) and phosphorylated heavy NFs have been observed in the cerebrospinal fluid and serum of ALS, together with lower mRNA NFL levels in MNs (Volkening et al., 2009; Gaiani et al., 2017; Feneberg et al., 2018). Accumulation of NF components in axons is a prominent feature of both human and animal ALS (Leigh and Swash, 1991; Rouleau et al., 1996; Morrison et al., 2000), and has been observed along both motor and sensory axons in the hSOD-1^{G93A} ALS mouse model (King et al., 2012; Gentil et al., 2015; Sassone et al., 2016). Alterations of ALS-key genes, such as *SOD1* and *TARDBP*, have been shown to affect the stability of NFL mRNA, suggesting that altered stoichiometry between the different NF components may contribute to the disease (Volkening et al., 2009). Mutations in *NEFL* and *NEFH,* leading to axonal CMT, promote protein aggregation, aberrant mitochondrial morphology and shortening of axonal length (Yoshihara et al., 2002; Rebelo et al., 2016; Nam et al., 2017). Tubulin Alpha 4a (*TUBA4A)*, encoding for α-tubulin, has been recently identified as a novel ALS gene (Smith et al., 2014). It has been shown that *TUBA4A* mutations are able to alter microtubule dynamics, with depolymerization and degradation of α-tubulin (Helferich et al., 2018). Although this gene has been described only in cohorts of ALS and FTD patients, a sensory and motor neuronopathy is observed in progressive motor neuronopathy mice mutated in tubulin binding cofactor E (*TBCE*), a known interactor of α-tubulin (Schafer et al., 2017). Profilin 1 (*PFN1*) has been found as a rare cause of familial ALS (Wu et al., 2012). It is a crucial protein for the conversion of monomeric (G)-actin to filamentous (F)-actin. Interestingly, although pathogenic variants do not disrupt actin dynamics (Freischmidt et al., 2015), alterations in microtubule growth and increased propensity to cytoplasmic TDP43 aggregation have been described (Matsukawa et al., 2016; Tanaka et al., 2016; Henty-Ridilla et al., 2017), revealing potential cell-type specific mechanisms of neurodegeneration.

Axonal Transport

Defects in axonal transport have been clearly observed both in ALS and dHMN. SOD1 and TDP43 mutants impair anterograde and retrograde axonal transport, leading to loss of essential synaptic components, mitochondrial abnormalities, and neurite shortening (Williamson and Cleveland, 1999; Perlson et al., 2009; Alami et al., 2014). Similarly abnormal transport of organelles, such as mitochondria and the endoplasmic reticulum (ER), is found in CMT models due to *NEFL* mutations (Tradewell et al., 2009). The failure of axon-cell body communication may dampen the activation of repair mechanisms, thus conferring an increase vulnerability of the peripheral compartment, more pronounced for the distal segments (Fischer et al., 2004; Dadon-Nachum et al., 2011). Strategies aiming at reinforcing axonal transport may thus confer protection to a broad range of pathogenic alterations, preserving the axonal routes and its endings.

Protein Folding

The highly differentiated and post-mitotic state of MNs exerts a relevant pressure over the protein quality control system of the cell, which is devoted to the correct folding, function and turnover of the whole proteome. HSPB1/27, HSPB8/22, and sigma non-opioid intracellular receptor 1 (SIGMAR1) are three chaperones with a significant expression levels in MNs (Mavlyutov et al., 2010). Mutations of the small heat shock proteins have been mostly associated with a dHMN or axonal CMT2 phenotype, while *SIGMAR1* gene variants have been reported also in rare ALS patients (Al-Saif et al., 2011; Rossor et al., 2012). HSPB1 mutants alter both its chaperone activity in NF organization and preservation of axonal stability

(Evgrafov et al., 2004; Ackerley et al., 2006). HSPB8 mutant mice develop a progressive motor neuropathy through specific neurite degeneration (Irobi et al., 2010; Bouhy et al., 2018). In addition, alterations in TDP-43 expression, and associated splicing is observed in the muscles of these mice (Cortese et al., 2018). Conversely, there is a significantly higher HSPB8 expression in surviving neurons in the hSOD-1^{G93A} mouse model of ALS (Crippa et al., 2010). Finally, SIGMAR1, together with MFN2 and vesicle-associated membrane protein-associated protein B/C (VAPB), are able to modulate unfolded protein response (UPR) sensors, including protein kinase RNA-like endoplasmic reticulum kinase (PERK) (Nguyen et al., 2015).

Mitochondrial/Associated Membranes (MAMs)

The ER and mitochondria have evolved complex sites of interactions, defined as mitochondrial/associated membranes (MAMs), which are important for essential cellular functions, such as calcium homeostasis, autophagy, regulation of mitochondrial dynamics, and axon survival (Giorgi et al., 2015). Both ALS and hereditary neuropathies display dysfunction in one or more of MAM components. A huge number of cellular proteins participate in MAMs, including MFN2 and VAPB, which act as tethering sites between the two organelles (Bernard-Marissal et al., 2015). VAPB mutants affect the interaction with the mitochondrial protein tyrosine phosphatase-interacting protein 51 (PTPIP5) (de Brito and Scorrano, 2008; De Vos et al., 2012). Interestingly, TDP43 and FUS mutants have been shown to decrease ER-mitochondria contacts by disrupting VAPB-PTPIP5 binding (Stoica et al., 2014). MFN2 mutants alter other MAM functions, such as mitochondrial transport, fusion, and autophagosome assembly (Kim et al., 2015). Loss-of function mutations in *SIGMAR1* display decreased ER-mitochondria association, resulting in MN death, and axonal degeneration due to impaired retrograde transport (Bernard-Marissal et al., 2015; Watanabe et al., 2016).

THERAPEUTIC PERSPECTIVES

Due to the high similarity between these neuromuscular disorders and the intimate relationship observed in the molecular pathways involved, it may not be surprising if functional proteins involved in either of the two diseases may provide benefits on their respective counterpart.

Preservation of NMJ is an attractive target to delay muscle denervation and disability in ALS. A drug-screening platform in *C. elegans* and zebrafish models of ALS identified pimozide, an already approved neuroleptic, as a NMJ stabilizer by blocking T-type Ca^{2+} channels (Patten et al., 2017). Despite some benefits in neurophysiologic measures were reported in hSOD-1^{G93A} mice and human patients in the first report, one study assessing long-term effects showed warned caution, as worsening of survival and muscle function were observed in mutant SOD1, and TDP-43 mice models (Pozzi et al., 2018). Notably, an agonist antibody directed toward muscle-specific kinase (MuSK), a post-synaptic tyrosine kinase receptor essential for NMJ maintenance, preserved motor synapses, delayed muscle denervation and extended lifespan in hSOD-1^{G93A} ALS mice (Cantor et al., 2018). The promising findings of this study still awaits confirmation and replication in other disease models.

Reinforcing the action of factors involved in axonal stability and transport may restore soma-axon communication, delaying NMJ loss and axonal degeneration. Pharmacological inhibition of histone deacetylase 6 (HDAC6), a known client protein of HSPB1, was able to increase the stability of microtubules, rescuing axonal loss, and improving outcome in a CMT2F mouse model with mutant HSPB1 (d'Ydewalle et al., 2011). Interestingly, HDAC6 inhibition was also able to restore axonal transport defects in patient-derived MNs mutated in FUS (Guo et al., 2017). Pharmacologic agents targeting microtubule dynamics, such as noscapine and vinblastine, delayed disease onset and prolonged survival in hSOD-1^{G93A} mice, by reducing microtubule turnover (Fanara et al., 2007).

Inhibition of p38/mitogen-associated protein kinase (MAPK) signaling in MNs was able to restore retrograde axonal transport defects *in vivo* (Gibbs et al., 2018), together with reduced microglia-induced neuroinflammation in the spinal cord (Kim and Choi, 2010; Zhan et al., 2015; Leal-Lasarte et al., 2017). However, it is remarkable that no significant improvement was observed on the clinical phenotype (Gibbs et al., 2018), suggesting that inhibition of this pathway alone is not sufficient to fully rescue MN function.

Reducing ER stress through either a potentiation of the chaperone system or inhibition of stress-related factors may limit the production rate of misfolded proteins. Enhancing HSPB8 activity has been shown to be protective against accumulation of TDP43 aggregates in MNs (Crippa et al., 2016; Rusmini et al., 2017), and to extend survival of hSOD-1^{G93A} mice (Aurelian et al., 2012). Trehalose, a chemical chaperone known to induce HSPB8, reduces ER stress levels and improve autophagy, delaying disease, and prolonging MN survival in a mouse model (Zhang et al., 2014; Li Y. et al., 2015). Upon UPR activation, cells switch to a state of translational repression and stimulation of chaperones' synthesis, processes regulated by phosphorylation of the eukaryotic translation initiation factor 2A (eiF2α). Salubrinal and guanabenz, two eiF2α phosphatase inhibitors, protected against MN degeneration in ALS mice (Saxena et al., 2009; Wang et al., 2014), although one study reported that guanabenz accelerated disease progression in an ALS model (Vieira et al., 2015).

Considering their importance in cell physiology, MAMs could represent an important target for therapy in ALS and may prove beneficial for a wide array of downstream signaling cascades relying upon these structures. An agonist of SIGMAR1 was shown to improve muscle activity and motor performance in pre-symptomatic hSOD-1^{G93A} ALS mice (Mancuso et al., 2012). Notably, overexpression of MFN2 prevented NMJ loss and delayed onset and progression in a murine model by raising calpastatin levels, a calpain inhibitor, essential for survival, and function of the NMJ (Wang et al., 2018).

Boosting axonal regeneration through either neuron- or Schwann-targeting factors may also be a valuable alternative strategy in order to preserve motor function (Gilley et al., 2017).

Studies modulating Neuregulin 1 activity, a neurotrophic factor involved in peripheral nerve development and regeneration, in murine hSOD-1^{G93A} ALS lead to increased re-innervation and MN survival (Mancuso et al., 2016; Modol-Caballero et al., 2017). However, other approaches aimed at slowing Wallerian degeneration, such as the mutations in the Slowed Wallerian degeneration (Wld^{S}) and Sterile Alpha and TIR Motif Containing 1 (Sarm1) proteins, gave only modest or no results in terms of progression and survival (Fischer et al., 2005; Peters et al., 2018), suggesting that alternative mechanisms may underlie the axonal loss observed in ALS.

CONCLUSION

Clinical and pathological studies in both human patients and rodent models suggest that the PNS is involved in ALS pathogenetic cascade. A better comprehension of the molecular events occurring into the PNS may prove essential for a better understanding of MND pathomechanisms and may pave the way to the discovery of novel therapeutic targets. Considering that in ALS neurodegeneration derives from the combination of several dysfunctional pathways, it might be possible that the development of combination therapies acting on multiple cascades located in both the central and the peripheral compartments may show synergistic neuroprotective effect in order to prevent axonal degeneration, MN cell death, und ultimately in prolonging patients' survival.

AUTHOR CONTRIBUTIONS

FG and SS contributed to the collection of available evidence and writing. YF, AQ, NR, CL, and LT contributed to the manuscript writing and review of intellectual content of the manuscript, and approved the final version of the manuscript. AQ and NR contributed to the manuscript design.

REFERENCES

Ackerley, S., James, P. A., Kalli, A., French, S., Davies, K. E., and Talbot, K. (2006). A mutation in the small heat-shock protein HSPB1 leading to distal hereditary motor neuronopathy disrupts neurofilament assembly and the axonal transport of specific cellular cargoes. *Hum. Mol. Genet.* 15, 347–354. doi: 10.1093/hmg/ddi452

Airoldi, G., Guidarelli, A., Cantoni, O., Panzeri, C., Vantaggiato, C., Bonato, S., et al. (2010). Characterization of two novel SETX mutations in AOA2 patients reveals aspects of the pathophysiological role of senataxin. *Neurogenetics* 11, 91–100. doi: 10.1007/s10048-009-0206-0

Alami, N. H., Smith, R. B., Carrasco, M. A., Williams, L. A., Winborn, C. S., Han, S. S. W., et al. (2014). Axonal transport of TDP-43 mRNA granules is impaired by ALS-causing mutations. *Neuron* 81, 536–543. doi: 10.1016/j.neuron.2013.12.018

Al-Chalabi, A., Andersen, P. M., Nilsson, P., Chioza, B., Andersson, J. L., Russ, C., et al. (1999). Deletions of the heavy neurofilament subunit tail in amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 8, 157–164. doi: 10.1093/hmg/8.2.157

Al-Saif, A., Al-Mohanna, F., and Bohlega, S. (2011). A mutation in sigma-1 receptor causes juvenile amyotrophic lateral sclerosis. *Ann. Neurol.* 70, 913–919. doi: 10.1002/ana.22534

Antonellis, A., Ellsworth, R. E., Sambuughin, N., Puls, I., Abel, A., Lee-Lin, S. Q., et al. (2003). Glycyl tRNA synthetase mutations in Charcot-Marie-Tooth disease type 2D and distal spinal muscular atrophy type V. *Am. J. Hum. Genet.* 72, 1293–1299. doi: 10.1086/375039

Arbour, D., Tremblay, E., Martineau, E., Julien, J. P., and Robitaille, R. (2015). Early and persistent abnormal decoding by glial cells at the neuromuscular junction in an ALS model. *J. Neurosci.* 35, 688–706. doi: 10.1523/JNEUROSCI.1379-14.2015

Arbour, D., Vande Velde, C., and Robitaille, R. (2017). New perspectives on amyotrophic lateral sclerosis: the role of glial cells at the neuromuscular junction. *J. Physiol.* 595, 647–661. doi: 10.1113/JP270213

Arendt, T. (2009). Synaptic degeneration in Alzheimer's disease. *Acta Neuropathol.* 118, 167–179.

Arning, L., Epplen, J. T., Rahikkala, E., Hendrich, C., Ludolph, A. C., and Sperfeld, A. D. (2013). The SETX missense variation spectrum as evaluated in patients with ALS4-like motor neuron diseases. *Neurogenetics* 14, 53–61. doi: 10.1007/s10048-012-0347-4

Arning, L., Schols, L., Cin, H., Souquet, M., Epplen, J. T., and Timmann, D. (2008). Identification and characterisation of a large senataxin (SETX) gene duplication in ataxia with ocular apraxia type 2 (AOA2). *Neurogenetics* 9, 295–299. doi: 10.1007/s10048-008-0139-z

Arnold, E. S., Ling, S. C., Huelga, S. C., Lagier-Tourenne, C., Polymenidou, M., Ditsworth, D., et al. (2013). ALS-linked TDP-43 mutations produce aberrant RNA splicing and adult-onset motor neuron disease without aggregation or loss of nuclear TDP-43. *Proc Natl Acad Sci U.S.A.* 110, E736–E745. doi: 10.1073/pnas.1222809110

Auer-Grumbach, M., Olschewski, A., Papic, L., Kremer, H., Mcentagart, M. E., Uhrig, S., et al. (2010). Alterations in the ankyrin domain of TRPV4 cause congenital distal SMA, scapuloperoneal SMA and HMSN2C. *Nat. Genet.* 42, 160–164. doi: 10.1038/ng.508

Aurelian, L., Laing, J. M., and Lee, K. S. (2012). H11/HspB8 and its herpes simplex virus type 2 homologue ICP10PK share functions that regulate cell life/death decisions and human disease. *Autoimmune Dis.* 2012:395329. doi: 10.1155/2012/395329

Avemaria, F., Lunetta, C., Tarlarini, C., Mosca, L., Maestri, E., Marocchi, A., et al. (2011). Mutation in the senataxin gene found in a patient affected by familial ALS with juvenile onset and slow progression. *Amyotroph. Lateral Scler.* 12, 228–230. doi: 10.3109/17482968.2011.566930

Azzedine, H., Zavadakova, P., Plante-Bordeneuve, V., Vaz Pato, M., Pinto, N., Bartesaghi, L., et al. (2013). PLEKHG5 deficiency leads to an intermediate form of autosomal-recessive Charcot-Marie-Tooth disease. *Hum. Mol. Genet.* 22, 4224–4232. doi: 10.1093/hmg/ddt274

Beers, D. R., Henkel, J. S., Zhao, W., Wang, J., and Appel, S. H. (2008). CD4+ T cells support glial neuroprotection, slow disease progression, and modify glial morphology in an animal model of inherited ALS. *Proc. Natl. Acad. Sci. U.S.A.* 105, 15558–15563. doi: 10.1073/pnas.0807419105

Benedetti, S., Previtali, S. C., Coviello, S., Scarlato, M., Cerri, F., Di Pierri, E., et al. (2010). Analyzing histopathological features of rare charcot-marie-tooth neuropathies to unravel their pathogenesis. *Arch. Neurol.* 67, 1498–1505. doi: 10.1001/archneurol.2010.303

Bernard-Marissal, N., Medard, J. J., Azzedine, H., and Chrast, R. (2015). Dysfunction in endoplasmic reticulum-mitochondria crosstalk underlies SIGMAR1 loss of function mediated motor neuron degeneration. *Brain* 138, 875–890. doi: 10.1093/brain/awv008

Bhatt, A., Farooq, M. U., Aburashed, R., Kassab, M. Y., Majid, A., Bhatt, S., et al. (2009). Hereditary neuropathy with liability to pressure palsies and amyotrophic lateral sclerosis. *Neurol. Sci.* 30, 241–245. doi: 10.1007/s10072-009-0034-x

Bouhy, D., Juneja, M., Katona, I., Holmgren, A., Asselbergh, B., De Winter, V., et al. (2018). A knock-in/knock-out mouse model of HSPB8-associated distal hereditary motor neuropathy and myopathy reveals toxic gain-of-function of mutant Hspb8. *Acta Neuropathol.* 135, 131–148. doi: 10.1007/s00401-017-1756-0

Bradley, W. G., Good, P., Rasool, C. G., and Adelman, L. S. (1983). Morphometric and biochemical studies of peripheral nerves in amyotrophic lateral sclerosis. *Ann. Neurol.* 14, 267–277. doi: 10.1002/ana.410140304

Braun, R. J., and Zischka, H. (2008). Mechanisms of Cdc48/VCP-mediated cell death: from yeast apoptosis to human disease. *Biochim. Biophys. Acta* 1783, 1418–1435. doi: 10.1016/j.bbamcr.2008.01.015

Brenner, D., Yilmaz, R., Muller, K., Grehl, T., Petri, S., Meyer, T., et al. (2018). Hot-spot KIF5A mutations cause familial ALS. *Brain* 141, 688–697. doi: 10.1093/brain/awx370

Brettschneider, J., Arai, K., Del Tredici, K., Toledo, J. B., Robinson, J. L., Lee, E. B., et al. (2014). TDP-43 pathology and neuronal loss in amyotrophic lateral sclerosis spinal cord. *Acta Neuropathol.* 128, 423–437. doi: 10.1007/s00401-014-1299-6

Brettschneider, J., Del Tredici, K., Toledo, J. B., Robinson, J. L., Irwin, D. J., Grossman, M., et al. (2013). Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. *Ann. Neurol.* 74, 20–38. doi: 10.1002/ana.23937

Bruneteau, G., Bauche, S., Gonzalez De Aguilar, J. L., Brochier, G., Mandjee, N., Tanguy, M. L., et al. (2015). Endplate denervation correlates with Nogo-A muscle expression in amyotrophic lateral sclerosis patients. *Ann. Clin. Transl. Neurol.* 2, 362–372. doi: 10.1002/acn3.179

Camdessanche, J. P., Belzil, V. V., Jousserand, G., Rouleau, G. A., Creac'h, C., Convers, P., et al. (2011). Sensory and motor neuronopathy in a patient with the A382P TDP-43 mutation. *Orphanet J. Rare Dis.* 6:4. doi: 10.1186/1750-1172-6-4

Campanari, M. L., Garcia-Ayllon, M. S., Ciura, S., Saez-Valero, J., and Kabashi, E. (2016). Neuromuscular junction impairment in amyotrophic lateral sclerosis: reassessing the role of acetylcholinesterase. *Front. Mol. Neurosci.* 9:160. doi: 10.3389/fnmol.2016.00160

Campeau, P. M., Lenk, G. M., Lu, J. T., Bae, Y., Burrage, L., Turnpenny, P., et al. (2013). Yunis-Varon syndrome is caused by mutations in FIG4, encoding a phosphoinositide phosphatase. *Am. J. Hum. Genet.* 92, 781–791. doi: 10.1016/j.ajhg.2013.03.020

Cantor, S., Zhang, W., Delestree, N., Remedio, L., Mentis, G. Z., and Burden, S. J. (2018). Preserving neuromuscular synapses in ALS by stimulating MuSK with a therapeutic agonist antibody. *eLife* 7:e34375

Ceroni, M., Curti, D., and Alimonti, D. (2001). Amyotrophic lateral sclerosis and SOD1 gene: an overview. *Funct. Neurol.* 16, 171–180.

Chen, Y. Z., Bennett, C. L., Huynh, H. M., Blair, I. P., Puls, I., Irobi, J., et al. (2004). DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). *Am. J. Hum. Genet.* 74, 1128–1135. doi: 10.1086/421054

Chia, R., Chio, A., and Traynor, B. J. (2018). Novel genes associated with amyotrophic lateral sclerosis: diagnostic and clinical implications. *Lancet Neurol.* 17, 94–102. doi: 10.1016/S1474-4422(17)30401-5

Chow, C. Y., Landers, J. E., Bergren, S. K., Sapp, P. C., Grant, A. E., Jones, J. M., et al. (2009). Deleterious variants of FIG4, a phosphoinositide phosphatase, in patients with ALS. *Am. J. Hum. Genet.* 84, 85–88. doi: 10.1016/j.ajhg.2008.12.010

Chow, C. Y., Zhang, Y., Dowling, J. J., Jin, N., Adamska, M., Shiga, K., et al. (2007). Mutation of FIG4 causes neurodegeneration in the pale tremor mouse and patients with CMT4J. *Nature* 448, 68–72. doi: 10.1038/nature05876

Chung, C. Y., Koprich, J. B., Siddiqi, H., and Isacson, O. (2009). Dynamic changes in presynaptic and axonal transport proteins combined with striatal neuroinflammation precede dopaminergic neuronal loss in a rat model of AAV α-synucleinopathy. *J. Neurosci.* 29, 3365–3373. doi: 10.1523/JNEUROSCI.5427-08.2009

Conforti, L., Gilley, J., and Coleman, M. P. (2014). Wallerian degeneration: an emerging axon death pathway linking injury and disease. *Nat. Rev. Neurosci.* 15, 394–409. doi: 10.1038/nrn3680

Corbo, M., Abouzahr, M. K., Latov, N., Iannaccone, S., Quattrini, A., Nemni, R., et al. (1997). Motor nerve biopsy studies in motor neuropathy and motor neuron disease. *Muscle Nerve* 20, 15–21. doi: 10.1002/(sici)1097-4598(199701)20:1<15::aid-mus2>3.0.co;2-k

Cortese, A., Laura, M., Casali, C., Nishino, I., Hayashi, Y. K., Magri, S., et al. (2018). Altered TDP-43-dependent splicing in HSPB8-related distal hereditary motor neuropathy and myofibrillar myopathy. *Eur. J. Neurol.* 25, 154–163. doi: 10.1111/ene.13478

Crimella, C., Baschirotto, C., Arnoldi, A., Tonelli, A., Tenderini, E., Airoldi, G., et al. (2012). Mutations in the motor and stalk domains of KIF5A in spastic paraplegia type 10 and in axonal Charcot-Marie-Tooth type 2. *Clin. Genet.* 82, 157–164. doi: 10.1111/j.1399-0004.2011.01717.x

Crippa, V., Cicardi, M. E., Ramesh, N., Seguin, S. J., Ganassi, M., Bigi, I., et al. (2016). The chaperone HSPB8 reduces the accumulation of truncated TDP-43 species in cells and protects against TDP-43-mediated toxicity. *Hum. Mol. Genet.* 25, 3908–3924. doi: 10.1093/hmg/ddw232

Crippa, V., Sau, D., Rusmini, P., Boncoraglio, A., Onesto, E., Bolzoni, E., et al. (2010). The small heat shock protein B8 (HspB8) promotes autophagic removal of misfolded proteins involved in amyotrophic lateral sclerosis (ALS). *Hum. Mol. Genet.* 19, 3440–3456. doi: 10.1093/hmg/ddq257

Dadon-Nachum, M., Melamed, E., and Offen, D. (2011). The "dying-back" phenomenon of motor neurons in ALS. *J. Mol. Neurosci.* 43, 470–477. doi: 10.1007/s12031-010-9467-1

Dalla Bella, E., Lombardi, R., Porretta-Serapiglia, C., Ciano, C., Gellera, C., Pensato, V., et al. (2016). Amyotrophic lateral sclerosis causes small fiber pathology. *Eur. J. Neurol.* 23, 416–420. doi: 10.1111/ene.12936

D'Amico, E., Factor-Litvak, P., Santella, R. M., and Mitsumoto, H. (2013). Clinical perspective on oxidative stress in sporadic amyotrophic lateral sclerosis. *Free Radic. Biol. Med.* 65, 509–527. doi: 10.1016/j.freeradbiomed.2013.06.029

Daoud, H., Zhou, S., Noreau, A., Sabbagh, M., Belzil, V., Dionne-Laporte, A., et al. (2012). Exome sequencing reveals SPG11 mutations causing juvenile ALS. *Neurobiol. Aging* 33:839.e5-9. doi: 10.1016/j.neurobiolaging.2011.11.012

Davenport, R. J., Swingler, R. J., Chancellor, A. M., and Warlow, C. P. (1996). Avoiding false positive diagnoses of motor neuron disease: lessons from the Scottish motor neuron disease register. *J. Neurol. Neurosurg. Psychiatry* 60, 147–151. doi: 10.1136/jnnp.60.2.147

de Brito, O. M., and Scorrano, L. (2008). Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* 456, 605–610. doi: 10.1038/nature07534

De Jonghe, P., Auer-Grumbach, M., Irobi, J., Wagner, K., Plecko, B., Kennerson, M., et al. (2002). Autosomal dominant juvenile amyotrophic lateral sclerosis and distal hereditary motor neuronopathy with pyramidal tract signs: synonyms for the same disorder? *Brain* 125, 1320–1325. doi: 10.1093/brain/awf127

De Vos, K. J., Morotz, G. M., Stoica, R., Tudor, E. L., Lau, K. F., Ackerley, S., et al. (2012). VAPB interacts with the mitochondrial protein PTPIP51 to regulate calcium homeostasis. *Hum. Mol. Genet.* 21, 1299–1311. doi: 10.1093/hmg/ddr559

DeJesus-Hernandez, M., Mackenzie, I. R., Boeve, B. F., Boxer, A. L., Baker, M., Rutherford, N. J., et al. (2011). Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 72, 245–256. doi: 10.1016/j.neuron.2011.09.011

Del Bo, R., Moggio, M., Rango, M., Bonato, S., D'angelo, M. G., Ghezzi, S., et al. (2008). Mutated mitofusin 2 presents with intrafamilial variability and brain mitochondrial dysfunction. *Neurology* 71, 1959–1966. doi: 10.1212/01.wnl.0000327095.32005.a4

DeLaBarre, B., Christianson, J. C., Kopito, R. R., and Brunger, A. T. (2006). Central pore residues mediate the p97/VCP activity required for ERAD. *Mol. Cell* 22, 451–462. doi: 10.1016/j.molcel.2006.03.036

Deng, H. X., Chen, W., Hong, S. T., Boycott, K. M., Gorrie, G. H., Siddique, N., et al. (2011). Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. *Nature* 477, 211–215. doi: 10.1038/nature10353

Dengler, R., Konstanzer, A., Kuther, G., Hesse, S., Wolf, W., and Struppler, A. (1990). Amyotrophic lateral sclerosis: macro-EMG and twitch forces of single motor units. *Muscle Nerve* 13, 545–550. doi: 10.1002/mus.880130612

Devigili, G., Uceyler, N., Beck, M., Reiners, K., Stoll, G., Toyka, K. V., et al. (2011). Vasculitis-like neuropathy in amyotrophic lateral sclerosis unresponsive to treatment. *Acta Neuropathol.* 122, 343–352. doi: 10.1007/s00401-011-0837-8

Di Trapani, G., David, P., La Cara, A., Tonali, P., and Laurienzo, P. (1987). Light and ultrastructural studies in sural biopsies of the pseudopolyneuropathic form of ALS. *Adv. Exp. Med. Biol.* 209, 111–119. doi: 10.1007/978-1-4684-5302-7_18

Duquette, A., Roddier, K., Mcnabb-Baltar, J., Gosselin, I., St-Denis, A., Dicaire, M. J., et al. (2005). Mutations in senataxin responsible for Quebec cluster of ataxia with neuropathy. *Ann. Neurol.* 57, 408–414. doi: 10.1002/ana.20408

Dyck, P. J., Stevens, J. C., Mulder, D. W., and Espinosa, R. E. (1975). Frequency of nerve fiber degeneration of peripheral motor and sensory neurons in amyotrophic lateral sclerosis. Morphometry of deep and superficial peroneal nerves. *Neurology* 25, 781–785.

d'Ydewalle, C., Krishnan, J., Chiheb, D. M., Van Damme, P., Irobi, J., Kozikowski, A. P., et al. (2011). HDAC6 inhibitors reverse axonal loss in a mouse model of mutant HSPB1-induced Charcot-Marie-Tooth disease. *Nat. Med.* 17, 968–974. doi: 10.1038/nm.2396

Engelfried, K., Vorgerd, M., Hagedorn, M., Haas, G., Gilles, J., Epplen, J. T., et al. (2006). Charcot-Marie-Tooth neuropathy type 2A: novel mutations in the mitofusin 2 gene (MFN2). *BMC Med. Genet.* 7:53. doi: 10.1186/1471-2350-7-53

England, J. D., and Asbury, A. K. (2004). Peripheral neuropathy. *Lancet* 363, 2151–2161.

Evgrafov, O. V., Mersiyanova, I., Irobi, J., Van Den Bosch, L., Dierick, I., Leung, C. L., et al. (2004). Mutant small heat-shock protein 27 causes axonal Charcot-Marie-Tooth disease and distal hereditary motor neuropathy. *Nat. Genet.* 36, 602–606. doi: 10.1038/ng1354

Fallini, C., Bassell, G. J., and Rossoll, W. (2012). The ALS disease protein TDP-43 is actively transported in motor neuron axons and regulates axon outgrowth. *Hum. Mol. Genet.* 21, 3703–3718. doi: 10.1093/hmg/dds205

Fanara, P., Banerjee, J., Hueck, R. V., Harper, M. R., Awada, M., Turner, H., et al. (2007). Stabilization of hyperdynamic microtubules is neuroprotective in amyotrophic lateral sclerosis. *J. Biol. Chem.* 282, 23465–23472. doi: 10.1074/jbc.m703434200

Farrer, M. J., Hulihan, M. M., Kachergus, J. M., Dachsel, J. C., Stoessl, A. J., Grantier, L. L., et al. (2009). DCTN1 mutations in Perry syndrome. *Nat. Genet.* 41, 163–165. doi: 10.1038/ng.293

Fecto, F., Yan, J., Vemula, S. P., Liu, E., Yang, Y., Chen, W., et al. (2011). SQSTM1 mutations in familial and sporadic amyotrophic lateral sclerosis. *Arch. Neurol.* 68, 1440–1446. doi: 10.1001/archneurol.2011.250

Feneberg, E., Oeckl, P., Steinacker, P., Verde, F., Barro, C., Van Damme, P., et al. (2018). Multicenter evaluation of neurofilaments in early symptom onset amyotrophic lateral sclerosis. *Neurology* 90, e22–e30. doi: 10.1212/WNL.0000000000004761

Ferrari, G., Grisan, E., Scarpa, F., Fazio, R., Comola, M., Quattrini, A., et al. (2014). Corneal confocal microscopy reveals trigeminal small sensory fiber neuropathy in amyotrophic lateral sclerosis. *Front. Aging Neurosci.* 6:278. doi: 10.3389/fnagi.2014.00278

Figlewicz, D. A., Krizus, A., Martinoli, M. G., Meininger, V., Dib, M., Rouleau, G. A., et al. (1994). Variants of the heavy neurofilament subunit are associated with the development of amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 3, 1757–1761. doi: 10.1093/hmg/3.10.1757

Fischer, L. R., Culver, D. G., Davis, A. A., Tennant, P., Wang, M., Coleman, M., et al. (2005). The WldS gene modestly prolongs survival in the SOD1G93A fALS mouse. *Neurobiol. Dis.* 19, 293–300. doi: 10.1016/j.nbd.2005.01.008

Fischer, L. R., Culver, D. G., Tennant, P., Davis, A. A., Wang, M., Castellano-Sanchez, A., et al. (2004). Amyotrophic lateral sclerosis is a distal axonopathy: evidence in mice and man. *Exp. Neurol.* 185, 232–240. doi: 10.1016/j.expneurol.2003.10.004

Fischer, L. R., and Glass, J. D. (2007). Axonal degeneration in motor neuron disease. *Neurodegener. Dis.* 4, 431–442. doi: 10.1159/000107704

Fischer, L. R., Igoudjil, A., Magrane, J., Li, Y., Hansen, J. M., Manfredi, G., et al. (2011). SOD1 targeted to the mitochondrial intermembrane space prevents motor neuropathy in the Sod1 knockout mouse. *Brain* 134, 196–209. doi: 10.1093/brain/awq314

Fischer, L. R., Li, Y., Asress, S. A., Jones, D. P., and Glass, J. D. (2012). Absence of SOD1 leads to oxidative stress in peripheral nerve and causes a progressive distal motor axonopathy. *Exp. Neurol.* 233, 163–171. doi: 10.1016/j.expneurol.2011.09.020

Fischer-Hayes, L. R., Brotherton, T., and Glass, J. D. (2013). Axonal degeneration in the peripheral nervous system: implications for the pathogenesis of amyotrophic lateral sclerosis. *Exp. Neurol.* 246, 6–13. doi: 10.1016/j.expneurol.2013.05.001

Fogel, B. L., Cho, E., Wahnich, A., Gao, F., Becherel, O. J., Wang, X., et al. (2014). Mutation of senataxin alters disease-specific transcriptional networks in patients with ataxia with oculomotor apraxia type 2. *Hum. Mol. Genet.* 23, 4758–4769. doi: 10.1093/hmg/ddu190

Fogel, B. L., and Perlman, S. (2006). Novel mutations in the senataxin DNA/RNA helicase domain in ataxia with oculomotor apraxia 2. *Neurology* 67, 2083–2084. doi: 10.1212/01.wnl.0000247661.19601.28

Fonknechten, N., Mavel, D., Byrne, P., Davoine, C. S., Cruaud, C., Bonsch, D., et al. (2000). Spectrum of SPG4 mutations in autosomal dominant spastic paraplegia. *Hum. Mol. Genet.* 9, 637–644. doi: 10.1093/hmg/9.4.637

Freischmidt, A., Schopflin, M., Feiler, M. S., Fleck, A. K., Ludolph, A. C., and Weishaupt, J. H. (2015). Profilin 1 with the amyotrophic lateral sclerosis associated mutation T109M displays unaltered actin binding and does not affect the actin cytoskeleton. *BMC Neurosci.* 16:77. doi: 10.1186/s12868-015-0214-y

Gaiani, A., Martinelli, I., Bello, L., Querin, G., Puthenparampil, M., Ruggero, S., et al. (2017). Diagnostic and prognostic biomarkers in amyotrophic lateral sclerosis: neurofilament light chain levels in definite subtypes of disease. *JAMA Neurol.* 74, 525–532. doi: 10.1001/jamaneurol.2016.5398

Garg, N., Park, S. B., Vucic, S., Yiannikas, C., Spies, J., Howells, J., et al. (2017). Differentiating lower motor neuron syndromes. *J. Neurol. Neurosurg. Psychiatry* 88, 474–483. doi: 10.1136/jnnp-2016-313526

Gentil, B. J., Tibshirani, M., and Durham, H. D. (2015). Neurofilament dynamics and involvement in neurological disorders. *Cell Tissue Res.* 360, 609–620. doi: 10.1007/s00441-014-2082-7

Gerevini, S., Agosta, F., Riva, N., Spinelli, E. G., Pagani, E., Caliendo, G., et al. (2016). MR imaging of brachial plexus and limb-girdle muscles in patients with amyotrophic lateral sclerosis. *Radiology* 279, 553–561. doi: 10.1148/radiol.2015150559

Geser, F., Brandmeir, N. J., Kwong, L. K., Martinez-Lage, M., Elman, L., Mccluskey, L., et al. (2008). Evidence of multisystem disorder in whole-brain map of pathological TDP-43 in amyotrophic lateral sclerosis. *Arch. Neurol.* 65, 636–641. doi: 10.1001/archneur.65.5.636

Gibbs, K. L., Kalmar, B., Rhymes, E. R., Fellows, A. D., Ahmed, M., Whiting, P., et al. (2018). Inhibiting p38 MAPK alpha rescues axonal retrograde transport defects in a mouse model of ALS. *Cell Death Dis.* 9:596.

Gilley, J., Ribchester, R. R., and Coleman, M. P. (2017). Sarm1 deletion, but not Wld(S), confers lifelong rescue in a mouse model of severe axonopathy. *Cell Rep.* 21, 10–16. doi: 10.1016/j.celrep.2017.09.027

Giorgi, C., Missiroli, S., Patergnani, S., Duszynski, J., Wieckowski, M. R., and Pinton, P. (2015). Mitochondria-associated membranes: composition, molecular mechanisms, and physiopathological implications. *Antioxid. Redox Signal.* 22, 995–1019. doi: 10.1089/ars.2014.6223

Gitcho, M. A., Baloh, R. H., Chakraverty, S., Mayo, K., Norton, J. B., Levitch, D., et al. (2008). TDP-43 A315T mutation in familial motor neuron disease. *Ann. Neurol.* 63, 535–538.

Gonzalez, M. A., Feely, S. M., Speziani, F., Strickland, A. V., Danzi, M., Bacon, C., et al. (2014). A novel mutation in VCP causes Charcot-Marie-Tooth Type 2 disease. *Brain* 137, 2897–2902. doi: 10.1093/brain/awu224

Gould, T. W., Buss, R. R., Vinsant, S., Prevette, D., Sun, W., Knudson, C. M., et al. (2006). Complete dissociation of motor neuron death from motor dysfunction by Bax deletion in a mouse model of ALS. *J. Neurosci.* 26, 8774–8786. doi: 10.1523/jneurosci.2315-06.2006

Gubbay, S. S., Kahana, E., Zilber, N., Cooper, G., Pintov, S., and Leibowitz, Y. (1985). Amyotrophic lateral sclerosis. A study of its presentation and prognosis. *J. Neurol.* 232, 295–300. doi: 10.1007/bf00313868

Guo, W., Naujock, M., Fumagalli, L., Vandoorne, T., Baatsen, P., Boon, R., et al. (2017). HDAC6 inhibition reverses axonal transport defects in motor neurons derived from FUS-ALS patients. *Nat. Commun.* 8:861. doi: 10.1038/s41467-017-00911-y

Guo, Y. S., Wu, D. X., Wu, H. R., Wu, S. Y., Yang, C., Li, B., et al. (2009). Sensory involvement in the SOD1-G93A mouse model of amyotrophic lateral sclerosis. *Exp. Mol. Med.* 41, 140–150.

Hammad, M., Silva, A., Glass, J., Sladky, J. T., and Benatar, M. (2007). Clinical, electrophysiologic, and pathologic evidence for sensory abnormalities in ALS. *Neurology* 69, 2236–2242. doi: 10.1212/01.wnl.0000286948.99150.16

Harms, M. B., Ori-Mckenney, K. M., Scoto, M., Tuck, E. P., Bell, S., Ma, D., et al. (2012). Mutations in the tail domain of DYNC1H1 cause dominant spinal muscular atrophy. *Neurology* 78, 1714–1720. doi: 10.1212/WNL.0b013e3182556c05

Heads, T., Pollock, M., Robertson, A., Sutherland, W. H., and Allpress, S. (1991). Sensory nerve pathology in amyotrophic lateral sclerosis. *Acta Neuropathol.* 82, 316–320.

Hegedus, J., Putman, C. T., and Gordon, T. (2007). Time course of preferential motor unit loss in the SOD1G93A mouse model of amyotrophic

lateral sclerosis. *Neurobiol. Dis.* 28, 154–164. doi: 10.1016/j.nbd.2007.07.003

Hehr, U., Bauer, P., Winner, B., Schule, R., Olmez, A., Koehler, W., et al. (2007). Long-term course and mutational spectrum of spatacsin-linked spastic paraplegia. *Ann. Neurol.* 62, 656–665. doi: 10.1002/ana.21310

Helferich, A. M., Brockmann, S. J., Reinders, J., Deshpande, D., Holzmann, K., Brenner, D., et al. (2018). Dysregulation of a novel miR-1825/TBCB/TUBA4A pathway in sporadic and familial ALS. *Cell Mol. Life Sci.* 75, 4301–4319. doi: 10.1007/s00018-018-2873-1

Henty-Ridilla, J. L., Juanes, M. A., and Goode, B. L. (2017). Profilin directly promotes microtubule growth through residues mutated in amyotrophic lateral sclerosis. *Curr. Biol.* 27, 3535.e4–3543.e4. doi: 10.1016/j.cub.2017.10.002

Herdewyn, S., Cirillo, C., Van Den Bosch, L., Robberecht, W., Vanden Berghe, P., and Van Damme, P. (2014). Prevention of intestinal obstruction reveals progressive neurodegeneration in mutant TDP-43 (A315T) mice. *Mol. Neurodegener.* 9:24. doi: 10.1186/1750-1326-9-24

Hirokawa, N., Noda, Y., Tanaka, Y., and Niwa, S. (2009). Kinesin superfamily motor proteins and intracellular transport. *Nat. Rev. Mol. Cell Biol.* 10, 682–696. doi: 10.1038/nrm2774

Hoffman, P. N., Cleveland, D. W., Griffin, J. W., Landes, P. W., Cowan, N. J., and Price, D. L. (1987). Neurofilament gene expression: a major determinant of axonal caliber. *Proc. Natl. Acad. Sci. U.S.A.* 84, 3472–3476. doi: 10.1073/pnas.84.10.3472

Huang, C., Xia, P. Y., and Zhou, H. (2010). Sustained expression of TDP-43 and FUS in motor neurons in rodent's lifetime. *Int. J. Biol. Sci.* 6, 396–406. doi: 10.7150/ijbs.6.396

Huang, C., Zhou, H., Tong, J., Chen, H., Liu, Y. J., Wang, D., et al. (2011). FUS transgenic rats develop the phenotypes of amyotrophic lateral sclerosis and frontotemporal lobar degeneration. *PLoS Genet.* 7:e1002011. doi: 10.1371/journal.pgen.1002011

Iapadre, G., Morana, G., Vari, M. S., Pinto, F., Lanteri, P., Tessa, A., et al. (2018). A novel homozygous MFN2 mutation associated with severe and atypical CMT2 phenotype. *Eur. J. Paediatr. Neurol.* 22, 563–567. doi: 10.1016/j.ejpn.2017.12.020

Irobi, J., Almeida-Souza, L., Asselbergh, B., De Winter, V., Goethals, S., Dierick, I., et al. (2010). Mutant HSPB8 causes motor neuron-specific neurite degeneration. *Hum. Mol. Genet.* 19, 3254–3265. doi: 10.1093/hmg/ddq234

Irobi, J., Van Impe, K., Seeman, P., Jordanova, A., Dierick, I., Verpoorten, N., et al. (2004). Hot-spot residue in small heat-shock protein 22 causes distal motor neuropathy. *Nat. Genet.* 36, 597–601. doi: 10.1038/ng1328

Isaacs, J. D., Dean, A. F., Shaw, C. E., Al-Chalabi, A., Mills, K. R., and Leigh, P. N. (2007). Amyotrophic lateral sclerosis with sensory neuropathy: part of a multisystem disorder? *J. Neurol. Neurosurg. Psychiatry* 78, 750–753. doi: 10.1136/jnnp.2006.098798

Isak, B., Pugdahl, K., Karlsson, P., Tankisi, H., Finnerup, N. B., Furtula, J., et al. (2017). Quantitative sensory testing and structural assessment of sensory nerve fibres in amyotrophic lateral sclerosis. *J. Neurol. Sci.* 373, 329–334. doi: 10.1016/j.jns.2017.01.005

Isak, B., Tankisi, H., Johnsen, B., Pugdahl, K., Torvin, M. A., Finnerup, N. B., et al. (2016). Involvement of distal sensory nerves in amyotrophic lateral sclerosis. *Muscle Nerve* 54, 1086–1092. doi: 10.1002/mus.25157

Iskender, C., Kartal, E., Akcimen, F., Kocoglu, C., Ozoguz, A., Kotan, D., et al. (2015). Turkish families with juvenile motor neuron disease broaden the phenotypic spectrum of SPG11. *Neurol. Genet.* 1:e25. doi: 10.1212/NXG.0000000000000025

Jacquier, A., Delorme, C., Belotti, E., Juntas-Morales, R., Sole, G., Dubourg, O., et al. (2017). Cryptic amyloidogenic elements in mutant NEFH causing Charcot-Marie-Tooth 2 trigger aggresome formation and neuronal death. *Acta Neuropathol. Commun.* 5:55. doi: 10.1186/s40478-017-0457-1

Jaiswal, M. K. (2013). Calcium, mitochondria, and the pathogenesis of ALS: the good, the bad, and the ugly. *Front. Cell Neurosci.* 7:199. doi: 10.3389/fncel.2013.00199

Jerath, N. U., Crockett, C. D., Moore, S. A., Shy, M. E., Weihl, C. C., Chou, T. F., et al. (2015). Rare Manifestation of a c.290 C > T, p.Gly97Glu VCP Mutation. *Case Rep. Genet.* 2015:239167. doi: 10.1155/2015/239167

Johnson, J. O., Mandrioli, J., Benatar, M., Abramzon, Y., Van Deerlin, V. M., Trojanowski, J. Q., et al. (2010). Exome sequencing reveals VCP mutations as a cause of familial ALS. *Neuron* 68, 857–864. doi: 10.1016/j.neuron.2010.11.036

Jordanova, A., De Jonghe, P., Boerkoel, C. F., Takashima, H., De Vriendt, E., Ceuterick, C., et al. (2003). Mutations in the neurofilament light chain gene (NEFL) cause early onset severe Charcot-Marie-Tooth disease. *Brain* 126, 590–597. doi: 10.1093/brain/awg059

Ju, J. S., Fuentealba, R. A., Miller, S. E., Jackson, E., Piwnica-Worms, D., Baloh, R. H., et al. (2009). Valosin-containing protein (VCP) is required for autophagy and is disrupted in VCP disease. *J. Cell Biol.* 187, 875–888. doi: 10.1083/jcb.200908115

Kabashi, E., Valdmanis, P. N., Dion, P., Spiegelman, D., Mcconkey, B. J., Vande Velde, C., et al. (2008). TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nat. Genet.* 40, 572–574. doi: 10.1038/ng.132

Kang, J., and Rivest, S. (2007). MyD88-deficient bone marrow cells accelerate onset and reduce survival in a mouse model of amyotrophic lateral sclerosis. *J. Cell Biol.* 179, 1219–1230. doi: 10.1083/jcb.200705046

Kato, S. (2008). Amyotrophic lateral sclerosis models and human neuropathology: similarities and differences. *Acta Neuropathol.* 115, 97–114. doi: 10.1007/s00401-007-0308-4

Kawahara, Y., and Mieda-Sato, A. (2012). TDP-43 promotes microRNA biogenesis as a component of the Drosha and Dicer complexes. *Proc. Natl. Acad. Sci. U.S.A.* 109, 3347–3352. doi: 10.1073/pnas.1112427109

Kawamura, Y., Dyck, P. J., Shimono, M., Okazaki, H., Tateishi, J., and Doi, H. (1981). Morphometric comparison of the vulnerability of peripheral motor and sensory neurons in amyotrophic lateral sclerosis. *J. Neuropathol. Exp. Neurol.* 40, 667–675. doi: 10.1097/00005072-198111000-00008

Kevenaar, J. T., and Hoogenraad, C. C. (2015). The axonal cytoskeleton: from organization to function. *Front. Mol. Neurosci.* 8:44. doi: 10.3389/fnmol.2015.00044

Kim, E. K., and Choi, E. J. (2010). Pathological roles of MAPK signaling pathways in human diseases. *Biochim. Biophys. Acta* 1802, 396–405. doi: 10.1016/j.bbadis.2009.12.009

Kim, H. J., Hong, Y. B., Park, J. M., Choi, Y. R., Kim, Y. J., Yoon, B. R., et al. (2013). Mutations in the PLEKHG5 gene is relevant with autosomal recessive intermediate Charcot-Marie-Tooth disease. *Orphanet J. Rare Dis.* 8:104. doi: 10.1186/1750-1172-8-104

Kim, H. J., Nagano, Y., Choi, S. J., Park, S. Y., Kim, H., Yao, T. P., et al. (2015). HDAC6 maintains mitochondrial connectivity under hypoxic stress by suppressing MARCH5/MITOL dependent MFN2 degradation. *Biochem. Biophys. Res. Commun.* 464, 1235–1240. doi: 10.1016/j.bbrc.2015.07.111

King, A. E., Blizzard, C. A., Southam, K. A., Vickers, J. C., and Dickson, T. C. (2012). Degeneration of axons in spinal white matter in G93A mSOD1 mouse characterized by NFL and alpha-internexin immunoreactivity. *Brain Res.* 1465, 90–100. doi: 10.1016/j.brainres.2012.05.018

Kolb, S. J., Snyder, P. J., Poi, E. J., Renard, E. A., Bartlett, A., Gu, S., et al. (2010). Mutant small heat shock protein B3 causes motor neuropathy: utility of a candidate gene approach. *Neurology* 74, 502–506. doi: 10.1212/WNL.0b013e3181cef84a

Kraft, A. D., Resch, J. M., Johnson, D. A., and Johnson, J. A. (2007). Activation of the Nrf2–ARE pathway in muscle and spinal cord during ALS-like pathology in mice expressing mutant SOD1. *Exp. Neurol.* 207, 107–117. doi: 10.1016/j.expneurol.2007.05.026

Kruger, S., Battke, F., Sprecher, A., Munz, M., Synofzik, M., Schols, L., et al. (2016). Rare variants in neurodegeneration associated genes revealed by targeted panel sequencing in a German ALS cohort. *Front. Mol. Neurosci.* 9:92. doi: 10.3389/fnmol.2016.00092

Kwiatkowski, T. J. Jr., Bosco, D. A., Leclerc, A. L., Tamrazian, E., Vanderburg, C. R., Russ, C., et al. (2009). Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* 323, 1205–1208. doi: 10.1126/science.1166066

Kwinter, D. M., Lo, K., Mafi, P., and Silverman, M. A. (2009). Dynactin regulates bidirectional transport of dense-core vesicles in the axon and dendrites of cultured hippocampal neurons. *Neuroscience* 162, 1001–1010. doi: 10.1016/j.neuroscience.2009.05.038

Lanfranconi, F., Ferri, A., Corna, G., Bonazzi, R., Lunetta, C., Silani, V., et al. (2017). Inefficient skeletal muscle oxidative function flanks impaired motor neuron recruitment in amyotrophic lateral sclerosis during exercise. *Sci. Rep.* 7:2951. doi: 10.1038/s41598-017-02811-z

Laurin, N., Brown, J. P., Morissette, J., and Raymond, V. (2002). Recurrent mutation of the gene encoding sequestosome 1 (SQSTM1/p62) in Paget disease of bone. *Am. J. Hum. Genet.* 70, 1582–1588. doi: 10.1086/340731

Leal, S. S., and Gomes, C. M. (2015). Calcium dysregulation links ALS defective proteins and motor neuron selective vulnerability. *Front. Cell. Neurosci.* 9:225. doi: 10.3389/fncel.2015.00225

Leal-Lasarte, M. M., Franco, J. M., Labrador-Garrido, A., Pozo, D., and Roodveldt, C. (2017). Extracellular TDP-43 aggregates target MAPK/MAK/MRK overlapping kinase (MOK) and trigger caspase-3/IL-18 signaling in microglia. *FASEB J.* 31, 2797–2816. doi: 10.1096/fj.201601163R

Leigh, P. N., and Swash, M. (1991). Cytoskeletal pathology in motor neuron diseases. *Adv. Neurol.* 56, 115–124.

Li, X., Hu, Z., Liu, L., Xie, Y., Zhan, Y., Zi, X., et al. (2015). A SIGMAR1 splice-site mutation causes distal hereditary motor neuropathy. *Neurology* 84, 2430–2437. doi: 10.1212/WNL.0000000000001680

Li, Y., Guo, Y., Wang, X., Yu, X., Duan, W., Hong, K., et al. (2015). Trehalose decreases mutant SOD1 expression and alleviates motor deficiency in early but not end-stage amyotrophic lateral sclerosis in a SOD1-G93A mouse model. *Neuroscience* 298, 12–25. doi: 10.1016/j.neuroscience.2015.03.061

Lieberburg, I., Spinner, N., Snyder, S., Anderson, J., Goldgaber, D., Smulowitz, M., et al. (1989). Cloning of a cDNA encoding the rat high molecular weight neurofilament peptide (NF-H): developmental and tissue expression in the rat, and mapping of its human homologue to chromosomes 1 and 22. *Proc. Natl. Acad. Sci. U.S.A.* 86, 2463–2467. doi: 10.1073/pnas.86.7.2463

Lin, M. J., Cheng, C. W., and Shen, C. K. (2011). Neuronal function and dysfunction of *Drosophila* dTDP. *PLoS One* 6:e20371. doi: 10.1371/journal.pone.0020371

Ling, S. C., Polymenidou, M., and Cleveland, D. W. (2013). Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. *Neuron* 79, 416–438. doi: 10.1016/j.neuron.2013.07.033

Liu, X., Yang, L., Tang, L., Chen, L., and Fan, D. (2017). DCTN1 gene analysis in Chinese patients with sporadic amyotrophic lateral sclerosis. *PLoS One* 12:e0182572. doi: 10.1371/journal.pone.0182572

Liu, Z. J., Li, H. F., Tan, G. H., Tao, Q. Q., Ni, W., Cheng, X. W., et al. (2014). Identify mutation in amyotrophic lateral sclerosis cases using HaloPlex target enrichment system. *Neurobiol Aging* 35, 2881.e11-2881.e15. doi: 10.1016/j.neurobiolaging.2014.07.003

Lobsiger, C. S., Boillee, S., Mcalonis-Downes, M., Khan, A. M., Feltri, M. L., Yamanaka, K., et al. (2009). Schwann cells expressing dismutase active mutant SOD1 unexpectedly slow disease progression in ALS mice. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4465–4470. doi: 10.1073/pnas.0813339106

Lopez, E., Casasnovas, C., Gimenez, J., Santamaria, R., Terrazas, J. M., and Volpini, V. (2015). Identification of two novel KIF5A mutations in hereditary spastic paraplegia associated with mild peripheral neuropathy. *J. Neurol. Sci.* 358, 422–427. doi: 10.1016/j.jns.2015.08.1529

Ludolph, A., Drory, V., Hardiman, O., Nakano, I., Ravits, J., Robberecht, W., et al. (2015). A revision of the El Escorial criteria - 2015. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 16, 291–292. doi: 10.3109/21678421.2015.1049183

Luigetti, M., Conte, A., Del Grande, A., Bisogni, G., Romano, A., and Sabatelli, M. (2012). Sural nerve pathology in ALS patients: a single-centre experience. *Neurol. Sci.* 33, 1095–1099. doi: 10.1007/s10072-011-0909-5

Magré, J., Delépine, M., Khallouf, E., Gedde-Dahl, T. Jr., Van Maldergem, L., Sobel, E., Papp, J. (2001). Identification of the gene altered in Berardinelli-Seip congenital lipodystrophy on chromosome 11q13. *Nat. Genet.* 28, 365–370. doi: 10.1038/ng585

Mancuso, R., Martinez-Muriana, A., Leiva, T., Gregorio, D., Ariza, L., Morell, M., et al. (2016). Neuregulin-1 promotes functional improvement by enhancing collateral sprouting in SOD1(G93A) ALS mice and after partial muscle denervation. *Neurobiol. Dis.* 95, 168–178. doi: 10.1016/j.nbd.2016.07.023

Mancuso, R., Olivan, S., Rando, A., Casas, C., Osta, R., and Navarro, X. (2012). Sigma-1R agonist improves motor function and motoneuron survival in ALS mice. *Neurotherapeutics* 9, 814–826. doi: 10.1007/s13311-012-0140-y

Marchesi, C., Ciano, C., Salsano, E., Nanetti, L., Milani, M., Gellera, C., et al. (2011). Co-occurrence of amyotrophic lateral sclerosis and Charcot-Marie-Tooth disease type 2A in a patient with a novel mutation in the mitofusin-2 gene. *Neuromuscul. Disord.* 21, 129–131. doi: 10.1016/j.nmd.2010.09.009

Martini, R., Berciano, J., and Van Broeckhoven, C. (2000). 5th Workshop of the European CMT Consortium, 69th ENMC International Workshop: therapeutic approaches in CMT neuropathies and related disorders 23-25 April 1999, Soestduinen, The Netherlands. *Neuromuscul. Disord.* 10, 69–74. doi: 10.1016/s0960-8966(99)00095-4

Maruyama, H., Morino, H., Ito, H., Izumi, Y., Kato, H., Watanabe, Y., et al. (2010). Mutations of optineurin in amyotrophic lateral sclerosis. *Nature* 465, 223–226. doi: 10.1038/nature08971

Matamala, J. M., Howells, J., Dharmadasa, T., Huynh, W., Park, S. B., Burke, D., et al. (2018). Excitability of sensory axons in amyotrophic lateral sclerosis. *Clin. Neurophysiol.* 129, 1472–1478. doi: 10.1016/j.clinph.2018.03.014

Matsukawa, K., Hashimoto, T., Matsumoto, T., Ihara, R., Chihara, T., Miura, M., et al. (2016). Familial amyotrophic lateral sclerosis-linked mutations in profilin 1 exacerbate TDP-43-induced degeneration in the retina of *Drosophila melanogaster* through an increase in the cytoplasmic localization of TDP-43. *J. Biol. Chem.* 291, 23464–23476. doi: 10.1074/jbc.m116.729152

Mavlyutov, T. A., Epstein, M. L., Andersen, K. A., Ziskind-Conhaim, L., and Ruoho, A. E. (2010). The sigma-1 receptor is enriched in postsynaptic sites of C-terminals in mouse motoneurons. An anatomical and behavioral study. *Neuroscience* 167, 247–255. doi: 10.1016/j.neuroscience.2010.02.022

Maystadt, I., Rezsohazy, R., Barkats, M., Duque, S., Vannuffel, P., Remacle, S., et al. (2007). The nuclear factor kappaB-activator gene PLEKHG5 is mutated in a form of autosomal recessive lower motor neuron disease with childhood onset. *Am. J. Hum. Genet.* 81, 67–76. doi: 10.1086/518900

McCluskey, L., Vandriel, S., Elman, L., Van Deerlin, V. M., Powers, J., Boller, A., et al. (2014). ALS-Plus syndrome: non-pyramidal features in a large ALS cohort. *J. Neurol. Sci.* 345, 118–124. doi: 10.1016/j.jns.2014.07.022

Menon, P., Kiernan, M. C., and Vucic, S. (2014). Cortical dysfunction underlies the development of the split-hand in amyotrophic lateral sclerosis. *PLoS One* 9:e87124. doi: 10.1371/journal.pone.0087124

Mersiyanova, I. V., Perepelov, A. V., Polyakov, A. V., Sitnikov, V. F., Dadali, E. L., Oparin, R. B., et al. (2000). A new variant of Charcot-Marie-Tooth disease type 2 is probably the result of a mutation in the neurofilament-light gene. *Am. J. Hum. Genet.* 67, 37–46. doi: 10.1086/302962

Meyer, T., Schwan, A., Dullinger, J. S., Brocke, J., Hoffmann, K. T., Nolte, C. H., et al. (2005). Early-onset ALS with long-term survival associated with spastin gene mutation. *Neurology* 65, 141–143. doi: 10.1212/01.wnl.0000167130.31618.0a

Miller, J. W., Smith, B. N., Topp, S. D., Al-Chalabi, A., Shaw, C. E., and Vance, C. (2012). Mutation analysis of VCP in British familial and sporadic amyotrophic lateral sclerosis patients. *Neurobiol. Aging* 33:2721.e1-2. doi: 10.1016/j.neurobiolaging.2012.06.003

Modol-Caballero, G., Santos, D., Navarro, X., and Herrando-Grabulosa, M. (2017). Neuregulin 1 reduces motoneuron cell death and promotes neurite growth in an in vitro model of motoneuron degeneration. *Front. Cell. Neurosci.* 11:431. doi: 10.3389/fncel.2017.00431

Moisse, K., Volkening, K., Leystra-Lantz, C., Welch, I., Hill, T., and Strong, M. J. (2009). Divergent patterns of cytosolic TDP-43 and neuronal progranulin expression following axotomy: implications for TDP-43 in the physiological response to neuronal injury. *Brain Res.* 1249, 202–211. doi: 10.1016/j.brainres.2008.10.021

Mondelli, M., Rossi, A., Passero, S., and Guazzi, G. C. (1993). Involvement of peripheral sensory fibers in amyotrophic lateral sclerosis: electrophysiological study of 64 cases. *Muscle Nerve* 16, 166–172. doi: 10.1002/mus.880160208

Montecchiani, C., Pedace, L., Lo Giudice, T., Casella, A., Mearini, M., Gaudiello, F., et al. (2016). ALS5/SPG11/KIAA1840 mutations cause autosomal recessive axonal Charcot-Marie-Tooth disease. *Brain* 139, 73–85. doi: 10.1093/brain/awv320

Moreira, M. C., Klur, S., Watanabe, M., Nemeth, A. H., Le Ber, I., Moniz, J. C., et al. (2004). Senataxin, the ortholog of a yeast RNA helicase, is mutant in ataxia-ocular apraxia 2. *Nat. Genet.* 36, 225–227. doi: 10.1038/ng1303

Morrison, B. M., Shu, I. W., Wilcox, A. L., Gordon, J. W., and Morrison, J. H. (2000). Early and selective pathology of light chain neurofilament in the spinal cord and sciatic nerve of G86R mutant superoxide dismutase transgenic mice. *Exp. Neurol.* 165, 207–220. doi: 10.1006/exnr.2000.7457

Muglia, M., Zappia, M., Timmerman, V., Valentino, P., Gabriele, A. L., Conforti, F. L., et al. (2001). Clinical and genetic study of a large Charcot-Marie-Tooth type 2A family from southern Italy. *Neurology* 56, 100–103. doi: 10.1212/wnl.56.1.100

Muller, H. P., Agosta, F., Riva, N., Spinelli, E. G., Comi, G., Ludolph, A. C., et al. (2018). Fast progressive lower motor neuron disease is an ALS variant: a two-centre tract of interest-based MRI data analysis. *Neuroimage Clin.* 17, 145–152. doi: 10.1016/j.nicl.2017.10.008

Munch, C., Rosenbohm, A., Sperfeld, A. D., Uttner, I., Reske, S., Krause, B. J., et al. (2005). Heterozygous R1101K mutation of the DCTN1 gene in a family with ALS and FTD. *Ann. Neurol.* 58, 777–780. doi: 10.1002/ana.20631

Munch, C., Sedlmeier, R., Meyer, T., Homberg, V., Sperfeld, A. D., Kurt, A., et al. (2004). Point mutations of the p150 subunit of dynactin (DCTN1) gene in ALS. *Neurology* 63, 724–726. doi: 10.1212/01.wnl.0000134608.83927.b1

Nam, D. E., Jung, S. C., Yoo, D. H., Choi, S. S., Seo, S. Y., Kim, G. H., et al. (2017). Axonal Charcot-Marie-Tooth neuropathy concurrent with distal and proximal weakness by translational elongation of the 3' UTR in NEFH. *J. Peripher. Nerv. Syst.* 22, 200–207. doi: 10.1111/jns.12223

Nardo, G., Trolese, M. C., De Vito, G., Cecchi, R., Riva, N., Dina, G., et al. (2016). Immune response in peripheral axons delays disease progression in SOD1(G93A) mice. *J. Neuroinflamm.* 13:261.

Nardo, G., Trolese, M. C., Verderio, M., Mariani, A., De Paola, M., Riva, N., et al. (2018). Counteracting roles of MHCI and CD8(+) T cells in the peripheral and central nervous system of ALS SOD1(G93A) mice. *Mol. Neurodegener.* 13:42. doi: 10.1186/s13024-018-0271-7

Naumenko, N., Pollari, E., Kurronen, A., Giniatullina, R., Shakirzyanova, A., Magga, J., et al. (2011). Gender-specific mechanism of synaptic impairment and its prevention by GCSF in a mouse model of ALS. *Front. Cell. Neurosci.* 5:26. doi: 10.3389/fncel.2011.00026

Nguyen, L., Lucke-Wold, B. P., Mookerjee, S. A., Cavendish, J. Z., Robson, M. J., Scandinaro, A. L., et al. (2015). Role of sigma-1 receptors in neurodegenerative diseases. *J. Pharmacol. Sci.* 127, 17–29.

Nicholson, G., Lenk, G. M., Reddel, S. W., Grant, A. E., Towne, C. F., Ferguson, C. J., et al. (2011). Distinctive genetic and clinical features of CMT4J: a severe neuropathy caused by mutations in the PI(3,5)P(2) phosphatase FIG4. *Brain* 134, 1959–1971. doi: 10.1093/brain/awr148

Nicholson, G. A., Magdelaine, C., Zhu, D., Grew, S., Ryan, M. M., Sturtz, F., et al. (2008). Severe early-onset axonal neuropathy with homozygous and compound heterozygous MFN2 mutations. *Neurology* 70, 1678–1681. doi: 10.1212/01.wnl.0000311275.89032.22

Nicolas, A., Kenna, K. P., Renton, A. E., Ticozzi, N., Faghri, F., Chia, R., et al. (2018). Genome-wide analyses identify KIF5A as a novel ALS gene. *Neuron* 97, 1268.e6–1283.e6. doi: 10.1016/j.neuron.2018.02.027

Nishihira, Y., Tan, C. -F., Onodera, O., Toyoshima, Y., Yamada, M., Morita, T., et al. (2008). Sporadic amyotrophic lateral sclerosis: two pathological patterns shown by analysis of distribution of TDP-43-immunoreactive neuronal and glial cytoplasmic inclusions. *Acta Neuropathol.* 116:169. doi: 10.1007/s00401-008-0385-z

Nolano, M., Provitera, V., Manganelli, F., Iodice, R., Caporaso, G., Stancanelli, A., et al. (2017). Non-motor involvement in amyotrophic lateral sclerosis: new insight from nerve and vessel analysis in skin biopsy. *Neuropathol. Appl. Neurobiol.* 43, 119–132. doi: 10.1111/nan.12332

Orlacchio, A., Babalini, C., Borreca, A., Patrono, C., Massa, R., Basaran, S., et al. (2010). SPATACSIN mutations cause autosomal recessive juvenile amyotrophic lateral sclerosis. *Brain* 133, 591–598. doi: 10.1093/brain/awp325

Osmanovic, A., Rangnau, I., Kosfeld, A., Abdulla, S., Janssen, C., Auber, B., et al. (2017). FIG4 variants in central European patients with amyotrophic lateral sclerosis: a whole-exome and targeted sequencing study. *Eur. J. Hum. Genet.* 25, 324–331. doi: 10.1038/ejhg.2016.186

Ozoguz, A., Uyan, O., Birdal, G., Iskender, C., Kartal, E., Lahut, S., et al. (2015). The distinct genetic pattern of ALS in Turkey and novel mutations. *Neurobiol. Aging* 36, 1764.e9–1764.e18. doi: 10.1016/j.neurobiolaging.2014.12.032

Park, S. B., Kiernan, M. C., and Vucic, S. (2017). Axonal excitability in amyotrophic lateral sclerosis : axonal excitability in ALS. *Neurotherapeutics* 14, 78–90. doi: 10.1007/s13311-016-0492-9

Patten, S. A., Aggad, D., Martinez, J., Tremblay, E., Petrillo, J., Armstrong, G. A., et al. (2017). Neuroleptics as therapeutic compounds stabilizing neuromuscular transmission in amyotrophic lateral sclerosis. *JCI Insight* 2: 97152

Perez-Branguli, F., Mishra, H. K., Prots, I., Havlicek, S., Kohl, Z., Saul, D., et al. (2014). Dysfunction of spatacsin leads to axonal pathology in SPG11-linked hereditary spastic paraplegia. *Hum. Mol. Genet.* 23, 4859–4874. doi: 10.1093/hmg/ddu200

Perlson, E., Jeong, G. B., Ross, J. L., Dixit, R., Wallace, K. E., Kalb, R. G., et al. (2009). A switch in retrograde signaling from survival to stress in rapid-onset neurodegeneration. *J. Neurosci.* 29, 9903–9917. doi: 10.1523/JNEUROSCI.0813-09.2009

Peters, O. M., Lewis, E. A., Osterloh, J. M., Weiss, A., Salameh, J. S., Metterville, J., et al. (2018). Loss of Sarm1 does not suppress motor neuron degeneration in the SOD1G93A mouse model of amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 27, 3761–3771. doi: 10.1093/hmg/ddy260

Philips, T., and Rothstein, J. D. (2015). Rodent models of amyotrophic lateral sclerosis. *Curr. Protoc. Pharmacol.* 69, 67.1–67.21.

Piccione, E. A., Sletten, D. M., Staff, N. P., and Low, P. A. (2015). Autonomic system and amyotrophic lateral sclerosis. *Muscle Nerve* 51, 676–679. doi: 10.1002/mus.24457

Picher-Martel, V., Valdmanis, P. N., Gould, P. V., Julien, J. P., and Dupre, N. (2016). From animal models to human disease: a genetic approach for personalized medicine in ALS. *Acta Neuropathol. Commun.* 4:70. doi: 10.1186/s40478-016-0340-5

Polke, J. M., Laura, M., Pareyson, D., Taroni, F., Milani, M., Bergamin, G., et al. (2011). Recessive axonal Charcot-Marie-Tooth disease due to compound heterozygous mitofusin 2 mutations. *Neurology* 77, 168–173. doi: 10.1212/WNL.0b013e3182242d4d

Pozzi, S., Thammisetty, S. S., and Julien, J. P. (2018). Chronic administration of pimozide fails to attenuate motor and pathological deficits in two mouse models of amyotrophic lateral sclerosis. *Neurotherapeutics* 15, 715–727. doi: 10.1007/s13311-018-0634-3

Pugdahl, K., Fuglsang-Frederiksen, A., De Carvalho, M., Johnsen, B., Fawcett, P. R., Labarre-Vila, A., et al. (2007). Generalised sensory system abnormalities in amyotrophic lateral sclerosis: a European multicentre study. *J. Neurol. Neurosurg. Psychiatry* 78, 746–749. doi: 10.1136/jnnp.2006.098533

Pugdahl, K., Fuglsang-Frederiksen, A., Johnsen, B., De Carvalho, M., Fawcett, P. R., Labarre-Vila, A., et al. (2008). A prospective multicentre study on sural nerve action potentials in ALS. *Clin. Neurophysiol.* 119, 1106–1110. doi: 10.1016/j.clinph.2008.01.010

Puls, I., Jonnakuty, C., Lamonte, B. H., Holzbaur, E. L., Tokito, M., Mann, E., et al. (2003). Mutant dynactin in motor neuron disease. *Nat. Genet.* 33, 455–456.

Pupillo, E., Messina, P., Giussani, G., Logroscino, G., Zoccolella, S., Chio, A., et al. (2014). Physical activity and amyotrophic lateral sclerosis: a European population-based case-control study. *Ann. Neurol.* 75, 708–716. doi: 10.1002/ana.24150

Rajabally, Y. A., and Jacob, S. (2008). Chronic inflammatory demyelinating polyneuropathy-like disorder associated with amyotrophic lateral sclerosis. *Muscle Nerve* 38, 855–860. doi: 10.1002/mus.21010

Ratti, A., and Buratti, E. (2016). Physiological functions and pathobiology of TDP-43 and FUS/TLS proteins. *J. Neurochem.* 138(Suppl. 1), 95–111. doi: 10.1111/jnc.13625

Rebelo, A. P., Abrams, A. J., Cottenie, E., Horga, A., Gonzalez, M., Bis, D. M., et al. (2016). Cryptic amyloidogenic elements in the 3' UTRs of neurofilament genes trigger axonal neuropathy. *Am. J. Hum. Genet.* 98, 597–614. doi: 10.1016/j.ajhg.2016.02.022

Reid, E., Kloos, M., Ashley-Koch, A., Hughes, L., Bevan, S., Svenson, I. K., et al. (2002). A kinesin heavy chain (KIF5A) mutation in hereditary spastic paraplegia (SPG10). *Am. J. Hum. Genet.* 71, 1189–1194. doi: 10.1086/344210

Renton, A. E., Majounie, E., Waite, A., Simon-Sanchez, J., Rollinson, S., Gibbs, J. R., et al. (2011). A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 72, 257–268. doi: 10.1016/j.neuron.2011.09.010

Rezaie, T., Child, A., Hitchings, R., Brice, G., Miller, L., Coca-Prados, M., et al. (2002). Adult-onset primary open-angle glaucoma caused by mutations in optineurin. *Science* 295, 1077–1079. doi: 10.1126/science.1066901

Riva, N., Agosta, F., Lunetta, C., Filippi, M., and Quattrini, A. (2016). Recent advances in amyotrophic lateral sclerosis. *J. Neurol.* 263, 1241–1254. doi: 10.1007/s00415-016-8091-6

Riva, N., Chaabane, L., Peviani, M., Ungaro, D., Domi, T., Dina, G., et al. (2014). Defining peripheral nervous system dysfunction in the SOD-1G93A transgenic rat model of amyotrophic lateral sclerosis. *J. Neuropathol. Exp. Neurol.* 73, 658–670. doi: 10.1097/NEN.0000000000000081

Riva, N., Gallia, F., Iannaccone, S., Corbo, M., Terenghi, F., Lazzerini, A., et al. (2011a). Chronic motor axonal neuropathy. *J. Peripher. Nerv. Syst.* 16, 341–346.

Riva, N., Iannaccone, S., Corbo, M., Casellato, C., Sferrazza, B., Lazzerini, A., et al. (2011b). Motor nerve biopsy: clinical usefulness and histopathological criteria. *Ann. Neurol.* 69, 197–201. doi: 10.1002/ana.22110

Rosen, D. R., Siddique, T., Patterson, D., Figlewicz, D. A., Sapp, P., Hentati, A., et al. (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 362, 59–62.

Rossor, A. M., Kalmar, B., Greensmith, L., and Reilly, M. M. (2012). The distal hereditary motor neuropathies. *J. Neurol. Neurosurg. Psychiatry* 83, 6–14. doi: 10.1136/jnnp-2011-300952

Rotem, N., Magen, I., Ionescu, A., Gershoni-Emek, N., Altman, T., Costa, C. J., et al. (2017). ALS along the axons - expression of coding and noncoding RNA differs in axons of ALS models. *Sci. Rep.* 7:44500. doi: 10.1038/srep44500

Rouaux, C., Panteleeva, I., René, F., Gonzalez De Aguilar, J.-L., Echaniz-Laguna, A., Dupuis, L., et al. (2007). Sodium valproate exerts neuroprotective effects *In Vivo* through CREB-Binding protein-dependent mechanisms but does not improve survival in an amyotrophic lateral sclerosis mouse model. *J. Neurosci.* 27, 5535–5545. doi: 10.1523/jneurosci.1139-07.2007

Rouleau, G. A., Clark, A. W., Rooke, K., Pramatarova, A., Krizus, A., Suchowersky, O., et al. (1996). SOD1 mutation is associated with accumulation of neurofilaments in amyotrophic lateral sclerosis. *Ann. Neurol.* 39, 128–131.

Rusmini, P., Cristofani, R., Galbiati, M., Cicardi, M. E., Meroni, M., Ferrari, V., et al. (2017). The role of the heat shock protein B8 (HSPB8) in motoneuron diseases. *Front. Mol. Neurosci.* 10:176. doi: 10.3389/fnmol.2017.00176

Sabatelli, M., Marangi, G., Conte, A., Tasca, G., Zollino, M., and Lattante, S. (2016). New ALS-Related genes expand the spectrum paradigm of amyotrophic lateral sclerosis. *Brain Pathol.* 26, 266–275. doi: 10.1111/bpa.12354

Saberi, S., Stauffer, J. E., Schulte, D. J., and Ravits, J. (2015). Neuropathology of amyotrophic lateral sclerosis and its variants. *Neurol. Clin.* 33, 855–876. doi: 10.1016/j.ncl.2015.07.012

Sassone, J., Taiana, M., Lombardi, R., Porretta-Serapiglia, C., Freschi, M., Bonanno, S., et al. (2016). ALS mouse model SOD1G93A displays early pathology of sensory small fibers associated to accumulation of a neurotoxic splice variant of peripherin. *Hum. Mol. Genet.* 25, 1588–1599. doi: 10.1093/hmg/ddw035

Sawa, N., Kataoka, H., Sugie, K., Kawahara, M., Horikawa, H., Kusunoki, S., et al. (2012). Clinical analysis and outcomes of amyotrophic lateral sclerosis with demyelinating polyneuropathy. *Amyotroph. Lateral Scler.* 13, 125–131. doi: 10.3109/17482968.2011.627590

Saxena, S., Cabuy, E., and Caroni, P. (2009). A role for motoneuron subtype-selective ER stress in disease manifestations of FALS mice. *Nat. Neurosci.* 12, 627–636. doi: 10.1038/nn.2297

Schafer, M. K., Bellouze, S., Jacquier, A., Schaller, S., Richard, L., Mathis, S., et al. (2017). Sensory neuropathy in progressive motor neuronopathy (pmn) mice is associated with defects in microtubule polymerization and axonal transport. *Brain Pathol.* 27, 459–471. doi: 10.1111/bpa.12422

Schwenk, B. M., Hartmann, H., Serdaroglu, A., Schludi, M. H., Hornburg, D., Meissner, F., et al. (2016). TDP-43 loss of function inhibits endosomal trafficking and alters trophic signaling in neurons. *EMBO J.* 35, 2350–2370. doi: 10.15252/embj.201694221

Sharma, A., Lyashchenko, A. K., Lu, L., Nasrabady, S. E., Elmaleh, M., Mendelsohn, M., et al. (2016). ALS-associated mutant FUS induces selective motor neuron degeneration through toxic gain of function. *Nat. Commun.* 7:10465. doi: 10.1038/ncomms10465

Shibuya, K., Misawa, S., Arai, K., Nakata, M., Kanai, K., Yoshiyama, Y., et al. (2011). Markedly reduced axonal potassium channel expression in human sporadic amyotrophic lateral sclerosis: an immunohistochemical study. *Exp. Neurol.* 232, 149–153. doi: 10.1016/j.expneurol.2011.08.015

Skourti-Stathaki, K., Proudfoot, N. J., and Gromak, N. (2011). Human senataxin resolves RNA/DNA hybrids formed at transcriptional pause sites to promote Xrn2-dependent termination. *Mol. Cell.* 42, 794–805. doi: 10.1016/j.molcel.2011.04.026

Smith, B. N., Ticozzi, N., Fallini, C., Gkazi, A. S., Topp, S., Kenna, K. P., et al. (2014). Exome-wide rare variant analysis identifies TUBA4A mutations associated with familial ALS. *Neuron* 84, 324–331. doi: 10.1016/j.neuron.2014.09.027

Sreedharan, J., Blair, I. P., Tripathi, V. B., Hu, X., Vance, C., Rogelj, B., et al. (2008). TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* 319, 1668–1672. doi: 10.1126/science.1154584

Stalekar, M., Yin, X., Rebolj, K., Darovic, S., Troakes, C., Mayr, M., et al. (2015). Proteomic analyses reveal that loss of TDP-43 affects RNA processing and intracellular transport. *Neuroscience* 293, 157–170. doi: 10.1016/j.neuroscience.2015.02.046

Stevanin, G., Santorelli, F. M., Azzedine, H., Coutinho, P., Chomilier, J., Denora, P. S., et al. (2007). Mutations in SPG11, encoding spatacsin, are a major cause of spastic paraplegia with thin corpus callosum. *Nat. Genet.* 39, 366–372. doi: 10.1038/ng1980

Stoica, R., De Vos, K. J., Paillusson, S., Mueller, S., Sancho, R. M., Lau, K. F., et al. (2014). ER-mitochondria associations are regulated by the VAPB-PTPIP51 interaction and are disrupted by ALS/FTD-associated TDP-43. *Nat. Commun.* 5:3996. doi: 10.1038/ncomms4996

Suraweera, A., Lim, Y., Woods, R., Birrell, G. W., Nasim, T., Becherel, O. J., et al. (2009). Functional role for senataxin, defective in ataxia oculomotor apraxia type 2, in transcriptional regulation. *Hum. Mol. Genet.* 18, 3384–3396. doi: 10.1093/hmg/ddp278

Suzuki, M., Mchugh, J., Tork, C., Shelley, B., Klein, S. M., Aebischer, P., et al. (2007). GDNF secreting human neural progenitor cells protect dying motor neurons, but not their projection to muscle, in a rat model of familial ALS. *PLoS One* 2:e689. doi: 10.1371/journal.pone.0000689

Swarup, V., Audet, J. N., Phaneuf, D., Kriz, J., and Julien, J. P. (2012). Abnormal regenerative responses and impaired axonal outgrowth after nerve crush in TDP-43 transgenic mouse models of amyotrophic lateral sclerosis. *J. Neurosci.* 32, 18186–18195. doi: 10.1523/JNEUROSCI.2267-12.2012

Tanaka, Y., Nonaka, T., Suzuki, G., Kametani, F., and Hasegawa, M. (2016). Gain-of-function profilin 1 mutations linked to familial amyotrophic lateral sclerosis cause seed-dependent intracellular TDP-43 aggregation. *Hum. Mol. Genet.* 25, 1420–1433. doi: 10.1093/hmg/ddw024

Tang, B. S., Zhao, G. H., Luo, W., Xia, K., Cai, F., Pan, Q., et al. (2005). Small heat-shock protein 22 mutated in autosomal dominant Charcot-Marie-Tooth disease type 2L. *Hum. Genet.* 116, 222–224. doi: 10.1007/s00439-004-1218-3

Tradewell, M. L., Durham, H. D., Mushynski, W. E., and Gentil, B. J. (2009). Mitochondrial and axonal abnormalities precede disruption of the neurofilament network in a model of charcot-marie-tooth disease type 2E and are prevented by heat shock proteins in a mutant-specific fashion. *J. Neuropathol. Exp. Neurol.* 68, 642–652. doi: 10.1097/NEN.0b013e3181a5deeb

Traynor, B. J., Codd, M. B., Corr, B., Forde, C., Frost, E., and Hardiman, O. M. (2000). Clinical features of amyotrophic lateral sclerosis according to the El Escorial and Airlie House diagnostic criteria: a population-based study. *Arch. Neurol.* 57, 1171–1176.

Tripathi, V. B., Baskaran, P., Shaw, C. E., and Guthrie, S. (2014). Tar DNA-binding protein-43 (TDP-43) regulates axon growth in vitro and in vivo. *Neurobiol. Dis.* 65, 25–34. doi: 10.1016/j.nbd.2014.01.004

Tripolszki, K., Torok, D., Goudenege, D., Farkas, K., Sulak, A., Torok, N., et al. (2017). High-throughput sequencing revealed a novel SETX mutation in a Hungarian patient with amyotrophic lateral sclerosis. *Brain Behav.* 7:e00669. doi: 10.1002/brb3.669

Truini, A., Biasiotta, A., Onesti, E., Di Stefano, G., Ceccanti, M., La Cesa, S., et al. (2015). Small-fibre neuropathy related to bulbar and spinal-onset in patients with ALS. *J. Neurol.* 262, 1014–1018. doi: 10.1007/s00415-015-7672-0

Vance, C., Rogelj, B., Hortobagyi, T., De Vos, K. J., Nishimura, A. L., Sreedharan, J., et al. (2009). Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* 323, 1208–1211. doi: 10.1126/science.1165942

Vandermoere, F., El Yazidi-Belkoura, I., Slomianny, C., Demont, Y., Bidaux, G., Adriaenssens, E., et al. (2006). The valosin-containing protein (VCP) is a target of Akt signaling required for cell survival. *J. Biol. Chem.* 281, 14307–14313. doi: 10.1074/jbc.m510003200

Vaughan, S. K., Kemp, Z., Hatzipetros, T., Vieira, F., and Valdez, G. (2015). Degeneration of proprioceptive sensory nerve endings in mice harboring amyotrophic lateral sclerosis-causing mutations. *J. Comp. Neurol.* 523, 2477–2494. doi: 10.1002/cne.23848

Vaughan, S. K., Sutherland, N. M., Zhang, S., Hatzipetros, T., Vieira, F., and Valdez, G. (2018). The ALS-inducing factors, TDP43(A315T) and SOD1(G93A),

directly affect and sensitize sensory neurons to stress. *Sci. Rep.* 8:16582. doi: 10.1038/s41598-018-34510-8

Verheijen, M. H., Peviani, M., Hendricusdottir, R., Bell, E. M., Lammens, M., Smit, A. B., et al. (2014). Increased axonal ribosome numbers is an early event in the pathogenesis of amyotrophic lateral sclerosis. *PLoS One* 9:e87255. doi: 10.1371/journal.pone.0087255

Vieira, F. G., Ping, Q., Moreno, A. J., Kidd, J. D., Thompson, K., Jiang, B., et al. (2015). Guanabenz treatment accelerates disease in a mutant SOD1 mouse model of ALS. *PLoS One* 10:e0135570. doi: 10.1371/journal.pone.0135570

Visser, J., Van Den Berg-Vos, R. M., Franssen, H., Van Den Berg, L. H., Vogels, O. J., Wokke, J. H., et al. (2002). Mimic syndromes in sporadic cases of progressive spinal muscular atrophy. *Neurology* 58, 1593–1596. doi: 10.1212/wnl.58.11.1593

Volkening, K., Leystra-Lantz, C., Yang, W., Jaffee, H., and Strong, M. J. (2009). Tar DNA binding protein of 43 kDa (TDP-43), 14-3-3 proteins and copper/zinc superoxide dismutase (SOD1) interact to modulate NFL mRNA stability. Implications for altered RNA processing in amyotrophic lateral sclerosis (ALS). *Brain Res.* 1305, 168–182. doi: 10.1016/j.brainres.2009.09.105

Waibel, S., Neumann, M., Rosenbohm, A., Birve, A., Volk, A. E., Weishaupt, J. H., et al. (2013). Truncating mutations in FUS/TLS give rise to a more aggressive ALS-phenotype than missense mutations: a clinico-genetic study in Germany. *Eur. J. Neurol.* 20, 540–546. doi: 10.1111/ene.12031

Wang, L., Gao, J., Liu, J., Siedlak, S. L., Torres, S., Fujioka, H., et al. (2018). Mitofusin 2 regulates axonal transport of calpastatin to prevent neuromuscular synaptic elimination in skeletal muscles. *Cell Metab.* 28, 400.e8–414.e8. doi: 10.1016/j.cmet.2018.06.011

Wang, L., Popko, B., Tixier, E., and Roos, R. P. (2014). Guanabenz, which enhances the unfolded protein response, ameliorates mutant SOD1-induced amyotrophic lateral sclerosis. *Neurobiol. Dis.* 71, 317–324. doi: 10.1016/j.nbd.2014.08.010

Wang, L., Pytel, P., Feltri, M. L., Wrabetz, L., and Roos, R. P. (2012). Selective knockdown of mutant SOD1 in Schwann cells ameliorates disease in G85R mutant SOD1 transgenic mice. *Neurobiol. Dis.* 48, 52–57. doi: 10.1016/j.nbd.2012.05.014

Wang, W., Wang, L., Lu, J., Siedlak, S. L., Fujioka, H., Liang, J., et al. (2016). The inhibition of TDP-43 mitochondrial localization blocks its neuronal toxicity. *Nat. Med.* 22, 869–878. doi: 10.1038/nm.4130

Watanabe, S., Ilieva, H., Tamada, H., Nomura, H., Komine, O., Endo, F., et al. (2016). Mitochondria-associated membrane collapse is a common pathomechanism in SIGMAR1- and SOD1-linked ALS. *EMBO Mol. Med.* 8, 1421–1437. doi: 10.15252/emmm.201606403

Watts, G. D., Wymer, J., Kovach, M. J., Mehta, S. G., Mumm, S., Darvish, D., et al. (2004). Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. *Nat. Genet.* 36, 377–381. doi: 10.1038/ng1332

Weedon, M. N., Hastings, R., Caswell, R., Xie, W., Paszkiewicz, K., Antoniadi, T., et al. (2011). Exome sequencing identifies a DYNC1H1 mutation in a large pedigree with dominant axonal Charcot-Marie-Tooth disease. *Am. J. Hum. Genet.* 89, 308–312. doi: 10.1016/j.ajhg.2011.07.002

Wegorzewska, I., Bell, S., Cairns, N. J., Miller, T. M., and Baloh, R. H. (2009). TDP-43 mutant transgenic mice develop features of ALS and frontotemporal lobar degeneration. *Proc. Natl. Acad. Sci. U.S.A.* 106, 18809–18814. doi: 10.1073/pnas.0908767106

Weihl, C. C., Pestronk, A., and Kimonis, V. E. (2009). Valosin-containing protein disease: inclusion body myopathy with Paget's disease of the bone and frontotemporal dementia. *Neuromuscul. Disord.* 19, 308–315. doi: 10.1016/j.nmd.2009.01.009

Weis, J., Katona, I., Muller-Newen, G., Sommer, C., Necula, G., Hendrich, C., et al. (2011). Small-fiber neuropathy in patients with ALS. *Neurology* 76, 2024–2029. doi: 10.1212/WNL.0b013e31821e553a

Williamson, T. L., and Cleveland, D. W. (1999). Slowing of axonal transport is a very early event in the toxicity of ALS-linked SOD1 mutants to motor neurons. *Nat. Neurosci.* 2, 50–56. doi: 10.1038/4553

Windpassinger, C., Auer-Grumbach, M., Irobi, J., Patel, H., Petek, E., Horl, G., et al. (2004). Heterozygous missense mutations in BSCL2 are associated with distal hereditary motor neuropathy and Silver syndrome. *Nat. Genet.* 36, 271–276. doi: 10.1038/ng1313

Wu, C. H., Fallini, C., Ticozzi, N., Keagle, P. J., Sapp, P. C., Piotrowska, K., et al. (2012). Mutations in the profilin 1 gene cause familial amyotrophic lateral sclerosis. *Nature* 488, 499–503. doi: 10.1038/nature11280

Yamashita, T., Hideyama, T., Hachiga, K., Teramoto, S., Takano, J., Iwata, N., et al. (2012). A role for calpain-dependent cleavage of TDP-43 in amyotrophic lateral sclerosis pathology. *Nat. Commun.* 3:1307. doi: 10.1038/ncomms2303

Yoshihara, T., Yamamoto, M., Hattori, N., Misu, K., Mori, K., Koike, H., et al. (2002). Identification of novel sequence variants in the neurofilament-light gene in a Japanese population: analysis of Charcot-Marie-Tooth disease patients and normal individuals. *J. Peripher. Nerv. Syst.* 7, 221–224. doi: 10.1046/j.1529-8027.2002.02028.x

Zhan, L., Xie, Q., and Tibbetts, R. S. (2015). Opposing roles of p38 and JNK in a *Drosophila* model of TDP-43 proteinopathy reveal oxidative stress and innate immunity as pathogenic components of neurodegeneration. *Hum. Mol. Genet.* 24, 757–772. doi: 10.1093/hmg/ddu493

Zhang, X., Chen, S., Song, L., Tang, Y., Shen, Y., Jia, L., et al. (2014). MTOR-independent, autophagic enhancer trehalose prolongs motor neuron survival and ameliorates the autophagic flux defect in a mouse model of amyotrophic lateral sclerosis. *Autophagy* 10, 588–602. doi: 10.4161/auto.27710

Zhao, Z. H., Chen, W. Z., Wu, Z. Y., Wang, N., Zhao, G. X., Chen, W. J., et al. (2009). A novel mutation in the senataxin gene identified in a Chinese patient with sporadic amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* 10, 118–122. doi: 10.1080/17482960802572673

Zuchner, S., De Jonghe, P., Jordanova, A., Claeys, K. G., Guergueltcheva, V., Cherninkova, S., et al. (2006). Axonal neuropathy with optic atrophy is caused by mutations in mitofusin 2. *Ann. Neurol.* 59, 276–281. doi: 10.1002/ana.20797

Zuchner, S., Mersiyanova, I. V., Muglia, M., Bissar-Tadmouri, N., Rochelle, J., Dadali, E. L., et al. (2004a). Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. *Nat. Genet.* 36, 449–451. doi: 10.1038/ng1341

Zuchner, S., Vorgerd, M., Sindern, E., and Schroder, J. M. (2004b). The novel neurofilament light (NEFL) mutation Glu397Lys is associated with a clinically and morphologically heterogeneous type of Charcot-Marie-Tooth neuropathy. *Neuromuscul. Disord.* 14, 147–157. doi: 10.1016/j.nmd.2003.10.003

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.

frontiers in Neuroscience

REVIEW
published: 10 September 2019
doi: 10.3389/fnins.2019.00951

Cognitive, Emotional and Psychological Manifestations in Amyotrophic Lateral Sclerosis at Baseline and Overtime: A Review

Soumia Benbrika, Béatrice Desgranges, Francis Eustache and Fausto Viader*

Neuropsychology and Imaging of Human Memory, Normandy University-PSL Research University-EPHE-INSERM U1077, Caen University Hospital, Caen, France

It is now well recognized that, in addition to motor impairment, amyotrophic lateral sclerosis (ALS) may cause extra-motor clinical signs and symptoms. These can include the alteration of certain cognitive functions, impaired social cognition, and changes in the perception and processing of emotions. Where these extra-motor manifestations occur in ALS, they usually do so from disease onset. In about 10% of cases, the cognitive and behavioral changes meet the diagnostic criteria for frontotemporal dementia. The timecourse of behavioral and cognitive involvement in ALS is unclear. Whereas longitudinal studies have failed to show cognitive decline over time, some cross-sectional studies have demonstrated poorer cognitive performances in the advanced stages of the disease. Neuroimaging studies show that in ALS, extra-motor signs and symptoms are associated with specific brain lesions, but little is known about how they change over time. Finally, patients with ALS appear less depressed than might be expected, given the prognosis. Moreover, many patients achieve satisfactory psychosocial adjustment throughout the course of the disease, regardless of their degree of motor disability. There are scant longitudinal data on extra-motor impairment in ALS, and to our knowledge, no systematic review on this subject has yet been published. Even so, a better understanding of patients' clinical trajectory is essential if they are to be provided with tailored care and given the best possible support. We therefore undertook to review the evidence for extra-motor changes and their time course in ALS, in both the cognitive, emotional and psychological domains, with a view to identifying mechanisms that may help these patients cope with their disease.

Keywords: amyotrophic lateral sclerosis, extra-motor manifestations, cognition, emotion, psychological adjustment, coping

OPEN ACCESS

Edited by:
Francesca Trojsi, University of Campania Luigi Vanvitelli, Italy

Reviewed by:
Judith Machts, Universitätsklinikum Magdeburg, Germany
Johannes Prudlo, Rostock University Hospital, Germany

****Correspondence:***
Béatrice Desgranges
beatrice.desgranges@unicaen.fr

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: *28 March 2019*
Accepted: *22 August 2019*
Published: *10 September 2019*

Citation:
Benbrika S, Desgranges B, Eustache F and Viader F (2019) Cognitive, Emotional and Psychological Manifestations in Amyotrophic Lateral Sclerosis at Baseline and Overtime: A Review. Front. Neurosci. 13:951. doi: 10.3389/fnins.2019.00951

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of upper and lower motor neurons. In all sporadic and most genetic cases, proteinaceous aggregates of TAR-DNA binding protein 43 (TDP43) are found in these upper and lower motor neurons, as well as in certain glial cells (Saberi et al., 2015). The disease worsens relentlessly, and death occurs after a median

duration of 3 years after onset and 2 years after diagnosis (Couratier et al., 2016).

All patients experience extensive and progressive muscle paralysis that results in severe functional disability, but as many as 50% may also have extra-motor signs and symptoms, including cognitive impairment (Abrahams et al., 2000; Murphy J. M. et al., 2007; Murphy J. et al., 2007) mainly affecting executive functions, emotion processing and social cognition, and behavior.

Despite being given such a gloomy prognosis, not all patients display symptoms of depression. Indeed, depression rates remain low, considering the severity of the disease (Rabkin et al., 2016), and patients' quality of life (QoL) is not as impaired as might be expected (Roach et al., 2009; Lulé et al., 2012; Jakobsson Larsson et al., 2017). This relatively good psychosocial adjustment raises important questions about the coping strategies used by patients.

While we now have substantial knowledge about extra-motor signs of ALS at baseline, data on their clinical course remain scarce. The results of cross-sectional studies attempting to correlate disease duration and/or physical symptom severity with cognitive/psychological status suggest that, unlike motor impairment, cognitive performances are not always significantly worse in the late stages of the disease than they were in the early stages (Ringholz et al., 2005). These observations suggest that the mechanisms of psychological involvement do not simply come down to degenerative changes in the neurons of the brain. Furthermore, the fact that some studies have described patients as retaining a relatively satisfactory level of wellbeing, despite the continuous worsening of their physical condition, may seem a little surprising and deserves further consideration.

We therefore set out to review the cognitive, emotional and psychological impairments that can emerge at the beginning of the disease, and then to address the question of how they change throughout the course of the disease. We focused on ALS cases that do not meet the clinical criteria for dementia, as ALS-dementia syndromes raise different issues, and may not be as similar to ALS without dementia as is usually claimed (Lulé et al., 2018). Longitudinal studies are the most appropriate way of assessing how these impairments change, but they have several drawbacks. First, the follow-up periods are often short, and a large proportion of patients are liable to drop out, owing to the rapid progression of ALS, making it difficult to detect significant changes in extra-motor signs and symptoms over time. Second, it is difficult to find a suitable control group that accurately matches the patient sample. Third and last, the tools used to assess cognitive functioning have to be carefully chosen in order to be both adequate and usable at all stages of the disease-even the most advanced-, in order to accommodate the motor impairments that invariably interfere with testing procedures.

To fulfill the objectives of our work, we conducted a bibliographical search on PUBMED prior to the 1st October 2018 with the following keywords: Cognition, emotion, psychological adjustment, neuroimaging, longitudinal, AND ALS. We retained the articles that met our keywords, and that were written in English or in French. 190 articles were finally included in our study.

COGNITIVE, EMOTIONAL, AND BEHAVIORAL CHANGES IN ALS

Cognition in ALS (Table 1)

Risk Factors for Developing Cognitive Deficits

Female sex, older age at onset, and low education level seem to increase the risk of cognitive impairment (Irwin et al., 2007; Montuschi et al., 2015; Flaherty et al., 2017). The presence of a *C9orf72* gene mutation is associated with more severe cognitive deficits, even in non-demented patients (Byrne et al., 2012; Montuschi et al., 2015). There are still conflicting data about the potential positive correlation between bulbar symptoms and cognitive decline, with some studies supporting this correlation (Giordana et al., 2011; Gordon et al., 2011; Strutt et al., 2012) while others don't (Sterling et al., 2010; Zalonis et al., 2012).

The presence of depressive symptoms also seems to exacerbate the executive deficit in patients, with a negative correlation between scores on depression scales and those on cognitive tests (Wei et al., 2016; Carelli et al., 2018). These findings are not surprising, given the negative impact that depression is known to have on cognitive functions, in terms of attention and memory (Bortolato et al., 2016).

Baseline

Frequency and profile

Cognitive impairment occurs in 30–50% of patients with ALS, depending on the study and the neuropsychological tools used to assess cognitive functions (Giordana et al., 2011; Zago et al., 2011; Byrne et al., 2012; Kasper et al., 2015; Beeldman et al., 2016; Murphy et al., 2016). The cognitive deficit profile includes impairment of executive functions, verbal fluency, language, social cognition and verbal memory (Phukan et al., 2012; Beeldman et al., 2016). In 6–14% of patients, the cognitive impairment meets the criteria for a behavioral variant of frontotemporal dementia (FTD) (Consonni et al., 2013; Montuschi et al., 2015; Murphy et al., 2016). In the study by Ringholz et al. (2005), 51% of patients were cognitively impaired, compared with 5% of controls, and 14% met the diagnostic criteria for FTD. A cluster analysis indicated four patient subgroups: 49% with intact cognition, 32% with mild cognitive impairment, 13% with moderate impairment, and 6% with severe impairment.

Executive alterations affect verbal fluency, attention monitoring, switching, working memory, cognitive flexibility and mental control, and reasoning and coordinating rules (Phukan et al., 2012; Beeldman et al., 2016). Other executive functions are also impaired, such as initiation and shifting (Kasper et al., 2015). A study by Palmieri et al. (2015) found that female patients with ALS were twice as likely as males to have dysexecutive dysfunction-an intriguing finding that is not clearly explained to date.

Cognitive alterations observed in ALS also include language impairments (Iwasaki et al., 1990; Raaphorst et al., 2010; Giordana et al., 2011). According to Woolley and Rush (2017), language may be altered in 30–40% of patients without dementia, regardless of executive dysfunction, dysarthria or respiratory

TABLE 1 | Cognition in ALS.

Authors/Years	Number of patients	Type of study	Outcomes measure	Main findings
Abdulla et al., 2014	58 pts / 29 NC	CS	Neuropsychological assessment Brain MRI	Global cognitive dysfunction on executive and verbal memory tests. Smaller right hippocampal volume in pts; left hippocampal volume correlates with verbal episodic memory
Abrahams et al., 2000	2 pts / 25 NC	CS	Verbal fluency Working memory	Verbal fluency impairments result from deficits in the central executive component of working memory
Abrahams et al., 2005	20 pts / 18 NC	LS : BL, 6 mo	Executive, memory, language, visuospatial functions, behavior, and emotion	Verbal fluency remains stable whereas other language abilities decreased overtime
Bock et al., 2017	49 pts	LS : BL, 7 mo	cognitive-behavioral assessment: ALS-CBS	No cognitive change whereas patients develop bi overtime
Braber, 2016	100 pts / 50 NC	LS : BL, 3 mo	Behavioral and cognitive evaluation Genetic testing	No changes over time. *C9orf72* repeat expansion has no influence on cognition
Burke et al., 2015	33 pts / 33 CG	CS	CG burden. Cognitive-behavioral profile of pts	Ci and bi (apathy and disinhibition) predict high level of CG burden
Burkhardt et al., 2017	24 pts : 21 : NC	LS : BL, 6 mo, 12-18 mo	Cognitive assessment with the ECAS and FAB	No significant alteration overtime in cognition and behavior
Byrne et al., 2012	191 pts	CS	Clinical, cognitive, behavioral, and survival data 3T high resolution MRI Screening for *C9orf72* mutation.	Mutated ALS-pts have lower age of disease onset, more often family history of FTD, more comorbid FTD, distinct pattern of non-motor cortex changes on MRI and shorter survival
Carelli et al., 2018	168 pts	CS	FAB, MoCA, ECAS BDI, STAI	Depression correlates negatively with ECAS and specially with executive functions
Consonni et al., 2013	23 pts / 39 NC	CS	Standard neuropsychological battery FBI	30%: executive ci, naming and short-term memory deficits; 20%: disorganization and mental rigidity 13%: comorbid dementia.
Dary-Auriol et al., 1997	26 pts / 26 NC	CS	Global cognition, memory, language, executive functions, MADRS	Global but subtle cognitive impairment of all neuropsychological tests with no specific profile
Elamin et al., 2011	139 pts	CS	Executive function, memory, language, visuospatial function	Executive dysfunction and comorbid FTD associated to shorter survival
Elamin et al., 2013	186: BL / 96: 2 assessments / 46: 3 assessments	LS :	Cognitive assessment	Cognitive function declines faster in patients cognitively impaired at BL.
Flaherty et al., 2017	161 pts	CS	Cognitive-behavioral profile × site of onset and gender relative to emerging FTD	Bulbar pts : worse letter fluency; Bulbar females : worse category fluency; females with low oestrogen levels: worse letter fluency
Gillingham et al., 2017	20 pts / 36 NC	CS	ALS-CFB	Executive dysfunction
Gordon et al., 2011	131 pts	Cross-sectional	Spectrum and clinical associations of ci impairment in ALS Effect of ci on survival	40% ci, 10%FTD Impaired patients: less education, more likely to have bulbar onset. Severe cognitive impairment predicts shorter survival
Govaarts et al., 2016	110 pts	CS	Behavioral and cognitive evaluation	Frontal syndrome correlates negatively to survival
Hervieu-Bègue et al., 2016	15 pts	CS	Semantic memory	60% of pts have semantic memory impairment
Hu et al., 2013	37 pts / 33 NC	CS	ALS-BCA and other neuropsychological tests	Shorter survival associated to dementia and behavioral impairment
Iwasaki et al., 1990	22 pts / 18 NNMC / 17 NC	CS	MMSE; immediate and delayed memory tests	Pts perform lower than NNMC and NC at MMSE and memory tasks. MMSE and memory correlates negatively to upper limb function
Kamminga et al., 2016	20 ALS pts / 15 ALS-FTD / 27 PNFA / 23 NC	CS	Syntax comprehension : Test for Reception of Grammar Brain volume by MRI-VBM	Syntactic comprehension impaired in 25% of ALS, 92.9% of FTD-ALS, and 81.5% of PNFA Impairment correlates with left peri-insular atrophy
Kasper et al., 2015	98 pts / 70 NC	CS	Executive cognitive, Executive behavior	70% of ci pts have executive dysfunction (initiation and shifting). Dominant bi is apathy
Kasper et al., 2016	93 pts : 73 NC	LS : BL and at three time every 3-6 mo	Executive functioning	No significant decline
Leslie et al., 2015	17 pts / 19 ALS-FTD, 22 SD / 26 NC	CS	Assessment of semantic deficits Brain volume by MRI-VBM	Significant semantic deficits in ALS and ALS-FTD compared to controls. Severity of semantic deficits varies across clinical phenotypes. Anterior temporal lobe atrophy correlates with semantic deficits

(Continued)

TABLE 1 | Continued

Authors/Years	Number of patients	Type of study	Outcomes measure	Main findings
Kilani et al., 2004	18 pts : 19 NC	LS : BL, 6 and 12 mo	Cognitive function	Executive alteration at baseline does not worsen at follow-up
Machts et al., 2018	31 pts and 29 NC	CS	Brain MRI	Reduction of left and right hippocampal volumes in patients' *cornu ammonis* field 1 (CA1)
Montuschi et al., 2015	207 pts / 127 NC	CS	Comprehensive neuropsychological assessment	49.7% cognitively normal, 12.6% ALS with FTD, 19.7% ALS-executive ci, 5.5% ALS-non executive ci, 16% ALS-bi and 6% non-classifiable ci. ALS-FTD older, lower educational level and shorter survival
Murphy J. M. et al., 2007	23 pts	CS	Neuropsychological, neurobehavioral assessment	No impairment: 11 pts; behavioral changes: 4; FTD: 5;other :3 (Alzheimer : 1)
Murphy et al., 2016	274 pts	CS	Neuropsychological and neurobehavioral assessment : ALS-CBS	54.2% ci, 14.1% bi and 6.5% FTD
Palmieri et al., 2015	260 pts / 134 NC	CS	Executive function, memory and language.	29% pts have executive ci and 18% non-executive ci; Females have 2-fold risk to have executive ci
Olney et al., 2005	81 pts	CS	Survival predictors	Younger age, limb onset and absence of comorbid FTD predicted higher survival
Phukan et al., 2012	160 pts / 110 NC	CS	Comprehensive neuropsychological battery	46% no ci; 14% FTD; 21% executive ci; 14% non-executive ci
Poletti et al., 2018	164 BL / 48 at 6 mo / 18 at 12 mo / 5 at 18 mo	LS : BL, 6 mo, 12 mo, 24 mo	Cognitive and behavioral examination : ECAS	No behavioral or cognitive worsening
Raaphorst et al., 2015	26 pts / 21 NC	CS	Neuropsychological assessment Brain MRI	Prose memory impairment correlates to hippocampal volume
Rabkin et al., 2016	247 pts	CS	Cognition-behavior : CBS Psychological : PHQ	40 % ci, 9% bi, 18% ci and bi, 12 % Major or minor depression; 12% Bi associated with depression
Ringholz et al., 2005	279 pts / 129 NC	CS	Neuropsychological testing	49% intact; 32% mild ci, 13% moderate ci, 6% severe ci, 15% FTD
Roberts-South et al., 2012	16 pts / 12 NC	LS : BL, 6-12-18-24 mo	Language testing with standardized tests and analysis of productivity and content	No alteration at standardized tests. Impairment of discourse content. Alteration of performances overtime
Robinson et al., 2006	19 pts / 8 CG	LS : BL, 6 mo	Neuropsychological assessment	No change overtime even if some patients develop abnormalities
Schreiber et al., 2005	52 pts	LS : BL and each 4 months until 18 mo	Executive functions, memory and attentional control.	No decline on follow-up
Stojkovic et al., 2016	58 pts	LS : BL and yearly	Executive function and correlation to survival	49.5 % executive ci and BL executive status might predict survival
Strong et al., 1999	13 pts	CS	Neuropsychological, language and speech testing	Mild impairment in several domains especially when bulbar onset
Strutt et al., 2012	44 pts	CS	Comprehensive pulmonary (vital capacity) and neuropsychological assessments	More respiratory-impairment when clinically significant impairments in frontal-lobe-mediated behaviors. Greater executive functioning deficits in patients with bulbar versus limb onset
Xu et al., 2017	108 pts / 60 NMC	CS	ACE-III, FAB, ECAS, ALS-FTD-Q, MiND-B	14 to 30% ci on ALS and 3.3 to 11.7 % on NMC. 32 % bi on ALS and 39 % on NMC. Ci and bi influence prognosis
Wei et al., 2016	91 pts	CS	Neuropsychiatric symptoms and cognition: NPI, ACE-R, FAB	Depression 59%, anxiety 41%, lability 26%. NPI correlates with ACE-R but not with FAB
Woolley et al., 2018	294 BL / 134 at follow up	LS : BL, 5-18 mo	Cognitive and behavioral changes	Worsening of behavior but not cognition
Zalonis et al., 2012	48 pts / 47 NC	CS	Executive function: TMT, SNST, WAIS, WCST	Pts worse than NC on TMT, SNST and WAIS Similarities. No difference between bulbar and spinal onset pts

ALS, Amyotrophic Lateral Sclerosis; ACE, Addenbrooke's Cognitive Examination; ALS-BCA, ALS Brief Cognitive Assessment; ALS-CBS, ALS Cognitive Behavioral Screen; ALS-CFB, ALS-Computerized Frontal Battery; ALS-FTD, ALS-Frontotemporal Dementia; ALS-FTD-Q, ALS-Frontotemporal Dementia Questionnaire; BDI, Beck's Depression Inventory; Bi, Behavioral Impairment; BL, Baseline; Ci, Cognitive Impairment; CG, Caregiver; Cibi, Cognitive and Behavioral Impairment; CS, Cross-Sectional Study; ECAS, Edinburgh Cognitive and Behavioral ALS Screen; FAB, Frontal Assessment Battery; FTD, Frontotemporal Dementia; LS, Longitudinal Study; MADRS, Montgomery-Asberg Depression Rating Scale; MiND-B, Motor Neuron Disease Behavioral instrument; MMSE, Mini Mental State Examination; MRI, Magnetic Resonance Imaging; NC, Normal Controls; NMC, Controls With Neuromuscular Disease, NNMC, Non Neurological Medical Controls; NPI, Neuropsychiatric Inventory; PHQ, Patient Health Questionnaire; PNFA, Progressive Nonfluent Aphasia; Pt, Patient; SD, Semantic Dementia; STAI, State-Trait Anxiety Inventory; SNST, Stroop Neuropsychological Screening Test; TMT, Trail Making Test; VBM, Voxel-Based Morphometry; WAIS, Similarities Subtest of The Wechsler Adult Intelligence Scale.

failure. Linguistic impairments may include deficits in syntactic processing, verb naming and action verb processing, semantic and verbal paraphasias, and syntactic comprehension deficits. Kamminga et al. (2016) found that syntactic comprehension was defective in 25% of patients with ALS without dementia. Leslie et al. (2015) observed semantic deficits in 35% of not demented ALS patients, and Hervieu-Bègue et al. (2016) in as many as 60% of such patients. Woolley and Rush (2017) suggested that language alterations observed in ALS reflect the fact that ALS and nonfluent/agrammatic primary progressive aphasia lie on a pathogenesis continuum.

Memory may also be altered to some extent in ALS (Dary-Auriol et al., 1997; Abrahams et al., 2000; Neary et al., 2000; Ringholz et al., 2005), but the nature of this impairment is subject to debate. It has been suggested that defective memory is mainly the consequence of executive dysfunction, especially since memory deficits very rarely occur in isolation in ALS (Strong, 2017). There is, however, some evidence in favor of hippocampal involvement. Abdulla et al. (2014) found reduced hippocampal volume in a series of patients with ALS relative to controls, and Machts et al. (2018) showed that this reduction mostly affects the anterior part, including the CA1 field-a critical structure for episodic memory. Furthermore, Raaphorst et al. (2015) found that immediate and delayed story recall scores were below normal in 23% of patients with ALS, and these performances were correlated with hippocampal gray-matter volume. Further studies are needed to try to determine the real status of memory in ALS.

The Strong (2017) revised diagnostic criteria of ALS-FTSD classify ALS into several categories according to the type and severity of neuropsychological impairments: pure ALS (no impairment), ALSci (cognitive impairment), ALSbi (behavioral impairment), ALScibi (cognitive and behavioral impairment), all three without dementia, and ALS-FTD. According to those criteria, the diagnosis of ALS-ci requires either executive dysfunction or language impairment or both, and the diagnosis of ALS-bi requires either apathy or two of the Rascovsky criteria for FTD.

Over Time

Most longitudinal studies of cognitive changes in patients with ALS have a follow-up period of 6 months. A number of authors have reported an absence of decline in cognitive functions during the course of the disease (Kilani et al., 2004; Schreiber et al., 2005; Braber, 2016; Kasper et al., 2016; Burkhardt et al., 2017; Poletti et al., 2018; Woolley et al., 2018). Although patients may initially have lower performances than controls, their deficits remain stable over time. It has been argued, however, that unlike normal controls, patients with ALS do not display a *practice effect* in repeated assessments with the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) battery, which has been interpreted as evidence of a *pre-symptomatic* cognitive decline (Burkhardt et al., 2017). Abrahams et al. (2005) found that while most cognitive scores remained stable over time (including written and spoken verbal fluency) patients with ALS performed a single word retrieval test increasingly slowly whereas controls performed it faster. Furthermore, the caregivers of patients report increasing cognitive dysfunction in daily life over time, unlike controls' partners. Robinson et al. (2006) found that cognitive changes occurred in 36% of patients with ALS over a 6-month period. Cognitive status seems to have a heterogeneous outcome in ALS. Behavioral symptoms may appear in patients with stable cognitive performances (Bock et al., 2017). The presence of even mild cognitive or behavioral impairment (as defined by the 2017 Strong criteria) at baseline seems to be a significant risk factor for the later appearance of a full-blown frontotemporal syndrome (Elamin et al., 2013). The choice of the neuropsychological tests used to assess cognitive status is of primary importance, as Roberts-South et al. (2012) suggested in their longitudinal study of discourse changes in a group of patients with ALS compared with healthy participants over 24 months. Subtle cognitive language deficits affecting discourse (content rather than productivity) were found to emerge early in ALS and worsen as the disease progressed. The authors concluded that language deficits are more thoroughly detected by the discourse analysis method than by standard language tasks. Gillingham et al. (2017) assessed patients with ALS at baseline and 9 months later using both standardized non-specific cognitive tests and the ALS-Computerized Frontal Battery (ALS-CFB), which was specifically designed for patients with ALS. While the basic cognitive tests failed to reveal any change over time, the ALS-CFB showed a significant decrease in cognitive performances in patients compared with controls (e.g., for verbal fluency). Finally, patients with bulbar onset seem to exhibit a progressive decline in cognitive functions (Strong et al., 1999).

It is therefore difficult to give a simple answer to the question "do cognitive functions decline over time in ALS?" owing to the heterogeneity of the patients and the difficulty of differentiating genuine cognitive deficits from the consequences of the steadily worsening motor impairment. A tentative, preliminary answer could be that when ALS is associated with a cognitive impairment at baseline, this impairment is likely to progress, and when dementia is present at diagnosis, decline is faster (Woolley and Katz, 2008). Normal cognition at baseline was associated with tendency to remain cognitively intact overtime (Elamin et al., 2013).

Consequences for Patients and Caregivers

Cognitive impairment in ALS is associated with a more rapid progression of the disease and a poorer prognosis, with reduced survival (Elamin et al., 2011; Giordana et al., 2011; Gordon et al., 2011; Hu et al., 2013; Xu et al., 2017). Stojkovic et al. (2016) found that the death risk was increased threefold by the presence of executive dysfunction in ALS. Poorer survival could be explained by patients' difficulty weighing up the benefits of non-invasive ventilation (Govaarts et al., 2016), or their reduced compliance in the use of medical devices (Olney et al., 2005). The correlation between the severity of bulbar symptoms and the cognitive deficit may also influence survival figures (Olney et al., 2005). Elamin et al. (2013) evaluated the clinical impact of cognitive deficit in patients with ALS in a longitudinal study. Executive impairment at the initial consultation was associated with significantly higher rates of attrition due to disability or death, and faster rates of motor functional decline, particularly

bulbar function. QoL in patients has been found to be worse in the case of cognitive impairment (Hu et al., 2013). Caregivers are the pillars of patient care. They may be the spouse, children, brothers or sisters. It is cognitive and behavioral impairment, rather than the patients' physical disability, that increases caregivers' burden and anxiety (Rabkin et al., 2009; Burke et al., 2015). This underscores the importance of screening patients with ALS for cognitive dysfunction, in order to predict disease progression and provide more adequate care.

Neuroimaging Correlates of Cognitive Deficits

Anatomical brain changes are more pronounced in patients with ALS who exhibit cognitive impairment than in those who do not. Gray matter volume in the frontal and temporal lobes is reduced when cognitive impairment is present (Abe et al., 1997; Giordana et al., 2011; Mioshi et al., 2013), as is that of the cerebellar cortex and basal ganglia (Christidi et al., 2018). Reduced cortical thickness is observed in the bilateral precentral gyrus, insular and cingulate cortices, and frontotemporal regions in the case of cognitive deficit (Schuster et al., 2014a; Agosta et al., 2016). Left peri-insular atrophy was found to correlate with scores on a syntactic comprehension task (Kamminga et al., 2016). There are greater white-matter changes in the corticospinal and corpus callosum tracts when cognitive functions are altered (Sarro et al., 2011), and these extend to extra-motor tracts, particularly within the frontal lobes and associative areas including the cingulum and the inferior longitudinal, inferior fronto-occipital, and uncinate fasciculi (Sarro et al., 2011; Kasper et al., 2014; Christidi et al., 2018). Verbal learning and memory test scores are correlated with white-matter values in the fornix (Sarro et al., 2011). Cerebral regional metabolism has been repeatedly studied with fluorodeoxyglucose positron emission tomography (18FDG-PET) in patients both with and without cognitive dysfunction. One of the most recent studies shows that cognitively impaired (but not demented) patients with ALS have relative hypometabolism in the right cingulate and frontal cortex, and bilaterally in the prefrontal cortex, compared with patients with no cognitive impairment (Canosa et al., 2016). These patients also have relative hypermetabolism in parts of the midbrain and corticospinal tracts (Canosa et al., 2016). In a combined MRI and PET study in patients with ALS, Buhour et al. (2017) found gray-matter atrophy, predominantly in the temporal poles, and hypometabolism in the left superior medial cortex. Hypermetabolism was also found in parts of the temporal lobes and the cerebellum. A series of negative correlations between cognitive performance and regional cerebral metabolism in functionally relevant areas suggest that hypermetabolism is more likely to reflect deleterious processes such as neuro-inflammation rather than compensatory neuronal activity.

Longitudinal studies of anatomical cerebral changes in ALS with cognitive modifications remain rare, for the same reasons as those dealing with purely clinical aspects of the disease. Floeter et al. (2016) assessed brain volume changes in patients who were either sporadic or carriers of the genetic mutation C9orf72 (C9+), which is often associated with FTD or cognitive impairment. These authors found that over a 6-month period, ventricular volume increased in C9+ versus sporadic cases, suggesting that subcortical involvement influences cognitive performances in this particular group of patients. Other microstructural changes over time in ALS have been documented. A significant decline in the cortical thickness of frontal, temporal and parietal regions is observed over time, whereas the reduced cortical thickness of the precentral gyrus at the beginning of the disease remains stable (Verstraete et al., 2012; Schuster et al., 2014b). Menke et al. (2018) assessed brain changes in patients with ALS over 2 years and reported widespread changes in both white and gray matter in the cingulate gyrus, thalami, caudate nuclei, pallidum, hippocampi and parahippocampal gyri, and insula. These results indicate that over time, cerebral changes extend into extra-motor areas, but it remains difficult to draw a link between these changes and clinical cognitive implications.

Changes in Emotion Perception and Social Cognition (Table 2)

Baseline

Perception of emotions

A number of studies have shown that emotion perception is impaired in ALS (Lulé et al., 2005; Zimmerman et al., 2007; Palmieri et al., 2010; Girardi et al., 2011), with patients exhibiting deficits in emotion recognition (facial or prosodic) and emotional valence attribution, and decreased excitability when emotional material is presented. By contrast, Cavallo et al. (2011) and Papps et al. (2005) found no deterioration in either facial emotional recognition or judgments of emotional valence. Clinical features such as type of onset and disease severity may explain the heterogeneity of patients' emotional deficit profiles, as suggested by Sedda (2014). Oh et al. (2016) confirmed the presence of facial emotion recognition deficits in ALS. Bora (2017) carried out a meta-analysis of 15 studies of emotion recognition in ALS and concluded that ALS is associated with significant impairments in facial emotion recognition, especially for disgust and surprise.

Patients' processing of their own emotions (alexithymia) also seems to be altered, although there have been very few studies so far in this area. Roy-Bellina et al. (2008) reported that patients with ALS are more often alexithymic than controls. Benbrika et al. (2018) assessed a group of patients with ALS and a matched control group on the three dimensions of alexithymia: Difficulty Identifying Feelings, Difficulty Describing Feelings, and Externally-Oriented Thinking. Patients were more often alexithymic than controls and had a higher level of alexithymia, especially on the Difficulty Identifying Feelings dimension, suggesting that they have difficulty with the first stage of own emotion processing, namely recognizing one's own emotions.

Social cognition

Social cognition is a set of cognitive processes used to encode, decode, store, retrieve and use information about people in social relationships. It has several dimensions, such as theory of mind (ToM), empathy, and moral reasoning. ToM refers to the ability to infer the mental and emotional states (i.e., beliefs, preferences and intentions) of oneself and others, and contributes to the understanding of other people's behavior. It can be divided into

TABLE 2 | Social cognition and emotion perception in ALS.

Authors/year	Pts/NC		Outcome measures	Main findings
Andrews et al., 2017b	33 pts / 22 NC	CS	Emotion processing multimodal tasks (facial affect and voice prosody) Executive, mood and functional tests	Difficulties in recognizing emotions both in faces and voices
Benbrika et al., 2018	28 pts / 30 NC	CS	20-item Toronto Alexithymia Scale Correlation / gray matter volume	Pts > NC. Alexithymia correlated with prefrontal cortex, right temporal pole and parahippocampal gyri
Burke et al., 2016	106 pts/50 NC	CS	RME and executive function in bulbar vs. spinal-onset ALS	Bulbar onset pts have more social cognition but not more executive impairment than spinal onset pts.
Carluer et al., 2015	23 pts / 23 NC	CS	An original false-belief task and executive tasks 18F-FDG PET-scan examination	ToM impairment only partially linked to executive dysfunction. Correlated with metabolism of dorso-medial and dorsolateral prefrontal cortices, and SMA
Cavallo et al., 2011	15 pts / 21 NC	CS	Social cognition (private vs. social intentions)	Impaired comprehension of social context
Gibbons et al., 2007	16 pts / 16 NC	CS	ToM and executive function	Abnormalities of social cognition linked to executive function
Gillingham et al., 2017	20 pts / 36 NC at baseline 11 pts / 20 NC after 9 months	LS	ALS- Computerised Frontal Battery	Impairment in social cognition, initiation of behavior, executive processing and response suppression. Decline in executive processing over time
Girardi et al., 2011	19 pts/20 NC	CS	Behavior (FSBS) Social cognition (modified IGT) Gaze, RME, emotion recognition	Increased apathy. Different profile from NC. Impaired emotion recognition
Lulé et al., 2005	12 pts / 18 NC	CS	Judgment of pictures from the IAPS	Pts more positive than NC
Meier et al., 2010	18 pts / 18 NC	CS	Orbitomedial prefrontal tasks (Faux Pas, emotional prosody recognition, reversal of behavior in response to changes in reward, decision making and Neuropsychiatric Inventory Dorsolateral prefrontal tasks (verbal and written fluency and planning)	Dissociations involving either one or two or both of the orbito-frontal or dorsolateral prefrontal regions. Variability and heterogeneity of cognitive involvement in ALS
Oh et al., 2016	24 pts / 24 NC	CS	K-MMSE,BDI, FAB Perception of emotional expression	Pts < NC
Palmieri et al., 2010	9 pts / 10 NC	CS	Two fMRI emotional attribution and memory tasks	Activation increased in the left hemisphere and reduced in the right one in both tasks
Papps et al., 2005	19 pts / 20 NC	CS	Facial expression recognition, Social judgement rating of faces, Memory for emotional words.	No enhanced recognition memory for emotional vs. neutral words
Roy-Bellina et al., 2008	14 pts / 9 NC		20-item Toronto Alexithymia Scale	Pts > NC
Trojsi et al., 2016	22 pts / 15 NC	CS	ToM: Emotion Attribution Task, Advanced Test of ToM, Eyes Task Executive, verbal comprehension, visuospatial tasks, behavior, and QoL	Impairment of both affective and cognitive ToM that impacts the "Mental Health" component of QoL
Trojsi et al., 2017	21 pts / 15 NC at baseline and after 6 months	LS	Affective and cognitive ToM and global neuropsychological assessment, Resting state MRI study	No impairment at baseline. At 6 months, impairment of both affective and cognitive ToM in bulbar onset pts, and of the cognitive subcomponent alone in spinal onset pts. Various changes in RSN connectivity.
van der Hulst et al., 2015	33 pts / 26 NC	CS	Social cognition (Cognitive–Affective Judgement of Preference Test), Measures of empathy and awareness	Affective and ToM deficit, poor empathy and self-awareness of their difficulties
Watermeyer et al., 2015	55 pts / 49 NC	CS	Social cognition, executive functions, mood, behavior and personality	Social cognition impairment mainly predicted by executive dysfunction
Zimmerman et al., 2007	13 bulbar pts / 12 NC	CS	Facial emotional and prosodic recognition task	Pts < NC

IGT, Iowa Gambling Task; fMRI, Functional MRI; FDG, Fluorodeoxyglucose; PET, Positron Emission Tomography; ToM, Theory of Mind; FSBS, Frontal Systems Behavior Scale; RME, Reading the Mind in The Eyes; K-MMSE, Korean Version of the Mini Mental State Examination; QoL, Quality of Life; RSN, Resting-State Network; SMA, Supplementary Motor Area. For more please refer to ***Table 1****.*

cognitive and affective ToM. Studies of ToM abilities in ALS have sometimes yielded conflicting results, owing to the heterogeneity of the tasks used and the patients' cognitive status, or the presence of depressive symptoms.

Early studies in this domain attested to a deficit in ToM, showing that patients with ALS perform more poorly than healthy individuals on the faux pas task (task assessing cognitive ToM through stories featuring appropriate and inappropriate social behavior), story comprehension task (strip stories presented to participants who have to choose the most appropriate picture) and decision making task (task evaluating decision making abilities under ambiguity in a card game) (Gibbons et al., 2007; Meier et al., 2010; Cavallo et al., 2011; Girardi et al., 2011).

More recently, van der Hulst et al. (2015) investigated whether the ToM deficit described in ALS could be further delineated as one of either affective or cognitive ToM, and explored the relationship between this social cognition deficit and the behavioral manifestations of empathy and self-awareness. Patients were evaluated on a neuropsychological battery that included a cognitive–affective judgment of preference test, a questionnaire to evaluate their self-awareness, and the Neuropsychiatric Inventory–Questionnaire (NPI-Q) to pick up any behavioral changes. The authors found deficits in both cognitive and affective ToM (affective + cognitive ToM deficit in 36% of patients, affective ToM deficit in 12%, and cognitive ToM deficit in 3%). Patients with a ToM deficit were more likely to display behavioral changes such as apathy, lack of empathy, and low self-awareness. When Carluer et al. (2015) compared 23 non-demented patients with controls matched for age, sex and educational level on cognitive ToM, by administering a false-belief task, they found a cognitive ToM deficit in patients with impaired executive functions. Trojsi et al. (2016) assessed ToM in patients with ALS using a comprehensive battery of specific tasks: the Advanced Task of ToM (ATM), in which participants listen to stories and then have to explain the protagonists' actions; the Emotional Attribution Task (EAT), in which participants have to identify the emotions experienced by the protagonists in a story; and the Reading the Mind in the Eyes task (Eyes test or RME). Patients also underwent a cognitive battery and a QoL assessment. Patients scored lower than controls on the EAT but not on the ATM, and EAT and RME correlated positively with the education, prose memory and mental health items of the QoL questionnaire. Patients were not very cognitively impaired. By contrast, Andrews et al. (2017b) reported impaired complex facial and prosodic emotion recognition, but intact simple facial affect recognition, in a group of non-demented patients with ALS. Finally, Woolley and Rush (2017) showed that the performances of patients without dementia may be impaired on tasks of complex facial affect recognition or affective prosody recognition, and may have difficulty interpreting the gaze direction of others.

To summarize findings on social cognition in ALS, even though results are quite divergent, affective ToM seems to be systematically affected, whereas scores on cognitive ToM seem to be underpinned by patients' cognitive profile. Studies including patients with severe cognitive impairment have reported cognitive ToM deficits (Carluer et al., 2015), whereas those among patients with only mild or moderate cognitive impairment point to preserved cognitive ToM (Trojsi et al., 2016). This prompts the question of a link between social cognition and other cognitive processes.

Interactions between cognitive status, social cognition, and emotion recognition

Most studies investigating the pattern of interaction between social cognition impairment and other cognitive functions support the idea of an interaction between executive functions and cognitive ToM, with positive correlations between executive functions (verbal fluency, composite executive score, executive score of the Frontal System Behavior Scale, etc.) and ToM (decision making task, faux pas task, etc.) (Gibbons et al., 2007; Meier et al., 2010; Watermeyer et al., 2015). In Carluer et al. (2015) study, cognitive ToM scores were only partially associated with executive performances, notably shifting, inhibition, and the ability to manipulate items in working memory. This led the authors to propose that the ToM deficit observed in some cases of ALS is not simply the consequence of an executive function impairment. Nevertheless, as studies do not generally exclude patients who have only a mild cognitive impairment, it is still unclear whether any ToM deficit is associated with executive deficit in ALS or whether ToM *per se* is disturbed.

Results on the interaction between emotion recognition and affective ToM performances and cognitive abilities have been somewhat conflicting. In a subset of patients with bulbar ALS, Zimmerman et al. (2007), found that 62% of them had emotion recognition defects, with no correlation with cognitive symptoms. However, the cognitive assessment was done using the Mini-Mental State Examination (MMSE), which is not well suited to patients with ALS. When they calculated a composite executive score, Watermeyer et al. (2015) also found no correlation between cognitive performances and RME test scores. By contrast, Girardi et al. (2011) reported a negative correlation between RME performances and *z* scores for verbal fluency. Burke et al. (2016) compared the RME test performances of three groups of patients: "no cognitive impairment," "defect in only one cognitive domain," and "multi-executive deficits/cognitively impaired." The patients with no cognitive impairment performed better than those with a defect in only one cognitive domain, who in turn performed better than those in the third group. Finally, Andrews et al. (2017b) found that crossmodal (facial and prosodic integration) emotion recognition correlated with executive functions, whereas the separate modalities of emotion recognition did not. No specific cognitive function seems to be related to either affective ToM or emotion recognition, and as Trojsi et al. (2016) study suggests, the link between ToM performances and cognitive impairment may only concern the cognitive aspect of ToM.

Over Time

To our knowledge, only two studies have addressed changes in emotional and ToM abilities and their neural correlates in the course of ALS. Gillingham et al. (2017) assessed cognitive and emotional changes in patients with ALS at baseline and 9 months later using a specific cognitive and emotional battery (ALS-CFB) and other standardized cognitive tests, including cognitive first-order and cognitive and affective second-order ToM tasks. At baseline, patients showed a deficit in emotion perception for happy emotions, and scored significantly lower than controls on the first-order cognitive ToM task. At 9 months, in the emotion perception task, patients were better at recognizing angry faces than controls were, but there were no changes over time in ToM. Trojsi et al. (2017) assessed cognitive and affective ToM at baseline and 6 months later. At baseline, no ToM abnormalities were found in patients, whereas at follow up, patients with bulbar onset exhibited a decline in both affective and cognitive ToM, whereas those with limb onset only displayed impairment of cognitive ToM over time. This study also included an fMRI investigation, only at baseline, that will be discussed in the next section about neuroimaging.

This result is in line with the more extensive prefrontal hypometabolism observed in patients with bulbar versus limb onset by Cistaro et al. (2012).

Neuroimaging Correlates of Emotional and Social Cognition Changes

Emotion recognition impairment is associated with alteration of white-matter integrity along the right inferior longitudinal fasciculus and inferior fronto-occipital fasciculus (Crespi et al., 2014). Defective emotional empathy attribution is correlated with reduced gray-matter density in the anterior cingulate cortex and right inferior frontal gyrus (Cerami et al., 2014). Palmieri et al. (2010) found a general increase in left-hemisphere activation and reduced right-hemisphere activation in patients when they were asked to attribute an emotional valence or remember an emotion. Aho-Özhan et al. (2016) assessed emotion recognition and its functional neural correlates in a group of patients with ALS and a matched control group. Patients recognized disgust and fear less accurately, and had lower activity in the hippocampus on both sides (brain regions involved in negative emotion processing) and more activity in the right inferior frontal gyrus.

Using a composite executive function score as a covariate, Carluer et al. (2015) found positive correlations between cognitive ToM performances and the metabolism of the bilateral superior frontal gyrus, bilateral middle frontal gyrus, and bilateral supplementary motor area. Buhour et al. (2017) sought to understand the metabolic dysfunction and its neurobehavioral consequences better by subjecting a sample of 37 patients with ALS to a comprehensive neuropsychological assessment and PET imaging. Significant negative correlations were found between metabolic activity within the left fusiform gyrus and performance on a false-belief task.

Longitudinal studies are very few and far between. Lulé et al. (2007) assessed emotional valence attribution, arousal, association of movement, and brain functioning when emotional material was presented to patients with ALS and healthy controls at baseline and 6 months later. Patients had an increased brain response in the right supramarginal area and a reduced brain response in extrastriatal visual areas at both measurement points, compared with healthy controls. In the patient group, a reduced brain response in the anterior insula at follow up was correlated with subjective arousal. This reduced response was tentatively interpreted as indicating reduced arousal during the course of the disease at the neural and behavioral levels. The reduced activity in extrastriatal visual areas could be similarly interpreted. The increased brain response in the right supramarginal area could represent altered sensitivity to social-emotional cues.

In the aforementioned study of ToM, Trojsi et al. (2017) assessed, only at baseline, the resting state functional connectivity with fMRI in 21 ALS patients compared to a matched group control. Subjects also underwent affective and cognitive ToM tasks both at baseline and after 6 months. Compared to controls, ALS patients exhibited abnormalities (1) within the DMN (Default mode network) with decreased connectivity in anterior node and increased connectivity in posterior node, (2) within the right FPN (Fronto-parietal network) with decreased connectivity in the supramarginal gyri and (3) within the left FPN and SLN (salient network) with decreased connectivity on the medial and dorsolateral prefrontal cortices. Positive correlations were found between affective ToM performances and functional connectivity of the posterior node of the DMN and the supramarginal gyri. ToM alterations were associated to decreased connectivity in the posterior cingulate cortex (PCC) and the occipital gyri of DMN. The authors suggest that these results support the hypothesis of the potential role of frontotemporoparietal network structures, such as the PCC and the supramarginal gyri in reasoning about the contents of another person's mind particularly in affective mentalizing and in empathetic face processing.

Behavioral Changes (Table 3)

Baseline

Frequency and typology

Behavioral changes are increasingly being recognized as a common feature in ALS, and may be similar to those observed in FTD. They occur in 24–69% of patients with ALS (Murphy J. M. et al., 2007; Gibbons et al., 2008; Witgert et al., 2010; Lillo et al., 2011; Bock et al., 2016; Burke et al., 2017), 6–25% of whom meet the criteria for FTD (Murphy J. et al., 2007; Lillo et al., 2011; Bock et al., 2016; Burke et al., 2017). In some patients, they may appear as early features, even prior to the development of the motor symptoms (Mioshi et al., 2014).

Apathy seems to be the most common behavioral change (Grossman et al., 2007; Witgert et al., 2010; Lillo et al., 2011; Kasper et al., 2015). The most frequently encountered subtype of apathy in ALS is lack of initiation, that is, a lack of motivation to self-generate thoughts (Radakovic et al., 2017). Patients in the advanced stage may display aggressiveness and obsessiveness (Marconi et al., 2012). Disinhibition, impulsivity, lack of foresight and planning, distractibility, reduced concern for hygiene, irritability, increased self-centeredness and reduced concern for the feelings and needs of others, new unusual habits, loss of insight, and blunting of the primary emotions of happiness, sadness, fear and anger have also been reported (Gibbons et al., 2008; Giordana et al., 2011; Burke et al., 2017). Finally, changes such as aspontaneity, disorganization, and mental rigidity have also been observed (Consonni et al., 2013). Insight on behavioral changes is altered in ALS-FTD, but not in ALS without dementia (Woolley et al., 2010).

Implications for the disease course

The presence of behavioral symptoms has practical implications, as it impacts the patients' psychological state (and that of their caregivers), with consequences for their QoL and indeed their prognosis.

Rabkin et al. (2016) showed that whereas cognitive status does not correlate with mood, patients with behavioral impairment report more depressive symptoms, greater hopelessness, negative mood, and more negative feedback from spouses or caregivers. Caga et al. (2018) found that apathy was associated with more depressive symptoms and a poorer QoL, especially for achievement in life and community connectedness. Aggressiveness and obsession in the advanced stages of the disease correlate with a high level of anxiety in both patients and caregivers (Marconi et al., 2012). Unglik et al. (2018) reported

TABLE 3 | Behavioral changes in ALS.

Authors/year	N	Type	Outcomes measures	Main findings
Andrews et al., 2017a	40 pts / 40CG and 27 NC & relatives	CS	Behavioral changes: CBI-R CG burden	Pts: disturbance on everyday skills, self-care, and sleep, mood and motivation. CG burden: pts' skills, motivation and memory
Bock et al., 2016	86 pts	CS	Cognitive-Behavioral Screen Patient QoL CG burden Disease stage	Cognitive impairment: 62%; Behavioral impairment: 37%; FTD: 5% Severity of deficits not associated with patient QoL; predicts higher CG burden. Self-reported QoL lower in pts with depressive symptoms and more advanced disease
Bock et al., 2017	49 pts BL & 7 mo	LS	Assessment of cognitive-behavioral function using the ALS-CBS	Cognitive status: no change over time; Pts initially classified as behaviorally normal show increased behavioral problems over time
Burke et al., 2017	317 pts / 66 NC	CS	Behavioral changes: BBI Cognitive assessment, Impact on survival	Behavioral changes: none, 57%; mild to moderate: 30%; severe (FTD) : 13% Behavioral changes predicted by social cognitive performances. No impact on survival
Caga et al., 2018	60 pts	Cs	Impact of apathy (AES) on QoL (PWI)	Apathy: 30 %. Pts with apathy have poorer overall QoL
Consonni et al., 2013	23 pts, 11 Lower MND, 39 NC	CS	Cognitive and behavioral assessment	Executive dysfunction: 30% of ALS pts disorganization and mental rigidity: 20% Dementia:13%. No correlation between cognitive and behavioral changes and clinical features
Crockford et al., 2018	161 pts, 80 NC	CS	Pts: ECAS; Disease stage : the King's Clinical Staging System CG behavioral interview	Behavioral impairment : 40% (firstly apathy). Higher number of behavioral features found across advancing stages.
Femiano et al., 2018	22 pts / 19 NC	CS	Apathy: AES, FrSBe, Global cognitive assessment, Brain imaging: DTI	No behavioral and cognitive impairment. Apathy inversely correlated to fractional anisotropy (FA) in several WM areas
Floeter et al., 2017	34 pts at BL, 6, 12, 18 mo	LS	Cognitive and behavioral manifestation in carriers of the mutation *C9orf72*: letter fluency and FBI-ALS	Symptomatic carriers decline at each evaluation on cognitive and behavioral functioning
Gibbons et al., 2008	16 pts	CS	The Manchester FTD Behavioral Interview of informants	Behavioral changes: 87%; FTD:8% Behavioral changes associated to bulbar palsy, but not to disease duration
Kasper et al., 2015	98 pts / 70 NC	CS	Executive cognitive, Executive behavior	70% of ci pts have executive dysfunction (initiation and shifting). Dominant bi is apathy
Grossman et al., 2007	45 pts	CS	FrSBe, Verbal fluency and DKEFS BDI	Changes in apathy scores. Apathy correlates with verbal fluency but not with depressive symptoms
Lillo et al., 2011	92 pts	CS	Self-report measures of motor function and mood CBI-R in 81 pts	Reduced motivation: 80 % (apathy in 41 %). Stereotypical and abnormal motor behaviors: 20 %; FTD: 11 %
Lillo et al., 2012	140 CG	CS	CG burden: Zarit Burden Interview CG mood: DASS, Pts behavioral changes: CBI-R	Behavioral changes in 10-40% of pts; Depression, anxiety in 20% of CG; high burden in 48% of CG; Strongest predictor of high CG burden = pts' abnormal behavior
Marconi et al., 2012	10 pts with tracheostomy and their CG	CS	Anxiety and depression with the HADS Personality of CG using the Big Five Questionnaire (BFQ)	A trend of aggression and high level of obsessiveness in ALS pts. High levels of anxiety in both pts and CG. Higher scores in the dimension of conscientiousness in CG
Mioshi et al., 2014	219 pts 20 pts at 6m	Co LS	MiND-B ALS-FRS	Neuropsychiatric symptoms appear before classic motor features. Not associated with survival. No significant change at 6 mo
Murphy J. M. et al., 2007	23 pts	CS	Neuropsychological and neurobehavioral assessment : ALS-CBS	No impairment: 11 pts; behavioral changes: 4; FTD: 5;other :3 (Alzheimer : 1)
Ohta et al., 2017	57 ALS, 5 ALS-FTD, 12 FTD, 35 NC	CS	Cognitive, behavioral, affective and activities of daily living assessment	FAB and MoCA useful to assess frontal cognitive impairments. ALS-FTD-Q useful to detect mild behavioral and affective disturbances.
Poletti et al., 2018	168 pts at BL 48 after 6 mo 18 after 12 mo 5 after 24 mo	LS	ECAS, FAB and MoCA BDI and STAI/Y	No cognitive deterioration across follow-ups. improvement of some ECAS scores over time due to possible practice effects. Apathy/Inertia = most common behavioral symptom, but no worsening over time.
Rabkin et al., 2016	247 pts	CS	Cognition-behavior : CBS Psychological : PHQ	40 % ci, 9% bi, 18% ci and bi, 12 % Major or minor depression; 12% Bi associated with depression
Radakovic et al., 2017	30 ALS pts / 29 NC	CS	Apathy subtypes with the self- and informant/carer-rated DAS, Cognition: ECAS Comprehensive neuropsychological battery	Increased Initiation apathy was the only significantly elevated subtype in ALS. Initiation apathy associated with verbal fluency deficit, and Emotional apathy, with emotional recognition deficits
Terada et al., 2011	24 pts	CS	Behavioral changes: the FrSBe, ALSFRS respiratory function, arterial blood gases	No correlation between FrSBe scores and ALSFRS, respiratory function, or arterial blood gases. Most frequent behavioral change: apathy

(Continued)

TABLE 3 | Continued

Authors/year	N	Type	Outcomes measures	Main findings
Tremolizzo et al., 2016	84 pts & CG	CS	Pts: ALSCBS-ci and –bi, FAB and BDI CG: BDI and CGBI.	CG burden correlates with pts behavioral but not cognitive changes. CG Burden correlates to CG depression
Unglik et al., 2018	152 pts	CS	EPN-31 (emotional feeling); HADS, The Marin's apathy evaluation scale Cognitive assessment: ALS-CBS scale.	Apathy: 42 %; related to negative emotions and negatively correlated to cognitive functioning and survival
Witgert et al., 2010	225 pts	CS	FrSBe, Comprehensive neuropsychological evaluation	Changes in the total score: 24.4% (firstly apathy). Cognitively impaired pts have worse total and apathy scores. Behavioral changes in 16 % of cognitively intact pts
Woolley et al., 2010	17 ALS 4 ALS-FTD	CS	Behavioral changes: FrSBe Pts' awareness of their behavioral changes	Not demented ALS pts have normal insight compared to FTD-ALS pts who have behavioral changes and no insight
Woolley et al., 2011	24 pts; 24 NC	CS	Apathy Brain imaging: DTI	Apathy correlated to FA in right anterior cingulum; not correlated with disease duration or respiratory dysfunction
Woolley et al., 2018	294 at BL 134 at 12 mo	LS	ALS-CBS, Verbal Fluency Index, Controlled Oral Word Association Test and FBI-ALS	No cognitive decline over time; Behavioral change, with increased disinhibition among patients with abnormal BL behavioral scores; BL behavioral problems associated with advanced, rapidly progressive disease

AES, Apathy Evaluation Scale; ALS-FRS, ALS Functional Rating Scale; BBI, Beaumont Behavioral Inventory;CBI, Cambridge Behavioral Inventory; CGBI, Caregiver Burden Inventory; DAS, Dimensional Apathy Scale; DASS, Depression,Anxiety and Stress Scale; DKEFS, Delis-Kaplan Executive Functioning Scales; EPN-31, Positive and Negative Emotionality Scale; HADS, Hospital Anxiety and Depression Scale; MND, Motor Neuron Disease; MoCA, Montreal Cognitive Assessment; PWI, Personal Wellbeing Index; WM, White Matter. For more please refer to previous tables.

that both apathetic and nonapathetic patients reported anxious and depressive symptoms, and the only significant difference between the two groups was that apathetic and anxious patients experienced more negative emotions, including sadness, shame and anger, than anxious patients without apathy.

The strongest predictor of high caregiver burden is patients' abnormal behavior (e.g., apathy and disinhibition), rather than physical disability (Lillo et al., 2012; Watermeyer et al., 2015; Tremolizzo et al., 2016). The level of depressive and anxious symptoms in caregivers is also correlated with behavioral changes (Watermeyer et al., 2015; Tremolizzo et al., 2016). Caregiver burden is further influenced by patients' everyday skills, motivation and memory, mostly because poor motivation, memory dysfunction, and difficulty performing activities of daily living require more support in the shape of direct supervision, prompting, or hands-on care (Andrews et al., 2017b).

Survival in ALS is highly influenced by the presence or absence of apathy, with a median survival time of 21.7 months in the case of moderate-to-severe apathy, 46.9 months in the case of mild apathy, and 51.9 months when apathy is absent (Caga et al., 2018). Apathy correlates negatively with survival time (Unglik et al., 2018).

Associated factors

Behavioral changes can occur either on their own or in the presence of a cognitive deficit (Murphy J. M. et al., 2007; Witgert et al., 2010). When cognitive impairment is present, behavioral symptoms seem to be greater (Witgert et al., 2010; Consonni et al., 2013). Apathy has been found to be associated with verbal fluency, leading some authors to suggest that apathy in ALS is underpinned by the medial prefrontal cortex (Grossman et al., 2007; Radakovic et al., 2017). Although Burke et al. (2017) found that social cognitive performances predicted behavioral changes, others have failed to do so (Terada et al., 2011).

Studies assessing relationships between behavioral changes and physical parameters have yielded somewhat conflicting results. Terada et al. (2011) found that apathy was correlated with the ALS condition *per se*, and not with the physical disability. Their population, however, consisted of only mildly disabled patients, who needed no assistance with activities of daily living, and all had normal blood gases. By contrast, in a larger sample of patients, with a wider range of physical impairments (although no details were given about their respiratory status), Ohta et al. (2017) did find a correlation between scores on the ALS-FTD Questionnaire and ALS Functional Rating Scale. Whereas the motor deficit may not directly impact behavior in mildly impaired patients, it is obviously mandatory to control for physical parameters, and first and foremost for blood gases, to avoid erroneously ascribing the consequences of hypercapnic encephalopathy to the cerebral neuronal involvement of ALS in the advanced stage.

Bock et al. (2017) found no association between bulbar or spinal onset and behavioral changes at baseline, whereas other authors (Gibbons et al., 2008; Crockford et al., 2018) have reported that bulbar palsy is associated with a higher rate of behavioral change.

Over Time

The way behavioral symptoms change as the disease progresses is still a matter of debate.

Most cross-sectional studies have shown no correlation between the severity of behavioral changes and time elapsed between disease onset and the time of study, suggesting that there is no significant decline over time (Woolley et al., 2011; Bock et al., 2017; Femiano et al., 2018). Crockford et al. (2018) assessed behavior in a sample of 149 patients using the ECAS caregiver behavioral interview and examined whether behavior was related to disease stages according to King's Clinical Staging System (stage 1 from stage 4 depending on the number of affected

bodily regions). Almost 40% of patients were behaviorally impaired (most of them with apathy) and a higher number of behavioral features was found across advancing stages. This result suggests that, contrary to what had been reported previously, behavioral and cognitive impairments are more severe in more severe disease stages.

Similarly, most of the longitudinal studies support the idea that behavioral manifestations over time may either increase (Bock et al., 2017) or appear as the illness progresses, with the emergence of frustration tolerance, reduced insight, mental rigidity, and lack of interest (Bock et al., 2017; Woolley et al., 2018). The severity of these disturbances increases faster over time when dementia is present in patients with the C9+ mutation (Floeter et al., 2017). Poletti et al. (2018) found no change over time in the prevalence of behavioral changes as measured with the ECAS, but did find an increase in behavioral disturbances as measured with the Frontal Behavioral Inventory, which is possibly a more sensitive tool, as it quantifies patients' performances, whereas the ECAS only indicates whether or not there are changes.

Neuroimaging Correlates

Most studies addressing the issue of the neural correlates of behavioral changes in ALS point to a significant correlation between apathy scores and prefrontal cortex atrophy, especially in the orbitofrontal and dorsolateral areas (Tsujimoto et al., 2011; Consonni et al., 2018), whereas disinhibition is negatively correlated with thickness of the right frontotemporal and cingulate cortices (Consonni et al., 2018).

Diffusion tensor imaging studies have shown a significant negative correlation between apathy scores and fractional anisotropy in the right anterior cingulate region, corpus callosum, bilateral amygdalae, left thalamus, and fornix, with atrophy of these brain regions (Woolley et al., 2011; Branco et al., 2018; Femiano et al., 2018). Correlations between apathy and the prefrontal cortex have also been found in other neurological diseases.

On the whole, the relationships between the anterior cingulate (and possibly some subcortical structures) and apathy, and between the anterior temporal lobe and disinhibition, appear quite consistent across studies.

PSYCHOLOGICAL ADJUSTMENT (TABLE 4)

Psychological Reactions and Wellbeing

Baseline

Chronic diseases induce a wide range of psychological responses, such as uncertainty about the future, anxiety, and depression. These psychological responses can have a major impact on health, through for example the perceived somatic symptom burden, adherence to treatment and compliance with care, malnutrition, and mortality (Cukor et al., 2006; Katon et al., 2007), and therefore need to be detected and supported.

Patients with ALS were initially described as *abnormally positive* (Brown and Mueller, 1970) and, in contrast to what is usually described in other serious chronic somatic diseases, had a relatively low prevalence of depressive disorders ranging from 0% (Rabkin et al., 2000; Bungener et al., 2005) to 10% (Hammer et al., 2008; McElhiney et al., 2009; Rabkin et al., 2016; Wei et al., 2016), according to their responses to semi-structured questionnaires based on international criteria for depression.

Nevertheless, these results have to be considered in the light of several additional factors. First, when patients are assessed at the extreme stages of the disease (either soon after diagnosis or at a very advanced stage), validated scales indicate that the prevalence of depressive symptoms is around 20% (Rabkin et al., 2005), and this figure rises to above 50% when self-report questionnaires are used (Wicks et al., 2007). Second, a history of depression before the onset of ALS increases the prevalence rate from 10 to 21% when patients are assessed with a semi-structured questionnaire (McElhiney et al., 2009; Ferentinos et al., 2011). An interesting study conducted by Roos et al. (2016) found that the risk of receiving a diagnosis of depression was increased during the year before and the year after the diagnosis of ALS. This is also true for other major psychiatric disorders, namely schizophrenia, bipolar disorder, and anxiety, some of which may predate the diagnosis of ALS by as much as 5 years (Turner et al., 2016). Self-report questionnaires designed to probe depressive symptoms may have their limits, insofar as they are not able to diagnose a depressive state with certainty, leading to potential overestimation of the rate of depression, but they do have the advantage of detecting the potential presence of depressive symptoms that reflect a degree of distress. Studies using this type of instrument report higher rates of depression of above 30% (Lulé et al., 2008; Atassi et al., 2011; Grehl et al., 2011; Carvalho et al., 2016). Thus, even when the official diagnostic criteria are not met, it does not mean that patients do not feel they are affected by their disease. One must thus be aware that depression rates in ALS vary greatly, depending on the tools used to assess it. Finally, studies comparing the rate of depression in patients with ALS versus other chronically and seriously ill patients (e.g., with neuromuscular disease or receiving palliative care for cancer) have failed to find any significant differences, with 8–10% of patients in each group having a diagnosis of major depression according to DSM-IV criteria, and 50% mild-to-moderate depressive symptoms, as measured with the Beck Depressive Inventory (Taylor et al., 2010; Lulé et al., 2012).

Furthermore, more than one third of patients with ALS are on antidepressants (Pisa et al., 2015). Not all studies assessing the prevalence of depression in ALS take this fact into account, which could induce a bias and result in underestimation of the prevalence of depression in this population.

Intuitively, one might assume that the worsening of physical disability increases signs of depression. However, many studies have found either no such link or an inverse link between the severity of the motor disability and scores on mood scales (Lulé et al., 2008; Jelsone-Swain et al., 2012; Chen et al., 2015; Thakore and Pioro, 2016; Wei et al., 2016). This is only true, however, for the consequences of spinal involvement, as the presence of bulbar symptoms, or of breathing difficulty, does increase depressive

TABLE 4 | Psychosocial adjustment and coping in ALS.

Authors/year	Number of pts	Type of study	Outcomes measures	Main findings
Albert et al., 2005	53 pts	CS	Prevalence of wish to die and its determinants	18.9% express the wish to die. More likely to have depression, less optimism, less comfort in religion, and greater hopelessness. 5.7% having hastened dying reported reduction in suffering in the final weeks of life
Atassi et al., 2011	127 pts	CS	Depression: ADI-12	29% moderate or severe depression, not correlated to disease duration
Brown and Mueller, 1970	10 pts/controls with chronic diseases	CS	IECS MAACL MMPI	Active masterful behavior. Exclusion of dysphoric affect from awareness. Independence and competent behavior
Bungener et al., 2005	27 pts	CS	DSM-IV, Covi anxiety scale, MADRS, Depressive Mood Scale	No severe depression or anxiety. Emotional reactions in the first 6 months after diagnostic disclosure
Caga et al., 2015	27 pts	CS	DASS-21	Lengthy diagnostic interval / higher depressive symptoms
Chen et al., 2015	93 pts and CG	CS	Depression and anxiety: Hamilton depression and anxiety scales	Depression and anxiety rates in pts correlate with CG but not with disease duration or physical incapacity
Chiò et al., 2005	60 pts / 60 CG	CS	CG burden: CBI QoL: MQoL Depression: ZDS Perceived Burden: SPBS	Depression: 18% pts,7% CG CG burden // CG's mood and pt's physical disability. Depression of CG/pt correlate
Cui et al., 2015	100 pts / 100 NC		Cognition: MMSE Anxiety: SAS Depression: SDS Functional state: ALSFRS	MMSE negatively correlated with disease duration and ALS-FRS. Higher depression and anxiety in pts than in NC
De Groot et al., 2007	73 pts / general population	Prospective cohort study BL, 6 & 12 mo	Measure of QoL: SF-36 Functional disability: ALS-FRS	QoL lower than controls (Physical Functioning, Role Physical, Social Functioning) but stable over time
Fang et al., 2008	6642 pts	Population-based cohort study	Suicide rate in ALS pts / general population	Suicide risk 6 x in ALS pts. Higher in younger pts and 1st year after 1st hospital stay
Ferentinos et al., 2011	37	CS	Depression: SCID-IV, BDI, HADS, ADI-12 and CES-D	21-25% major depression with SCID, CES-D and BDI
Ganzini et al., 1998	100 pts / CG	Prospective cohort	Determination of pts and caregivers' attitude toward assisted suicide	56% would consider suicide. Men, higher education, less religiosity, higher scores for hopelessness, lower QoL increase positive attitude towards assisted suicide 73%: pts and caregivers have the same point of view
Gauthier et al., 2007	31 pts / 31 CG	LS: BL – 9 mo	Depression: ZDS QoL: (MQoL) Caregivers' burden: CBI Perceived burden: SPBS	Depression and QoL stable over time in pts but Depression and burden increase in CG
Goldstein et al., 2006b	50 CG at BL, 21 on follow-up	LS BL-follow-up 6 mo intervals	Mood, burden and strain, Social support and marital relationship	Main predictor of distress in ALS pts CG over time is poor social support
Goldstein et al., 2006a	50 at BL 26 over time	LS: BL, 6 and 11 mo	Predictors of psychological distress	Affective state and self-esteem predicted by social support and pre-illness marital intimacy
Goldstein et al., 1998	19 pts / 19 CG	CS	Psychological distress in pts and CG and their determinants	In pts: anxiety and depression correlate to physical disability In CG: distress depends on pts' funct. impairment, and intimacy loss. Perceived good social support correlates to future ability to cope
Grehl et al., 2011	41 pts / 41 relatives	CS	Depression with ADL-12; QoL with MLDL in pts and relatives	Mood and QoL correlate between pts and relatives but not to functional impairment
Hammer et al., 2008	39 pts	CS	Assessment of depression by DSMIV, BDI and ADI-12 scales	10 % depressed by SCID ADI-12 recommended for screening depression in ALS
Hillemacher et al., 2004	41 pts	CS	Depressive symptoms Correlation to the ALS-FRS, disease duration, age and sex	Depression correlated with swallowing and breathing but not with age, sex or ALS-FRS; depression correlated with duration
Jakobsson Larsson et al., 2016	36 pts	LS: BL and at 5 time over a period of 2 years	Coping strategies: with Motor Neuron Disease Coping Scale Well-being: Hospital Anxiety and Depression Scale Physical abilities	No changes over time in coping strategies; Psychological state correlates with some coping items (e.g. negative correlation with depressive symptoms and "positive action, positive thinking and independence")

(Continued)

TABLE 4 | Continued

Authors/year	Number of pts	Type of study	Outcomes measures	Main findings
Jakobsson Larsson et al., 2017	36 pts	Longitudinal with a follow up periode of 2 years	QoL: SEIQoL-DW; Emotional distress: HADS	Anxiety 11%, depression 5% early on after diagnosis Anxiety decreases over time QoL related to depression soon after diagnosis
Jelsone-Swain et al., 2012	22 pts / 17 NC	CS	Neuropsychological assessement Depression: GDS, BDI	Cognitive tests: Pts < NC. No influence of depression. Depression correlated with limb function
Houpt et al., 1977	40 pts	CS	Patient's control: IECS; Depression: BDI, MAACL Denial	Depression 22%. Dysphoria frequently found. No specific use of denial or internal locus of control
Lillo et al., 2012	140 CG	CS	CG burden: Zarit Burden Interview CG mood: DASS-21 Pts; behavior: CBI-R	Behavioral changes in 10–40% of pts; Depression, anxiety in 20% of CG; high burden in 48% of CG; Strongest predictor of high CG burden = pts' abnormal behavior
Lou et al., 2003	25 ALS / 22 NC	CS	Fatigue and depression: MQoL CES-D	Fatigue and depression higher in pts/NC Associated with poorer QoL
Lulé et al., 2008	1: 39 pts 2: 30 pts / 30 NC	CS: pts / NC LS: BL and 80 - 100 days later	Depression with ADI-12; QoL with SEIQoL-DW	Depression 28% not correlated to physical impairment QoL = NC and not correlated to physical impairment
Lulé et al., 2012	30 ALS pts 29 cancer pts 29 NC	CS	Depression: BDI; QoL: SEIQoL-DW Coping strategy: Jerusalem Coping scale	Good psychosocial adjustment and subjective QoL in both patient groups
Matuz et al., 2010	27 pts	CS	Depression, QoL Predictors: social support, cognitive appraisal, coping strategies	Perceived social support predicts depression and QoL. Appraisal of coping potential predicts depression. No impact of physical status
Matuz et al., 2015	27 pts	Longitudinal with four evaluation in 2 years	QoL; Depression Social support, cognitive appraisals, and coping strategies	Social support, cognitive appraisals, coping strategies are the best predictors of QoL and depression
McElhiney et al., 2009	223 at BL 113 at 3 mo 65 final visit	LS	Fatigue and depression prevalence at BL, 3 and 6 mo y PHQ-9 interview	Fatigue associated to severity and more prevalent and persistent than depression
McElhiney et al., 2014	81 pts / 81 CG	LS: BL, 3 mo, 6 mo	ALS-FRS, QoL and Goal Assessment Scale (GAS)	QoL, GAS: no consistent correlations with ALSFRS-R change
Miglioretti et al., 2008	74 pts	CS	ALS -FRS Illness representation: common sense model	QoL, mood, and illness representation correlate with functional state and respiratory capacity.
Rabkin et al., 2000	56 pts, 31 CG	LS BL / 3-8 mo	Pts: DSM-IV, BDI, STAI, QoL, outlook about future and ZARIT caregiver burden	Pts: major depression 2% by DSM-IV and 28% by BDI. Psychological distress not related to illness progression CG: low rate of depression but high perceived burden
Rabkin et al., 2005	80 pts BL / 61 pts follow-up	LS / monthly for 15 mo	Prevalence of depression over time: PHQ and BDI	20% depression increasing to 31% before death
Rabkin et al., 2009	71 pts / 71 CG	LS: BL and monthly for 51 mo	Depressive symptoms, DSM-IV disorders, Coping strategies Caregiver burden satisfaction with care-giving	CG burden & depression // Reliance on avoidance, perceived burden, fatigue, feeling that pt critical and unappreciative; long-term mechanical ventilation; pts' plans and supportiveness
Rabkin et al., 2015	329 pts	CS	Prevalence of depression and wish to die at BL	Depression 12%, related to ALSFRS and motor strength. Wish to die 19% but only 1/3 of which depressed
Rabkin et al., 2016	247 pts	CS	Cognitive, behavioral or, mood impairment by CBS and PHQ9	Cognitive impairment:40 %; Behavioral impairment:9%; Both:18%; depression:12 % Behavioral impairment associated to depression
Roach et al., 2009	55 pts / 53 CG	LS	QoL: MQoL	Pt's Qol: no change over time Total QoL and QoL related to physical symptoms decline in CGs; younger CG = lower QoL
Roos et al., 2016	1752 ALS pts and 8760 NC	R	Depression: ICD-10 and use of antidepressants	Higher risk of depression the year before and the year after the diagnosis of ALS
Sandstedt et al., 2016	60 pts	CS	HRQL/ disease severity, fatigue, anxiety, depression, social activities, coping and mechanical ventilation	Severe disease, weak coping capacity, fatigue, mechanical ventilator and anxiety and/or depression associated with worse HRQL
Siciliano et al., 2017	96 CG	CG	Burden, depression and anxiety Coping strategy: CISS Pts' cognition/behavior	Burden, anxiety, depression in CG related to: emotion-oriented coping strategy and Pts' functional dependence

(Continued)

TABLE 4 | Continued

Authors/year	Number of pts	Type of study	Outcomes measures	Main findings
Taylor et al., 2010	51 ALS pts 39 other neuromuscular disorders		Depression: BDI, HADS and MDI	Same depression rates in both groups
Thakore and Pioro, 2016	964	Retrospective cohort	Depression: PHQ-9 and its associated factor	Depression 49 %. High PHQ-9 scores predict mortality. PHQ-9 correlates with QoL. Depression correlated with pseudobulbar symptoms and advanced disease
Turner et al., 2016	National psychiatric database / reference cohort	R	Evaluation of the risk to develop ALS in psychiatric pts	Psychiatric disease, especially bipolar disorder and schizophrenia = higher risk to develop ALS, mainly the year after psychiatric illness onset
Verschueren et al., 2018	71 pts	Prospective, observational cohort study	Depression: BDI Columbia Suicide severity rating scale Reasons for Living inventory for adults.	39% express either passive or active suicidal ideation. Depressive symptoms, worse disability and coping beliefs scores more present in pts expressing suicidal ideation
Vignola et al., 2008	75 pts / CG	LS	Depression: ZDS Anxiety: STAI	High anxiety in pts and CG during the diagnostic phase QoL decreases in CG but not in pts at follow-up
Wei et al., 2016	91 pts	CS	Neuropsychiatric symptoms and cognition: NPI, ACE-R, FAB	Depression 59%, anxiety 41%, lability 26%. NPI correlates with ACE-R but not with FAB
Wicks et al., 2007	104	CS	Depression: BDI, HADS Anxiety: STAi	Depression: 54% with BDI, 25% with HADS Anxiety 35% state 8% trait

ADI, Assessment of Depression Inventory; CES-D, Center For Epidemiologic Studies Depression Scale; CISS, Coping Inventory For Stressful Situations; DSM, Diagnostic and Statistical Manual of Mental Disorders; GDS, Global Deterioration Scale; ICD-10, International Classification of Diseases; IECS, Internal-External Control Scale; HRQL, Health-Related Quality of Life; MAACL, Multiple Affect Adjective Check List; MLDL, Munich Quality-of-Life Dimensions List; MMSE, Mini-Mental State Examination; MQoL, Mcgill Quality-of-Life Questionnaire; SAS, Self-Rating Anxiety Scale; SCID, Structured Clinical Interview For DSM-IV; SDS, Zung Self-Rating Depression Scale; SeiQoL-DW, Schedule For The Evaluation of Individual Quality of Life; SPBS, Self-Perceived Burden Scale; ZDS, Zung Depression Scale. For more please refer to previous tables.

symptoms (Hillemacher et al., 2004; Goldstein et al., 2006a; Miglioretti et al., 2008; Jelsone-Swain et al., 2012).

Studies looking for a link between disease duration and the severity of depressive symptoms have reported contradictory results, with some finding a positive correlation, some a negative one (Cui et al., 2015; Hillemacher et al., 2004, and others no link at all (Atassi et al., 2011; Caga et al., 2015).

Severe somatic diseases may induce a number of other psychological reactions, such as anxiety, hopelessness, or suicidal thoughts. Kurt et al. (2007) found that the prevalence of anxiety in ALS ranged from 0 to 30%. Vignola et al. (2008) reported that almost 75% of patients experienced moderate-to-severe state anxiety at baseline, which was correlated with trait anxiety. However, Pagnini et al. (2012) found that only 20% of patients had scores above the anxiety cut off. Suicidal thoughts are not rare in patients, ranging from 19 to 39% across studies (Albert et al., 2005; Rabkin et al., 2015; Verschueren et al., 2018). This rate rises to more than 50% when assisted suicide is considered (Ganzini et al., 1998). Patients have an almost 6-fold higher risk of suicide, especially in the first year after symptom onset and when they are younger (Fang et al., 2008). A wish to die is not always associated with a depressive state (Albert et al., 2005) but it is linked to less optimism, less comfort in religion, and greater hopelessness (Albert et al., 2005; Verschueren et al., 2018).

In addition to psychological signs of distress such as depression or anxiety, the estimation of wellbeing is based on the person's satisfaction with his or her QoL. In patients with ALS, QoL is found to be high, especially when the measurement scales are adapted to the disease (Norris et al., 2010; Jakobsson Larsson et al., 2017) and avoid lending too much importance to the patient's physical state (Norris et al., 2010; Jakobsson Larsson et al., 2017). The Amyotrophic Lateral Sclerosis Quality of Life (ALSSQOL) was specifically designed for ALS, and has a revised and a short form (Felgoise et al., 2018) that give a balanced appraisal. Qol depends not only on patients' physical disabilities, but also on their religiosity/spirituality and sociability (Norris et al., 2010; Simmons, 2015). Other factors influencing QoL are anxiety and depression, the ability to cope with physical disabilities, fatigue, and hopelessness (Lou et al., 2003; Abe, 2004; Pagnini et al., 2012; Sandstedt et al., 2016; Jakobsson Larsson et al., 2017).

Over Time

Surprisingly, longitudinal studies of changes in patients' mood as the disease progresses report either stability (Rabkin et al., 2005; Gauthier et al., 2007; McElhiney et al., 2009; Matuz et al., 2015) or a decrease in the depression rate (McElhiney et al., 2014). When Goldstein et al. (2006a) assessed psychosocial factors influencing patients' psychological state (level of depression and anxiety) at baseline and 6 and 11 months later, they found that the quality of premarital intimacy and social support at disease onset influenced the psychological wellbeing of patients in the more advanced stages of the disease. Anxiety is particularly high in the diagnostic phase, but tends to decrease thereafter in patients, though not in caregivers (Vignola et al., 2008). Importantly, these authors also found that in caregivers, state anxiety was linked to trait anxiety, whereas in patients, it was correlated with clinical features such as a shorter disease course and the presence of depression.

Together with other factors, anxiety negatively impacts QoL (Jakobsson Larsson et al., 2017).

Caregivers' psychological distress and perceived burden is often reported to increase over time (Goldstein et al., 2006b; Gauthier et al., 2007). The burden is determined by a combination of factors, including both the caregiver's own personality and the patient's characteristics. Regarding the latter, Lillo et al. (2012) found that behavioral changes are a greater determinant of caregivers' wellbeing than physical disability, although motor impairment also plays a part, according to Chiò et al. (2005). Behavioral changes may include apathy, loss of empathy, and a lack of appreciation of the efforts made to satisfy their needs (Rabkin et al., 2009). As the disease progresses, patients' plans for future, particularly regarding life support, may also play a part and influence their caregivers' QoL. A longitudinal study by Rabkin et al. (2009) found that caregivers tended to be less depressed as the disease advanced, regardless of the outcome (death or tracheostomy), whereas the depression scores of patients remained stable.

Quality of life as reported by patients remains stable even in the advanced stages of the disease (Rabkin et al., 2000; De Groot et al., 2007; Roach et al., 2009). Religiosity, social support, level of anxiety and depression remain the mean determinants of wellbeing, despite the increase in motor impairment (Jakobsson Larsson et al., 2017).

Adaptive Mechanisms in the Face of the Disease

To explain the fact that patients with ALS have a relatively good adaptive reaction to their illness, some authors have suggested that they are in denial of the disease, thus protecting them from depression (Brown and Mueller, 1970; Miglioretti et al., 2008), but this hypothesis has not been confirmed (Houpt et al., 1977).

Psychological adjustment refers to the psychological processes that take place in response to a stressful situation like chronic illness and associated treatment. Various models have been developed, including the stress coping model (Lazarus and Folkman, 1984), illness representation model, adaptive tasks and coping model (Moos and Holahan, 2007), and adjustment model (Moss-Morris, 2013). Based on Lazarus and Folkman's theoretical framework, Matuz et al. (2010) developed an interesting integrative model of patients' adaptation in the face of the disease, whereby patients' mood state and QoL are influenced by social support, cognitive appraisal and coping strategies. The latter include problem management, problem appraisal, emotion regulation, and emotional avoidance. *Social support* encompasses perceived social support, received social support, and need for social support. Matuz et al. (2010) found that patients used emotion regulation and looked for social support, and the more they used emotion-focused strategies, the lower they scored on a depression scale. When Lulé et al. (2012) compared patients with ALS and patients with cancer on psychological adjustment, they failed to find any significant differences in the use of coping strategies, even if, on average, the ALS group scored lower than the cancer group on a scale measuring active coping strategies (thinking about the situation and trying to solve it, taking an adequate step to deal with their condition). By contrast, depressive symptoms and a high level of burden are more often present in caregivers if they use emotion-focused coping strategies (Siciliano et al., 2017). Factors that have been shown to help caregivers cope with the impact of the disease include social support, as well as anticipatory coping with foreseeable difficulties (Goldstein et al., 1998).

Studies addressing changes in psychological adjustment strategies in ALS over time have yielded conflicting results. Jakobsson Larsson et al. (2016) found that the patients used the same coping strategies throughout the disease course, namely support seeking, positive action, independence, and positive thinking. By contrast, Matuz et al. (2015) reported that while patients used both problem- and emotion-focused coping strategies at disease onset, they used less emotion regulation later on. On the other hand, they continued to have higher perceived social support and an accurate assessment of their own coping potential. These two factors were correlated with depressive scores at follow up. *Perceived social support*, which reflects patients' view about the amount and quality of support they receive, can help patients use effective coping strategies, encourage positive health behaviors, and reduce physiological reactivity to stress. A positive subjective appraisal of one's coping potential indicates that patients feel they are keeping control over their state, which probably decreases their anxiety over physical loss.

At a time when some people advocate legalizing euthanasia for intractable disease, accurate knowledge of the mechanisms by which many patients with ALS succeed in coping with such a dreadful disease is crucial, and could greatly enhance the assistance given to both patients and caregivers in their daily struggle.

CONCLUSION

The objective of this review was to provide readers with a clear picture of all the manifestations of ALS both at baseline and throughout the course of the disease. Unlike the relentless physical deterioration, cognitive, behavioral and psychological changes are extremely variable. The heterogeneity of clinical situations, and in particular the variety of symptoms present at baseline, seems to strongly influence patients' clinical course. Regarding their psychological reactions, even if they rarely develop a severe psychiatric illness, they can experience considerable distress, especially at the time of diagnosis and in the advanced stage of the disease. Caution must be taken not to minimize these psychological reactions and to give patients the best possible personalized support at these times. A careful examination of the psychological trajectory shows that the increase in motor disability is not the only determinant of psychological wellbeing. The quality of social support and the use of appropriate adaptive strategies enable patients to cope with their condition and to maintain good psychosocial functioning as the disease progresses.

This review also illustrates the theoretical and practical difficulties that may arise when investigating cognitive, behavioral

and psychological aspects of ALS. The first concerns the tools that are to be used. First, these must be adapted to the physical disability of patients with ALS. Potentially confounding variables, such as respiratory insufficiency, have to be controlled. As we have seen, a large number of tests have been used by the different authors. Such a diversity makes comparisons difficult and may at least partially account for the discrepancies that are often observed across studies. Investigators will have eventually to try to agree about which tests should be used, either as routine tests (such as the ECAS for the cognitive assessment, or ALS-FRS or the bulbar Norris scale for physical parameters) or for more specific purposes. It will also be interesting to further study less extensively explored cognitive domains such as memory or social cognition.

Another difficulty is that of longitudinal studies. These are particularly challenging, because of the major physical changes that occur over time. It is not clearly known, for example, whether a cognitive impairment, if any, worsens in line with the physical disability or not. Also, the short life expectancy of many patients is responsible for a substantial drop-out rate. These difficulties can be addressed by recruiting as many patients as possible, preferably in the setting of ALS centers. Bigger samples may also help to tackle the problem of physical and neuropsychological heterogeneity. For example, the seemingly simple question as to whether bulbar-onset patients are more cognitively affected than spinal-onset ones or not is still debated. Likewise, there have been very few studies about the relationships between the cognitive (or psychological, or behavioral, for that matter) and physical profiles of patients. Finally, morphological and functional cerebral imaging studies should be increasingly undertaken in order to learn more about the mechanisms of neuropsychological impairment, and to improve as much as possible the care of patients with ALS.

AUTHOR CONTRIBUTIONS

BD, SB, and FV contributed to conceptualization and methodology. SB wrote the first draft of the manuscript. BD, FV, and FE reviewed the manuscript. SB, FV, and FE edited the manuscript.

ACKNOWLEDGMENTS

We would like to thank Mrs. Elizabeth Portier for assisting in language editing.

REFERENCES

Abdulla, S., Machts, J., Kaufmann, J., Patrick, K., Kollewe, K., Dengler, R., et al. (2014). Hippocampal degeneration in patients with amyotrophic lateral sclerosis. *Neurobiol. Aging* 35, 2639–2645. doi: 10.1016/j.neurobiolaging.2014.05.035

Abe, K. (2004). Fatigue and depression are associated with poor quality of life in ALS. *Neurology* 60, 122–123. doi: 10.1212/01.wnl.0000042781.22278.0a

Abe, K., Fujimura, H., Toyooka, K., Sakoda, S., Yorifuji, S., and Yanagihara, T. (1997). Cognitive function in amyotrophic lateral sclerosis. *J. Neurol. Sci.* 148, 95–100.

Abrahams, S., Leigh, P. N., and Goldstein, L. H. (2005). Cognitive change in ALS: a prospective study. *Neurology* 64, 1222–1226. doi: 10.1212/01.WNL.0000156519.41681.27

Abrahams, S., Leigh, P. N., Harvey, A., Vythelingum, G. N., Grisé, D., and Goldstein, L. H. (2000). Verbal fluency and executive dysfunction in amyotrophic lateral sclerosis (ALS). *Neuropsychologia* 38, 734–747. doi: 10.1016/s0028-3932(99)00146-3

Agosta, F., Ferraro, P. M., Riva, N., Spinelli, E. G., Chiò, A., Canu, E., et al. (2016). Structural brain correlates of cognitive and behavioral impairment in MND. *Hum. Brain Mapp.* 37, 1614–1626. doi: 10.1002/hbm.23124

Aho-Özhan, H. E. A., Keller, J., Heimrath, J., Uttner, I., Kassubek, J., Birbaumer, N., et al. (2016). Perception of emotional facial expressions in amyotrophic lateral sclerosis (ALS) at behavioural and brain metabolic level. *PLoS One* 11:e0164655. doi: 10.1371/journal.pone.0164655

Albert, S. M., Rabkin, J. G., Del Bene, M. L., Tider, T., O'Sullivan, I., Rowland, L. P., et al. (2005). Wish to die in end-stage ALS. *Neurology* 65, 68–74. doi: 10.1212/01.wnl.0000168161.54833.bb

Andrews, S. C., Pavlis, A., Staios, M., and Fisher, F. (2017a). Which behaviours? Identifying the most common and burdensome behaviour changes in amyotrophic lateral sclerosis. *Psychol. Health Med.* 22, 483–492. doi: 10.1080/13548506.2016.1164871

Andrews, S. C., Staios, M., Howe, J., Reardon, K., and Fisher, F. (2017b). Multimodal emotion processing deficits are present in amyotrophic lateral sclerosis. *Neuropsychology* 31, 304–310. doi: 10.1037/neu0000323

Atassi, N., Cook, A., Pineda, C. M. E., Yerramilli-Rao, P., Pulley, D., and Cudkowicz, M. (2011). Depression in amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* 12, 109–112. doi: 10.3109/17482968.2010.536839

Beeldman, E., Raaphorst, J., Klein Twennaar, M., de Visser, M., Schmand, B. A., and de Haan, R. J. (2016). The cognitive profile of ALS: a systematic review and meta-analysis update. *J. Neurol. Neurosurg. Psychiatr.* 87, 611–619. doi: 10.1136/jnnp-2015-310734

Benbrika, S., Doidy, F., Carluer, L., Mondou, A., Buhour, M.-S., Eustache, F., et al. (2018). Alexithymia in amyotrophic lateral sclerosis and its neural correlates. *Front. Neurol.* 9:566. doi: 10.3389/fneur.2018.00566

Bock, M., Duong, Y.-N., Kim, A., Allen, I., Murphy, J., and Lomen-Hoerth, C. (2016). Cognitive-behavioral changes in amyotrophic lateral sclerosis: screening prevalence and impact on patients and caregivers. *Amyotroph. Lateral Scler. Front. Degener.* 17, 366–373. doi: 10.3109/21678421.2016.1165257

Bock, M., Duong, Y.-N., Kim, A., Allen, I., Murphy, J., and Lomen-Hoerth, C. (2017). Progression and effect of cognitive-behavioral changes in patients with amyotrophic lateral sclerosis. *Neurol. Clin. Pract.* 7, 488–498. doi: 10.1212/CPJ.0000000000000397

Bora, E. (2017). Meta-analysis of social cognition in amyotrophic lateral sclerosis. *Cortex J. Devoted Study Nerv. Syst. Behav.* 88, 1–7. doi: 10.1016/j.cortex.2016.11.012

Bortolato, B., Miskowiak, K. W., Köhler, C. A., Maes, M., Fernandes, B. S., Berk, M., et al. (2016). Cognitive remission: a novel objective for the treatment of major depression? *BMC Med.* 14:9. doi: 10.1186/s12916-016-0560-3

Braber, W. (2016). *Measurements of Cognitive Functioning in ALS Patients: a Longitudinal Study [WWW Document]*. Available at: http://dspace.library.uu.nl/handle/1874/340869 (accessed February.19, 2019)

Branco, L. M. T., de Rezende, T. J. R., Roversi, C. O., Zanao, T., Casseb, R. F., de Campos, B. M., et al. (2018). Brain signature of mild stages of cognitive and behavioral impairment in amyotrophic lateral sclerosis. *Psychiatr. Res. Neuroimaging* 272, 58–64. doi: 10.1016/j.pscychresns.2017.11.010

Brown, W. A., and Mueller, P. S. (1970). Psychological function in individuals with amyotrophic lateral sclerosis (ALS). *Psychosom. Med.* 32, 141–152. doi: 10.1097/00006842-197003000-00002

Buhour, M.-S., Doidy, F., Mondou, A., Pélerin, A., Carluer, L., Eustache, F., et al. (2017). Voxel-based mapping of grey matter volume and glucose metabolism

profiles in amyotrophic lateral sclerosis. *EJNMMI Res.* 7, 21. doi: 10.1186/s13550-017-0267-2

Bungener, C., Piquard, A., Pradat, P.-F., Salachas, F., Meininger, V., and Lacomblez, L. (2005). Psychopathology in amyotrophic lateral sclerosis: a preliminary study with 27 ALS patients. *Amyotroph. Lateral Scler. Mot. Neuron Disord.* 6, 221–225. doi: 10.1080/14660820510037863

Burke, T., Elamin, M., Galvin, M., Hardiman, O., and Pender, N. (2015). Caregiver burden in amyotrophic lateral sclerosis: a cross-sectional investigation of predictors. *J. Neurol.* 262, 1526–1532. doi: 10.1007/s00415-015-7746-z

Burke, T., Pinto-Grau, M., Lonergan, K., Bede, P., O'Sullivan, M., Heverin, M., et al. (2017). A Cross-sectional population-based investigation into behavioral change in amyotrophic lateral sclerosis: subphenotypes, staging, cognitive predictors, and survival. *Ann. Clin. Transl. Neurol.* 4, 305–317. doi: 10.1002/acn3.407

Burke, T., Pinto-Grau, M., Lonergan, K., Elamin, M., Bede, P., Costello, E., et al. (2016). Measurement of social cognition in amyotrophic lateral sclerosis: a population based study. *PLoS One* 11:e0160850. doi: 10.1371/journal.pone.0160850

Burkhardt, C., Neuwirth, C., and Weber, M. (2017). Longitudinal assessment of the edinburgh cognitive and behavioural amyotrophic lateral sclerosis screen (ECAS): lack of practice effect in ALS patients? *Amyotroph. Lateral Scler. Front. Degener.* 18, 202–209. doi: 10.1080/21678421.2017.1283418

Byrne, S., Elamin, M., Bede, P., Shatunov, A., Walsh, C., Corr, B., et al. (2012). Cognitive and clinical characteristics of patients with amyotrophic lateral sclerosis carrying a C9orf72 repeat expansion: a population-based cohort study. *Lancet Neurol.* 11, 232–240. doi: 10.1016/S1474-4422(12)70014-5

Caga, J., Hsieh, S., Highton-Williamson, E., Zoing, M. C., Ramsey, E., Devenney, E., et al. (2018). Apathy and its impact on patient outcome in amyotrophic lateral sclerosis. *J. Neurol.* 265, 187–193. doi: 10.1007/s00415-017-8688-4

Caga, J., Ramsey, E., Hogden, A., Mioshi, E., and Kiernan, M. C. (2015). A longer diagnostic interval is a risk for depression in amyotrophic lateral sclerosis. *Palliat. Support. Care* 13, 1019–1024. doi: 10.1017/S1478951514000881

Canosa, A., Pagani, M., Cistaro, A., Montuschi, A., Iazzolino, B., Fania, P., et al. (2016). 18F-FDG-PET correlates of cognitive impairment in ALS. *Neurology* 86, 44–49. doi: 10.1212/WNL.0000000000002242

Carelli, L., Solca, F., Faini, A., Madotto, F., Lafronza, A., Monti, A., et al. (2018). The complex interplay between depression/anxiety and executive functioning: insights from the ECAS in a LARGE ALS population. *Front. Psychol.* 9:450. doi: 10.3389/fpsyg.2018.00450

Carluer, L., Mondou, A., Buhour, M.-S., Laisney, M., Pélerin, A., Eustache, F., et al. (2015). Neural substrate of cognitive theory of mind impairment in amyotrophic lateral sclerosis. *Cortex J. Devoted Study Nerv. Syst. Behav.* 65, 19–30. doi: 10.1016/j.cortex.2014.12.010

Carvalho, T. L., de Almeida, L. M. S., Lorega, C. M. A., Barata, M. F. O., Ferreira, M. L. B., de Brito-Marques, P. R., et al. (2016). Depression and anxiety in individuals with amyotrophic lateral sclerosis: a systematic review. *Trends Psychiatry Psychother.* 38, 1–5. doi: 10.1590/2237-6089-2015-0030

Cavallo, M., Adenzato, M., Macpherson, S. E., Karwig, G., Enrici, I., and Abrahams, S. (2011). Evidence of social understanding impairment in patients with amyotrophic lateral sclerosis. *PLoS One* 6:e25948. doi: 10.1371/journal.pone.0025948

Cerami, C., Dodich, A., Canessa, N., Crespi, C., Iannaccone, S., Corbo, M., et al. (2014). Emotional empathy in amyotrophic lateral sclerosis: a behavioural and voxel-based morphometry study. *Amyotroph. Lateral Scler. Front. Degener.* 15, 21–29. doi: 10.3109/21678421.2013.785568

Chen, D., Guo, X., Zheng, Z., Wei, Q., Song, W., Cao, B., et al. (2015). Depression and anxiety in amyotrophic lateral sclerosis: correlations between the distress of patients and caregivers. *Muscle Nerve* 51, 353–357. doi: 10.1002/mus.24325

Chiò, A., Gauthier, A., Calvo, A., Ghiglione, P., and Mutani, R. (2005). Caregiver burden and patients' perception of being a burden in ALS. *Neurology* 64, 1780–1782. doi: 10.1212/01.WNL.0000162034.06268.37

Christidi, F., Karavasilis, E., Riederer, F., Zalonis, I., Ferentinos, P., Velonakis, G., et al. (2018). Gray matter and white matter changes in non-demented amyotrophic lateral sclerosis patients with or without cognitive impairment: a combined voxel-based morphometry and tract-based spatial statistics whole-brain analysis. *Brain Imag. Behav.* 12, 547–563. doi: 10.1007/s11682-017-9722-y

Cistaro, A., Valentini, M. C., Chiò, A., Nobili, F., Calvo, A., Moglia, C., et al. (2012). Brain hypermetabolism in amyotrophic lateral sclerosis: a FDG PET study in ALS of spinal and bulbar onset. *Eur. J. Nucl. Med. Mol. Imag.* 39, 251–259. doi: 10.1007/s00259-011-1979-6

Consonni, M., Cappa, S. F., Dalla Bella, E., Contarino, V. E., and Lauria, G. (2018). Cortical correlates of behavioural change in amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatr.* [Epub ahead of print].

Consonni, M., Iannaccone, S., Cerami, C., Frasson, P., Lacerenza, M., Lunetta, C., et al. (2013). The cognitive and behavioural profile of amyotrophic lateral sclerosis: application of the consensus criteria. *Behav. Neurol.* 27, 143–153. doi: 10.3233/BEN-2012-110202

Couratier, P., Corcia, P., Lautrette, G., Nicol, M., Preux, P.-M., and Marin, B. (2016). Epidemiology of amyotrophic lateral sclerosis: a review of literature. *Rev. Neurol.* 172, 37–45. doi: 10.1016/j.neurol.2015.11.002

Crespi, C., Cerami, C., Dodich, A., Canessa, N., Arpone, M., Iannaccone, S., et al. (2014). Microstructural white matter correlates of emotion recognition impairment in amyotrophic lateral sclerosis. *Cortex J. Devoted Study Nerv. Syst. Behav.* 53, 1–8. doi: 10.1016/j.cortex.2014.01.002

Crockford, C., Newton, J., Lonergan, K., Chiwera, T., Booth, T., Chandran, S., et al. (2018). ALS-specific cognitive and behavior changes associated with advancing disease stage in ALS. *Neurology* 91, e1370–e1380. doi: 10.1212/WNL.0000000000006317

Cui, F., Zhu, W., Zhou, Z., Ren, Y., Li, Y., Li, M., et al. (2015). Frequency and risk factor analysis of cognitive and anxiety-depressive disorders in patients with amyotrophic lateral sclerosis/motor neuron disease. *Neuropsychiatr. Dis. Treat.* 11, 2847–2854. doi: 10.2147/NDT.S90520

Cukor, D., Peterson, R. A., Cohen, S. D., and Kimmel, P. L. (2006). Depression in end-stage renal disease hemodialysis patients. *Nat. Clin. Pract. Nephrol.* 2, 678–687. doi: 10.1038/ncpneph0359

Dary-Auriol, M., Ingrand, P., Bonnaud, V., Dumas, P., Neau, J. P., and Gil, R. (1997). [Amyotrophic lateral sclerosis and cognition disorders. neuropsychological study of a population of 26 patients]. *Rev. Neurol.* 153, 244–250.

De Groot, I. J. M., Post, M. W. M., van Heuveln, T., Van den Berg, L. H., and Lindeman, E. (2007). Cross-sectional and longitudinal correlations between disease progression and different health-related quality of life domains in persons with amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* 8, 356–361. doi: 10.1080/17482960701553949

Elamin, M., Bede, P., Byrne, S., Jordan, N., Gallagher, L., Wynne, B., et al. (2013). Cognitive changes predict functional decline in ALS: a population-based longitudinal study. *Neurology* 80, 1590–1597. doi: 10.1212/WNL.0b013e31828f18ac

Elamin, M., Phukan, J., Bede, P., Jordan, N., Byrne, S., Pender, N., et al. (2011). Executive dysfunction is a negative prognostic indicator in patients with ALS without dementia. *Neurology* 76, 1263–1269. doi: 10.1212/WNL.0b013e318214359f

Fang, F., Valdimarsdóttir, U., Fürst, C. J., Hultman, C., Fall, K., Sparén, P., et al. (2008). Suicide among patients with amyotrophic lateral sclerosis. *Brain J. Neurol.* 131, 2729–2733. doi: 10.1093/brain/awn161

Felgoise, S. H., Feinberg, R., Stephens, H. E., Barkhaus, P., Boylan, K., Caress, J., et al. (2018). Amyotrophic lateral sclerosis-specific quality of life-short form (ALSSQOL-SF): a brief, reliable, and valid version of the ALSSQOL-R. *Muscle Nerve* 58, 646–654. doi: 10.1002/mus.26203

Femiano, C., Trojsi, F., Caiazzo, G., Siciliano, M., Passaniti, C., Russo, A., et al. (2018). apathy is correlated with widespread diffusion tensor imaging (DTI) impairment in amyotrophic lateral sclerosis. *Behav. Neurol.* 2018:2635202. doi: 10.1155/2018/2635202

Ferentinos, P., Paparrigopoulos, T., Rentzos, M., Zouvelou, V., Alexakis, T., and Evdokimidis, I. (2011). Prevalence of major depression in ALS: comparison of a semi-structured interview and four self-report measures. *Amyotroph. Lateral Scler.* 12, 297–302. doi: 10.3109/17482968.2011.556744

Flaherty, C., Kraft, J., Brothers, A., Harrison, M., Legro, R. S., Manni, A., et al. (2017). The relationship between oestrogen and executive functioning in ALS females with emerging frontotemporal lobar degeneration (FTLD) supports a

neuroendocrine model of FTLD attenuation. *Amyotroph. Lateral Scler. Front. Degener.* 18, 74–85. doi: 10.1080/21678421.2016.1249487

Floeter, M. K., Bageac, D., Danielian, L. E., Braun, L. E., Traynor, B. J., and Kwan, J. Y. (2016). Longitudinal imaging in C9orf72 mutation carriers: relationship to phenotype. *NeuroImage Clin.* 12, 1035–1043. doi: 10.1016/j.nicl.2016.10.014

Floeter, M. K., Traynor, B. J., Farren, J., Braun, L. E., Tierney, M., Wiggs, E. A., et al. (2017). Disease progression in C9orf72 mutation carriers. *Neurology* 89, 234–241. doi: 10.1212/WNL.0000000000004115

Ganzini, L., Johnston, W. S., McFarland, B. H., Tolle, S. W., and Lee, M. A. (1998). Attitudes of patients with amyotrophic lateral sclerosis and their care givers toward assisted suicide. *N. Engl. J. Med.* 339, 967–973. doi: 10.1056/NEJM199810013391406

Gauthier, A., Vignola, A., Calvo, A., Cavallo, E., Moglia, C., Sellitti, L., et al. (2007). A longitudinal study on quality of life and depression in ALS patient-caregiver couples. *Neurology* 68, 923–926. doi: 10.1212/01.wnl.0000257093.53430.a8

Gibbons, Z. C., Richardson, A., Neary, D., and Snowden, J. S. (2008). Behaviour in amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* 9, 67–74. doi: 10.1080/17482960701642437

Gibbons, Z. C., Snowden, J. S., Thompson, J. C., Happé, F., Richardson, A., and Neary, D. (2007). Inferring thought and action in motor neurone disease. *Neuropsychologia* 45, 1196–1207. doi: 10.1016/j.neuropsychologia.2006.10.008

Gillingham, S. M., Yunusova, Y., Ganda, A., Rogaeva, E., Black, S. E., Stuss, D. T., et al. (2017). Assessing cognitive functioning in ALS: a focus on frontal lobe processes. *Amyotroph. Lateral Scler. Front. Degener.* 18, 182–192. doi: 10.1080/21678421.2016.1248977

Giordana, M. T., Ferrero, P., Grifoni, S., Pellerino, A., Naldi, A., and Montuschi, A. (2011). Dementia and cognitive impairment in amyotrophic lateral sclerosis: a review. *Neurol. Sci.* 32, 9–16. doi: 10.1007/s10072-010-0439-6

Girardi, A., MacPherson, S. E., and Abrahams, S. (2011). Deficits in emotional and social cognition in amyotrophic lateral sclerosis. *Neuropsychology* 25, 53–65. doi: 10.1037/a0020357

Goldstein, L. H., Adamson, M., Jeffrey, L., Down, K., Barby, T., Wilson, C., et al. (1998). The psychological impact of MND on patients and carers. *J. Neurol. Sci.* 160(Suppl. 1), S114–S121.

Goldstein, L. H., Atkins, L., Landau, S., Brown, R., and Leigh, P. N. (2006b). Predictors of psychological distress in carers of people with amyotrophic lateral sclerosis: a longitudinal study. *Psychol. Med.* 36, 865–875. doi: 10.1017/S0033291706007124

Goldstein, L. H., Atkins, L., Landau, S., Brown, R. G., and Leigh, P. N. (2006a). Longitudinal predictors of psychological distress and self-esteem in people with ALS. *Neurology* 67, 1652–1658. doi: 10.1212/01.wnl.0000242886.91786.47

Gordon, P. H., Delgadillo, D., Piquard, A., Bruneteau, G., Pradat, P.-F., Salachas, F., et al. (2011). The range and clinical impact of cognitive impairment in French patients with ALS: a cross-sectional study of neuropsychological test performance. *Amyotroph. Lateral Scler.* 12, 372–378. doi: 10.3109/17482968.2011.580847

Govaarts, R., Beeldman, E., Kampelmacher, M. J., van Tol, M.-J., van den Berg, L. H., van der Kooi, A. J., et al. (2016). The frontotemporal syndrome of ALS is associated with poor survival. *J. Neurol.* 263, 2476–2483. doi: 10.1007/s00415-016-8290-1

Grehl, T., Rupp, M., Budde, P., Tegenthoff, M., and Fangerau, H. (2011). Depression and QOL in patients with ALS: how do self-ratings and ratings by relatives differ? *Qual. Life Res. Int. J. Qual. Life Asp. Treat. Care Rehabil.* 20, 569–574. doi: 10.1007/s11136-010-9781-7

Grossman, A. B., Woolley-Levine, S., Bradley, W. G., and Miller, R. G. (2007). Detecting neurobehavioral changes in amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* 8, 56–61. doi: 10.1080/17482960601044106

Hammer, E. M., Häcker, S., Hautzinger, M., Meyer, T. D., and Kübler, A. (2008). Validity of the ALS-Depression-Inventory (ADI-12)–a new screening instrument for depressive disorders in patients with amyotrophic lateral sclerosis. *J. Affect. Disord.* 109, 213–219. doi: 10.1016/j.jad.2007.11.012

Hervieu-Bègue, M., Rouaud, O., Graule Petot, A., Catteau, A., and Giroud, M. (2016). Semantic memory assessment in 15 patients with amyotrophic lateral sclerosis. *Rev. Neurol.* 172, 307–312. doi: 10.1016/j.neurol.2015.10.009

Hillemacher, T., Grässel, E., Tigges, S., Bleich, S., Neundörfer, B., Kornhuber, J., et al. (2004). Depression and bulbar involvement in amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Mot. Neuron Disord.* 5, 245–249. doi: 10.1080/14660820410021294

Houpt, J. L., Gould, B. S., and Norris, F. H. (1977). Psychological characteristics of patients with amyotrophic lateral sclerosis (ALS). *Psychosom. Med.* 39, 299–303.

Hu, W. T., Shelnutt, M., Wilson, A., Yarab, N., Kelly, C., Grossman, M., et al. (2013). Behavior matters–cognitive predictors of survival in amyotrophic lateral sclerosis. *PLoS One* 8:e57584. doi: 10.1371/journal.pone.0057584

Irwin, D., Lippa, C. F., and Swearer, J. M. (2007). Cognition and amyotrophic lateral sclerosis (ALS). *Am. J. Alzheimers Dis. Other Demen.* 22, 300–312. doi: 10.1177/1533317507301613

Iwasaki, Y., Kinoshita, M., Ikeda, K., Takamiya, K., and Shiojima, T. (1990). Neuropsychological dysfunctions in amyotrophic lateral sclerosis: relation to motor disabilities. *Int. J. Neurosci.* 54, 191–195. doi: 10.3109/00207459008986635

Jakobsson Larsson, B., Nordin, K., and Nygren, I. (2016). Coping with amyotrophic lateral sclerosis; from diagnosis and during disease progression. *J. Neurol. Sci.* 361, 235–242. doi: 10.1016/j.jns.2015.12.042

Jakobsson Larsson, B., Ozanne, A. G., Nordin, K., and Nygren, I. (2017). A prospective study of quality of life in amyotrophic lateral sclerosis patients. *Acta Neurol. Scand.* 136, 631–638. doi: 10.1111/ane.12774

Jelsone-Swain, L., Persad, C., Votruba, K. L., Weisenbach, S. L., Johnson, T., Gruis, K. L., et al. (2012). The relationship between depressive symptoms, disease state, and cognition in amyotrophic lateral sclerosis. *Front. Psychol.* 3:542. doi: 10.3389/fpsyg.2012.00542

Kamminga, J., Leslie, F. V. C., Hsieh, S., Caga, J., Mioshi, E., Hornberger, M., et al. (2016). Syntactic comprehension deficits across the FTD-ALS continuum. *Neurobiol. Aging* 41, 11–18. doi: 10.1016/j.neurobiolaging.2016.02.002

Kasper, E., Schuster, C., Machts, J., Bittner, D., Vielhaber, S., Benecke, R., et al. (2015). Dysexecutive functioning in ALS patients and its clinical implications. *Amyotroph. Lateral Scler. Front. Degener.* 16, 160–171. doi: 10.3109/21678421.2015.1026267

Kasper, E., Schuster, C., Machts, J., Kaufmann, J., Bittner, D., Vielhaber, S., et al. (2014). Microstructural white matter changes underlying cognitive and behavioural impairment in ALS–an in vivo study using DTI. *PLoS One* 9:e114543. doi: 10.1371/journal.pone.0114543

Kasper, E., Zydatiss, K., Schuster, C., Machts, J., Bittner, D., Kaufmann, J., et al. (2016). No change in executive performance in ALS patients: a longitudinal neuropsychological study. *Neurodegener. Dis.* 16, 184–191. doi: 10.1159/000440957

Katon, W., Lin, E. H. B., and Kroenke, K. (2007). The association of depression and anxiety with medical symptom burden in patients with chronic medical illness. *Gen. Hosp. Psychiatr.* 29, 147–155. doi: 10.1016/j.genhosppsych.2006.11.005

Kilani, M., Micallef, J., Soubrouillard, C., Rey-Lardiller, D., Dematteï, C., Dib, M., et al. (2004). A longitudinal study of the evolution of cognitive function and affective state in patients with amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Mot. Neuron Disord.* 5, 46–54. doi: 10.1080/14660820310017560

Kurt, A., Nijboer, F., Matuz, T., and Kübler, A. (2007). Depression and anxiety in individuals with amyotrophic lateral sclerosis: epidemiology and management. *CNS Drugs* 21, 279–291. doi: 10.2165/00023210-200721040-00003

Lazarus, R. S., and Folkman, S. (1984). *Stress, Appraisal, and Coping*. New York, NY: Springer Publishing Company.

Leslie, F. V. C., Hsieh, S., Caga, J., Savage, S. A., Mioshi, E., Hornberger, M., et al. (2015). Semantic deficits in amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Front. Degener.* 16, 46–53. doi: 10.3109/21678421.2014.987301

Lillo, P., Mioshi, E., and Hodges, J. R. (2012). Caregiver burden in amyotrophic lateral sclerosis is more dependent on patients' behavioral changes than physical disability: a comparative study. *BMC Neurol.* 12:156. doi: 10.1186/1471-2377-12-156

Lillo, P., Mioshi, E., Zoing, M. C., Kiernan, M. C., and Hodges, J. R. (2011). How common are behavioural changes in amyotrophic lateral sclerosis? *Amyotroph. Lateral Scler.* 12, 45–51. doi: 10.3109/17482968.2010.520718

Lou, J.-S., Reeves, A., Benice, T., and Sexton, G. (2003). Fatigue and depression are associated with poor quality of life in ALS. *Neurology* 60, 122–123. doi: 10.1212/01.wnl.0000042781.22278.0a

Lulé, D., Diekmann, V., Anders, S., Kassubek, J., Kübler, A., Ludolph, A. C., et al. (2007). Brain responses to emotional stimuli in patients with amyotrophic

lateral sclerosis (ALS). *J. Neurol.* 254, 519–527. doi: 10.1007/s00415-006-0409-3

Lulé, D., Häcker, S., Ludolph, A., Birbaumer, N., and Kübler, A. (2008). Depression and quality of life in patients with amyotrophic lateral sclerosis. *Dtsch. Arzteblatt Int.* 105, 397–403. doi: 10.3238/arztebl.2008.0397

Lulé, D., Kurt, A., Jürgens, R., Kassubek, J., Diekmann, V., Kraft, E., et al. (2005). Emotional responding in amyotrophic lateral sclerosis. *J. Neurol.* 252, 1517–1524. doi: 10.1007/s00415-005-0907-8

Lulé, D., Pauli, S., Altintas, E., Singer, U., Merk, T., Uttner, I., et al. (2012). Emotional adjustment in amyotrophic lateral sclerosis (ALS). *J. Neurol.* 259, 334–341. doi: 10.1007/s00415-011-6191-x

Lulé, D. E., Aho-Özhan, H. E. A., Vázquez, C., Weiland, U., Weishaupt, J. H., Otto, M., et al. (2018). Story of the ALS-FTD continuum retold: rather two distinct entities. *J. Neurol. Neurosurg. Psychiatr.* 90, 586–589. doi: 10.1136/jnnp-2018-318800

Machts, J., Vielhaber, S., Kollewe, K., Petri, S., Kaufmann, J., and Schoenfeld, M. A. (2018). global hippocampal volume reductions and local CA1 shape deformations in amyotrophic lateral sclerosis. *Front. Neurol.* 9:565. doi: 10.3389/fneur.2018.00565

Marconi, A., Meloni, G., Fossati, F., Lunetta, C., Bastianello, S., Melazzini, M., et al. (2012). Aggressiveness, sexuality, and obsessiveness in late stages of ALS patients and their effects on caregivers. *Amyotroph. Lateral Scler.* 13, 452–458. doi: 10.3109/17482968.2012.696658

Matuz, T., Birbaumer, N., Hautzinger, M., and Kübler, A. (2010). Coping with amyotrophic lateral sclerosis: an integrative view. *J. Neurol. Neurosurg. Psychiatr.* 81, 893–898. doi: 10.1136/jnnp.2009.201285

Matuz, T., Birbaumer, N., Hautzinger, M., and Kübler, A. (2015). Psychosocial adjustment to ALS: a longitudinal study. *Front. Psychol.* 6:1197. doi: 10.3389/fpsyg.2015.01197

McElhiney, M., Rabkin, J. G., Goetz, R., Katz, J., Miller, R. G., Forshew, D. A., et al. (2014). Seeking a measure of clinically meaningful change in ALS. *Amyotroph. Lateral Scler. Front. Degener.* 15, 398–405. doi: 10.3109/21678421.2014.942668

McElhiney, M. C., Rabkin, J. G., Gordon, P. H., Goetz, R., and Mitsumoto, H. (2009). Prevalence of fatigue and depression in ALS patients and change over time. *J. Neurol. Neurosurg. Psychiatr.* 80, 1146–1149. doi: 10.1136/jnnp.2008.163246

Meier, S. L., Charleston, A. J., and Tippett, L. J. (2010). Cognitive and behavioural deficits associated with the orbitomedial prefrontal cortex in amyotrophic lateral sclerosis. *Brain J. Neurol.* 133, 3444–3457. doi: 10.1093/brain/awq254

Menke, R. A. L., Proudfoot, M., Talbot, K., and Turner, M. R. (2018). The two-year progression of structural and functional cerebral MRI in amyotrophic lateral sclerosis. *NeuroImage Clin.* 17, 953–961. doi: 10.1016/j.nicl.2017.12.025

Miglioretti, M., Mazzini, L., Oggioni, G. D., Testa, L., and Monaco, F. (2008). Illness perceptions, mood and health-related quality of life in patients with amyotrophic lateral sclerosis. *J. Psychosom. Res.* 65, 603–609. doi: 10.1016/j.jpsychores.2008.05.012

Mioshi, E., Caga, J., Lillo, P., Hsieh, S., Ramsey, E., Devenney, E., et al. (2014). Neuropsychiatric changes precede classic motor symptoms in ALS and do not affect survival. *Neurology* 82, 149–155. doi: 10.1212/WNL.0000000000000023

Mioshi, E., Lillo, P., Yew, B., Hsieh, S., Savage, S., Hodges, J. R., et al. (2013). Cortical atrophy in ALS is critically associated with neuropsychiatric and cognitive changes. *Neurology* 80, 1117–1123. doi: 10.1212/WNL.0b013e31828869da

Montuschi, A., Iazzolino, B., Calvo, A., Moglia, C., Lopiano, L., Restagno, G., et al. (2015). Cognitive correlates in amyotrophic lateral sclerosis: a population-based study in Italy. *J. Neurol. Neurosurg. Psychiatr.* 86, 168–173. doi: 10.1136/jnnp-2013-307223

Moos, R. H., and Holahan, C. J. (2007). "Adaptive tasks and methods of coping with illness and disability," in *Coping with Chronic Illness and Disability – Theoretical, Empirical, and Clinical Aspects*. ed E. Martz and H. Livneh. (New York, NY: Springer), 107–126.

Moss-Morris, R. (2013). Adjusting to chronic illness: time for a unified theory. *Br. J. Health Psychol.* 18, 681–686. doi: 10.1111/bjhp.12072

Murphy, J., Factor-Litvak, P., Goetz, R., Lomen-Hoerth, C., Nagy, P. L., Hupf, J., et al. (2016). Cognitive-behavioral screening reveals prevalent impairment in a large multicenter ALS cohort. *Neurology* 86, 813–820. doi: 10.1212/WNL.0000000000002305

Murphy, J., Henry, R., and Lomen-Hoerth, C. (2007). Establishing subtypes of the continuum of frontal lobe impairment in amyotrophic lateral sclerosis. *Arch. Neurol.* 64, 330–334. doi: 10.1001/archneur.64.3.330

Murphy, J. M., Henry, R. G., Langmore, S., Kramer, J. H., Miller, B. L., and Lomen-Hoerth, C. (2007). Continuum of frontal lobe impairment in amyotrophic lateral sclerosis. *Arch. Neurol.* 64, 530–534. doi: 10.1001/archneur.64.4.530

Neary, D., Snowden, J. S., and Mann, D. M. (2000). Cognitive change in motor neurone disease/amyotrophic lateral sclerosis (MND/ALS). *J. Neurol. Sci.* 180, 15–20. doi: 10.1016/s0022-510x(00)00425-1

Norris, L., Que, G., and Bayat, E. (2010). Psychiatric aspects of amyotrophic lateral sclerosis (ALS). *Curr. Psychiatry Rep.* 12, 239–245. doi: 10.1007/s11920-010-0118-6

Oh, S. I., Oh, K. W., Kim, H. J., Park, J. S., and Kim, S. H. (2016). Impaired perception of emotional expression in amyotrophic lateral sclerosis. *J. Clin. Neurol. Seoul Korea* 12, 295–300. doi: 10.3988/jcn.2016.12.3.295

Ohta, Y., Sato, K., Takemoto, M., Takahashi, Y., Morihara, R., Nakano, Y., et al. (2017). Behavioral and affective features of amyotrophic lateral sclerosis patients. *J. Neurol. Sci.* 381, 119–125. doi: 10.1016/j.jns.2017.08.024

Olney, R. K., Murphy, J., Forshew, D., Garwood, E., Miller, B. L., Langmore, S., et al. (2005). The effects of executive and behavioral dysfunction on the course of ALS. *Neurology* 65, 1774–1777. doi: 10.1212/01.wnl.0000188759.87240.8b

Pagnini, F., Simmons, Z., Corbo, M., and Molinari, E. (2012). Amyotrophic lateral sclerosis: time for research on psychological intervention? *Amyotroph. Lateral Scler.* 13, 416–417. doi: 10.3109/17482968.2011.653572

Palmieri, A., Mento, G., Calvo, V., Querin, G., D'Ascenzo, C., Volpato, C., et al. (2015). Female gender doubles executive dysfunction risk in ALS: a case-control study in 165 patients. *J. Neurol. Neurosurg. Psychiatr.* 86, 574–579. doi: 10.1136/jnnp-2014-307654

Palmieri, A., Naccarato, M., Abrahams, S., Bonato, M., D'Ascenzo, C., Balestreri, S., et al. (2010). Right hemisphere dysfunction and emotional processing in ALS: an fMRI study. *J. Neurol.* 257, 1970–1978. doi: 10.1007/s00415-010-5640-2

Papps, B., Abrahams, S., Wicks, P., Leigh, P. N., and Goldstein, L. H. (2005). Changes in memory for emotional material in amyotrophic lateral sclerosis (ALS). *Neuropsychologia* 43, 1107–1114. doi: 10.1016/j.neuropsychologia.2004.11.027

Phukan, J., Elamin, M., Bede, P., Jordan, N., Gallagher, L., Byrne, S., et al. (2012). The syndrome of cognitive impairment in amyotrophic lateral sclerosis: a population-based study. *J. Neurol. Neurosurg. Psychiatr.* 83, 102–108. doi: 10.1136/jnnp-2011-300188

Pisa, F. E., Logroscino, G., Casetta, A., Cecotti, L., Verriello, L., Bratina, A., et al. (2015). The use of antidepressant medication before and after the diagnosis of amyotrophic lateral sclerosis: a population-based cohort study. *Neuroepidemiology* 44, 91–98. doi: 10.1159/000374119

Poletti, B., Solca, F., Carelli, L., Faini, A., Madotto, F., Lafronza, A., et al. (2018). Cognitive-behavioral longitudinal assessment in ALS: the Italian Edinburgh Cognitive and Behavioral ALS screen (ECAS). *Amyotroph. Lateral Scler. Front. Degener.* 19, 387–395. doi: 10.1080/21678421.2018.1473443

Raaphorst, J., de Visser, M., Linssen, W. H. J. P., de Haan, R. J., and Schmand, B. (2010). The cognitive profile of amyotrophic lateral sclerosis: a meta-analysis. *Amyotroph. Lateral Scler.* 11, 27–37. doi: 10.3109/17482960802645008

Raaphorst, J., van Tol, M. J., de Visser, M., van der Kooi, A. J., Majoie, C. B., van den Berg, L. H., et al. (2015). Prose memory impairment in amyotrophic lateral sclerosis patients is related to hippocampus volume. *Eur. J. Neurol.* 22, 547–554. doi: 10.1111/ene.12615

Rabkin, J., Goetz, R., Murphy, J. M., Factor-Litvak, P., Mitsumoto, H., and Als Cosmos Study Group, (2016). Cognitive impairment, behavioral impairment, depression, and wish to die in an ALS cohort. *Neurology* 87, 1320–1328. doi: 10.1212/WNL.0000000000003035

Rabkin, J. G., Albert, S. M., Del Bene, M. L., O'Sullivan, I., Tider, T., Rowland, L. P., et al. (2005). Prevalence of depressive disorders and change over time in late-stage ALS. *Neurology* 65, 62–67. doi: 10.1212/01.wnl.0000167187.14501.0c

Rabkin, J. G., Albert, S. M., Rowland, L. P., and Mitsumoto, H. (2009). How common is depression among ALS caregivers? a longitudinal study. *Amyotroph. Lateral Scler.* 10, 448–455. doi: 10.1080/17482960802459889

Rabkin, J. G., Goetz, R., Factor-Litvak, P., Hupf, J., McElhiney, M., Singleton, J., et al. (2015). Depression and wish to die in a multicenter cohort of ALS patients.

Amyotroph. Lateral Scler. Front. Degener. 16, 265–273. doi: 10.3109/21678421.2014.980428

Rabkin, J. G., Wagner, G. J., and Del Bene, M. (2000). Resilience and distress among amyotrophic lateral sclerosis patients and caregivers. *Psychosom. Med.* 62, 271–279. doi: 10.1097/00006842-200003000-00020

Radakovic, R., Stephenson, L., Newton, J., Crockford, C., Swingler, R., Chandran, S., et al. (2017). Multidimensional apathy and executive dysfunction in amyotrophic lateral sclerosis. *Cortex J. Devoted Study Nerv. Syst. Behav.* 94, 142–151. doi: 10.1016/j.cortex.2017.06.023

Ringholz, G. M., Appel, S. H., Bradshaw, M., Cooke, N. A., Mosnik, D. M., and Schulz, P. E. (2005). Prevalence and patterns of cognitive impairment in sporadic ALS. *Neurology* 65, 586–590. doi: 10.1212/01.wnl.0000172911.39167.b6

Roach, A. R., Averill, A. J., Segerstrom, S. C., and Kasarskis, E. J. (2009). The dynamics of quality of life in ALS patients and caregivers. *Ann. Behav. Med. Publ. Soc. Behav. Med.* 37, 197–206. doi: 10.1007/s12160-009-9092-9

Roberts-South, A., Findlater, K., Strong, M. J., and Orange, J. B. (2012). Longitudinal changes in discourse production in amyotrophic lateral sclerosis. *Semin. Speech Lang.* 33, 79–94. doi: 10.1055/s-0031-1301165

Robinson, K. M., Lacey, S. C., Grugan, P., Glosser, G., Grossman, M., and McCluskey, L. F. (2006). Cognitive functioning in sporadic amyotrophic lateral sclerosis: a six month longitudinal study. *J. Neurol. Neurosurg. Psychiatr.* 77, 668–670. doi: 10.1136/jnnp.2005.073403

Roos, E., Mariosa, D., Ingre, C., Lundholm, C., Wirdefeldt, K., Roos, P. M., et al. (2016). Depression in amyotrophic lateral sclerosis. *Neurology* 86, 2271–2277. doi: 10.1212/WNL.0000000000002671

Roy-Bellina, S., Brunel, H., Almohsen, C., Gely-Nargeot, M., Carton, S., and Camu, W. (2008). Alexithymia in amyotrophic lateral sclerosis. *Neuropsychol Approach. J. Neurol.* 255:51.

Saberi, S., Stauffer, J. E., Schulte, D. J., and Ravits, J. (2015). neuropathology of amyotrophic lateral sclerosis and its variants. *Neurol. Clin.* 33, 855–876. doi: 10.1016/j.ncl.2015.07.012

Sandstedt, P., Johansson, S., Ytterberg, C., Ingre, C., Holmqvist, L. W., and Kierkegaard, M. (2016). Predictors of health-related quality of life in people with amyotrophic lateral sclerosis. *J. Neurol. Sci.* 370, 269–273. doi: 10.1016/j.jns.2016.09.034

Sarro, L., Agosta, F., Canu, E., Riva, N., Prelle, A., Copetti, M., et al. (2011). Cognitive functions and white matter tract damage in amyotrophic lateral sclerosis: a diffusion tensor tractography study. *AJNR Am. J. Neuroradiol.* 32, 1866–1872. doi: 10.3174/ajnr.A2658

Schreiber, H., Gaigalat, T., Wiedemuth-Catrinescu, U., Graf, M., Uttner, I., Muche, R., et al. (2005). Cognitive function in bulbar- and spinal-onset amyotrophic lateral sclerosis. a longitudinal study in 52 patients. *J. Neurol.* 252, 772–781. doi: 10.1007/s00415-005-0739-6

Schuster, C., Kasper, E., Dyrba, M., Machts, J., Bittner, D., Kaufmann, J., et al. (2014a). Cortical thinning and its relation to cognition in amyotrophic lateral sclerosis. *Neurobiol. Aging* 35, 240–246. doi: 10.1016/j.neurobiolaging.2013.07.020

Schuster, C., Kasper, E., Machts, J., Bittner, D., Kaufmann, J., Benecke, R., et al. (2014b). Longitudinal course of cortical thickness decline in amyotrophic lateral sclerosis. *J. Neurol.* 261, 1871–1880. doi: 10.1007/s00415-014-7426-4

Sedda, A. (2014). Disorders of emotional processing in amyotrophic lateral sclerosis. *Curr. Opin. Neurol.* 27, 659–665. doi: 10.1097/WCO.0000000000000147

Siciliano, M., Santangelo, G., Trojsi, F., Di Somma, C., Patrone, M., Femiano, C., et al. (2017). Coping strategies and psychological distress in caregivers of patients with amyotrophic lateral sclerosis (ALS). *Amyotroph. Lateral Scler. Front. Degener.* 18, 367–377. doi: 10.1080/21678421.2017.1285316

Simmons, Z. (2015). Patient-perceived outcomes and quality of life in ALS. *Neurother. J. Am. Soc. Exp. Neurother.* 12, 394–402. doi: 10.1007/s13311-014-0322-x

Sterling, L. E., Jawaid, A., Salamone, A. R., Murthy, S. B., Mosnik, D. M., McDowell, E., et al. (2010). Association between dysarthria and cognitive impairment in ALS: a prospective study. *Amyotroph. Lateral Scler.* 11, 46–51. doi: 10.3109/17482960903207997

Stojkovic, T., Stefanova, E., Pekmezovic, T., Peric, S., and Stevic, Z. (2016). Executive dysfunction and survival in patients with amyotrophic lateral sclerosis: preliminary report from a serbian centre for motor neuron disease. *Amyotroph. Lateral Scler. Front. Degener.* 17, 543–547. doi: 10.1080/21678421.2016.1211148

Strong, M. J. (2017). Revisiting the concept of amyotrophic lateral sclerosis as a multisystems disorder of limited phenotypic expression. *Curr. Opin. Neurol.* 30, 599–607. doi: 10.1097/WCO.0000000000000488

Strong, M. J., Grace, G. M., Orange, J. B., Leeper, H. A., Menon, R. S., and Aere, C. (1999). A prospective study of cognitive impairment in ALS. *Neurology* 53, 1665–1670.

Strutt, A. M., Palcic, J., Wager, J. G., Titus, C., Macadam, C., Brown, J., et al. (2012). Cognition, behavior, and respiratory function in amyotrophic lateral sclerosis. *ISRN Neurol.* 2012:912123. doi: 10.5402/2012/912123

Taylor, L., Wicks, P., Leigh, P. N., and Goldstein, L. H. (2010). Prevalence of depression in amyotrophic lateral sclerosis and other motor disorders. *Eur. J. Neurol.* 17, 1047–1053. doi: 10.1111/j.1468-1331.2010.02960.x

Terada, T., Obi, T., Yoshizumi, M., Murai, T., Miyajima, H., and Mizoguchi, K. (2011). Frontal lobe-mediated behavioral changes in amyotrophic lateral sclerosis: are they independent of physical disabilities? *J. Neurol. Sci.* 309, 136–140. doi: 10.1016/j.jns.2011.06.049

Thakore, N. J., and Pioro, E. P. (2016). Depression in ALS in a large self-reporting cohort. *Neurology* 86, 1031–1038. doi: 10.1212/WNL.0000000000002465

Tremolizzo, L., Pellegrini, A., Susani, E., Lunetta, C., Woolley, S. C., Ferrarese, C., et al. (2016). Behavioural but not cognitive impairment is a determinant of caregiver burden in amyotrophic lateral sclerosis. *Eur. Neurol.* 75, 191–194. doi: 10.1159/000445110

Trojsi, F., Di Nardo, F., Santangelo, G., Siciliano, M., Femiano, C., Passaniti, C., et al. (2017). Resting state fMRI correlates of Theory of Mind impairment in amyotrophic lateral sclerosis. *Cortex* 97, 1–16. doi: 10.1016/j.cortex.2017.09.016

Trojsi, F., Siciliano, M., Russo, A., Passaniti, C., Femiano, C., Ferrantino, T., et al. (2016). Theory of mind and its neuropsychological and quality of life correlates in the early stages of amyotrophic lateral sclerosis. *Front. Psychol.* 7:1934. doi: 10.3389/fpsyg.2016.01934

Tsujimoto, M., Senda, J., Ishihara, T., Niimi, Y., Kawai, Y., Atsuta, N., et al. (2011). Behavioral changes in early ALS correlate with voxel-based morphometry and diffusion tensor imaging. *J. Neurol. Sci.* 307, 34–40. doi: 10.1016/j.jns.2011.05.025

Turner, M. R., Goldacre, R., Talbot, K., and Goldacre, M. J. (2016). Psychiatric disorders prior to amyotrophic lateral sclerosis. *Ann. Neurol.* 80, 935–938. doi: 10.1002/ana.24801

Unglik, J., Bungener, C., Delgadillo, D., Salachas, F., Pradat, P. F., Bruneteau, G., et al. (2018). Emotional feeling in patients suffering from amyotrophic lateral sclerosis. *Geriatr. Psychol. Neuropsychiatr. Vieil.* 16, 414–422. doi: 10.1684/pnv.2018.0762

van der Hulst, E.-J., Bak, T. H., and Abrahams, S. (2015). Impaired affective and cognitive theory of mind and behavioural change in amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* 86, 1208–1215. doi: 10.1136/jnnp-2014-309290

Verschueren, A., Kianimehr, G., Belingher, C., Salort-Campana, E., Loundou, A., Grapperon, A.-M., et al. (2018). Wish to die and reasons for living among patients with amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Front. Degener.* 20, 68–73. doi: 10.1080/21678421.2018.1530265

Verstraete, E., Veldink, J. H., Hendrikse, J., Schelhaas, H. J., van den Heuvel, M. P., and van den Berg, L. H. (2012). Structural MRI reveals cortical thinning in amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* 83, 383–388. doi: 10.1136/jnnp-2011-300909

Vignola, A., Guzzo, A., Calvo, A., Moglia, C., Pessia, A., Cavallo, E., et al. (2008). Anxiety undermines quality of life in ALS patients and caregivers. *Eur. J. Neurol.* 15, 1231–1236. doi: 10.1111/j.1468-1331.2008.02303.x

Watermeyer, T. J., Brown, R. G., Sidle, K. C. L., Oliver, D. J., Allen, C., Karlsson, J., et al. (2015). Executive dysfunction predicts social cognition impairment in amyotrophic lateral sclerosis. *J. Neurol.* 262, 1681–1690. doi: 10.1007/s00415-015-7761-0

Wei, Q., Chen, X., Cao, B., Ou, R., Zhao, B., Wu, Y., et al. (2016). Associations between neuropsychiatric symptoms and cognition in Chinese patients with amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Front. Degener.* 17, 358–365. doi: 10.3109/21678421.2016.1154574

Wicks, P., Abrahams, S., Masi, D., Hejda-Forde, S., Leigh, P. N., and Goldstein, L. H. (2007). Prevalence of depression in a 12-month consecutive sample of patients with ALS. *Eur. J. Neurol.* 14, 993–1001. doi: 10.1111/j.1468-1331.2007.01843.x

Witgert, M., Salamone, A. R., Strutt, A. M., Jawaid, A., Massman, P. J., Bradshaw, M., et al. (2010). Frontal-lobe mediated behavioral dysfunction in amyotrophic lateral sclerosis. *Eur. J. Neurol.* 17, 103–110. doi: 10.1111/j.1468-1331.2009.02801.x

Woolley, S., Goetz, R., Factor-Litvak, P., Murphy, J., Hupf, J., Lomen-Hoerth, C., et al. (2018). Longitudinal screening detects cognitive stability and behavioral deterioration in ALS patients. *Behav. Neurol.* 2018:5969137. doi: 10.1155/2018/5969137

Woolley, S. C., and Katz, J. S. (2008). Cognitive and behavioral impairment in amyotrophic lateral sclerosis. *Phys. Med. Rehabil. Clin. N. Am.* 19, 607–617. doi: 10.1016/j.pmr.2008.04.002

Woolley, S. C., Moore, D. H., and Katz, J. S. (2010). Insight in ALS: awareness of behavioral change in patients with and without FTD. *Amyotroph. Lateral Scler.* 11, 52–56. doi: 10.3109/17482960903171110

Woolley, S. C., and Rush, B. K. (2017). Considerations for clinical neuropsychological evaluation in amyotrophic lateral sclerosis. *Arch. Clin. Neuropsychol.* 32, 906–916. doi: 10.1093/arclin/acx089

Woolley, S. C., Zhang, Y., Schuff, N., Weiner, M. W., and Katz, J. S. (2011). Neuroanatomical correlates of apathy in ALS using 4 Tesla diffusion tensor MRI. *Amyotroph. Lateral Scler.* 12, 52–58. doi: 10.3109/17482968.2010.521842

Xu, Z., Alruwaili, A. R. S., Henderson, R. D., and McCombe, P. A. (2017). Screening for cognitive and behavioural impairment in amyotrophic lateral sclerosis: frequency of abnormality and effect on survival. *J. Neurol. Sci.* 376, 16–23. doi: 10.1016/j.jns.2017.02.061

Zago, S., Poletti, B., Morelli, C., Doretti, A., and Silani, V. (2011). Amyotrophic lateral sclerosis and frontotemporal dementia (ALS-FTD). *Arch. Ital. Biol.* 149, 39–56. doi: 10.4449/aib.v149i1.1263

Zalonis, I., Christidi, F., Paraskevas, G., Zabelis, T., Evdokimidis, I., and Kararizou, E. (2012). Can executive cognitive measures differentiate between patients with spinal- and bulbar-onset amyotrophic lateral sclerosis? *Arch. Clin. Neuropsychol.* 27, 348–354. doi: 10.1093/arclin/acs031

Zimmerman, E. K., Eslinger, P. J., Simmons, Z., and Barrett, A. M. (2007). Emotional perception deficits in amyotrophic lateral sclerosis. *Cogn. Behav. Neurol.* 20, 79–82. doi: 10.1097/WNN.0b013e31804c700b

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.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership