MULTIFACETED GENES IN AMYOTROPHIC LATERAL SCLEROSIS-FRONTOTEMPORAL DEMENTIA

EDITED BY: Henry Houlden, Alan Edward Renton and Francesca Luisa Conforti
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MULTIFACETED GENES IN AMYOTROPHIC LATERAL SCLEROSIS-FRONTOTEMPORAL DEMENTIA

Topic Editors:
Henry Houlden, University College London, United Kingdom
Alan Edward Renton, Icahn School of Medicine at Mount Sinai New York, United States
Francesca Luisa Conforti, University of Calabria Arcavacata di Rende, Italy

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Table of Contents

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EDITORIAL
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Editorial: Multifaceted Genes in Amyotrophic Lateral Sclerosis-Frontotemporal Dementia

Francesca Luisa Conforti[1]*, *Alan Edward Renton*[2] and *Henry Houlden*[3]

[1] Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Rende, Italy, [2] Ronald M. Loeb Center for Alzheimer L Disease, Icahn School of Medicine at Mount Sinai, New York, NY, United States, [3] Department of Neuromuscular Disorders, Institute of Neurology, University College London, London, United Kingdom

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Editorial on the Research Topic

Multifaceted Genes in Amyotrophic Lateral Sclerosis-Frontotemporal Dementia

Amyotrophic lateral sclerosis-frontotemporal dementia (ALS-FTD) is a heterogeneous, multi-factorial, and multi-system disease spectrum currently lacking effective drug treatments. The fields of ALS-FTD genetics and genomics have greatly expanded since the first disease gene *SOD1* was identified in 1993 (Rosen et al., 1993). The advent of high-throughput next generation sequencing technologies has enabled systematic genome-wide interrogation of genetic variation, implicating disease-causing and disease-modifying genetic loci and improving our understanding of the diverse pathogenic basis of ALS-FTD. Over 30 genetic loci have been reproducibly linked or associated with ALS-FTD and novel loci continue to be identified (Chia et al., 2018; Guerreiro et al., 2020). It is now recognized that ALS and FTD constitute a disease spectrum or syndrome rather than distinct disorders. This scenario exemplifies the emerging observation of phenotypic pleiotropy, where mutations in the same gene give rise to diverse phenotypes, further increasing the complexity of genotype-phenotype correlation.

In 2011, the discovery that the *C9orf72* GGGGCC repeat expansion (C9-RE) is the most frequent genetic cause of ALS and FTD definitively consolidated the hypothesis that the two diseases belong to the same clinicopathological spectrum (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Repeat expansions have emerged in recent years as major contributors to motor neuron degeneration and with the advent of long-read sequencing, further expansions are likely to be discovered. Intermediate-length CAG repeat expansions in both *ATXN1* (Conforti et al., 2012; Tazelaar et al., 2020) and *ATXN2* (Elden et al., 2010) have also been associated with an increased risk of developing ALS. Mutations in *OPTN*, *VCP*, *SQTM1*, *MATR3*, and *NEK1* have offered insight into the connections between ALS-FTD and seemingly unrelated clinical disorders such as Paget's disease and myopathy (Chia et al., 2018). Recently, *KIF5A*, a gene previously linked to two rare neurodegenerative disorders, hereditary spastic paraplegia type 10 and Charcot-Marie-Tooth type 2, has been definitively linked to ALS (Brenner et al., 2018; Nicolas et al., 2018). Taken together, these and other genes have highlighted the complex genetic architecture of ALS-FTD, with many genes in seemingly unrelated or distantly related physiological pathways producing a similar phenotype.

This Research Topic includes significant focus on the C9-RE in ALS and FTD patients. Trojsi et al. studied the C9-RE in a large Italian ALS cohort. They reported C9-RE carriers exhibit ALS symptoms clinically distinct from sporadic ALS (sALS) patients and, found male but not female expansion carriers have decreased survival, suggesting a potential link between sex and disease progression. Esselin et al. described a large French ALS-FTD cohort with C9-RE. They observed

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Ka Wan Li,
Vrije Universiteit
Amsterdam, Netherlands

***Correspondence:**
Francesca Luisa Conforti
francescaluisa.conforti@cnr.it;
francescaluisa.conforti@unical.it

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C9 patients have an earlier age of onset compared to sALS patients, familial index cases and their siblings have an earlier age of onset compared to their parental generation suggesting anticipation, a predominant female transmission, and a high frequency of suicides in relatives. Trageser et al. reviewed the role of immune cell activation in ALS-FTD in the context of the C9-RE, providing an overview of C9-linked ALS-FTD pathogenesis and the interplay of these cellular events with the immune system. The authors suggested the C9-RE mediates neuroinflammatory mechanisms that significantly contribute to pathogenesis and represent promising new therapeutic approaches.

This Topic also concentrates on overlapping and discordant genetics across ALS, FTD and other disorders. Tripolszki et al. contributed with their first comprehensive genetic analysis of the Hungarian ALS population, highlighting the necessity for large-scale studies to distinguish true causative genetic variants from irrelevant ones and accurately uncover the genetic pattern of ALS. Abramzon et al. described genes involved both in ALS and FTD as key players in dysfunctional pathways such as RNA processing, autophagy, vesicle trafficking, mitochondria, and protein homeostasis. Due to such significant genetic overlap between ALS and FTD, the authors recommended looking in FTD cases for mutations in ALS genes and vice-versa. On the other hand, Ranganathan et al. highlighted that some genes are linked with only ALS or FTD, such as *SOD1* and *MAPT*. This distinction is reflected in the neuropathology, because most types of monogenic ALS, C9-FTD, and GRN-FTD are characterized by TDP-43-positive inclusions but *SOD1*-ALS and *MAPT*-FTD are not, underscoring the need to consider disease subtypes when conducting biomarker and therapeutic research. The authors discussed using next generation sequencing to identify multiple variants in disease-associated genes within an individual, emphasizing the importance of genomic data to facilitate a precision medicine approach for treating ALS-FTD. Furthermore, Broce et al. discussed how shifting our focus from studying ALS and FTD in isolation to identifying the common and distinct biological mechanisms that drive these diseases will improve treatment discovery and therapeutic development. Hence whole genome sequencing of large international ALS-FTD cohorts will begin to fully understand the genetic contribution to disease, particularly when large collaborative cohorts are sequenced such as in project MinE. Rich et al. suggested genome-wide association studies and rare variant association studies represent an attractive option for novel gene discovery because they do not require prior knowledge or hypotheses. Lower-penetrance alleles identified via association studies may inform important components of future combinatorial gene-targeted therapies.

Additionally, the field shows increasing interest in omics bioinformatic analysis to elucidate ALS complex molecular architecture and its role in clinical heterogeneity. Lin et al. compared gene expression profiles of sALS and control motor neurons to discover differentially expressed genes then identified pathways and regulators underlying sALS. They found differentially expressed genes are enriched for the extracellular matrix and implicated the NF-κB regulatory pathway in sALS pathogenesis. Finally, Morello et al. discussed the most significant contributions of omics approaches (genomics, transcriptomics, proteomics, and metabolomics) in unraveling the biological complexity of ALS, highlighting how holistic systems biology approaches and multi-omics data integration are ideal to provide comprehensive characterization of patient-specific molecular signatures that could potentially guide therapeutic decisions.

The 10 articles in this Research Topic provide an overview of the current state of the art in ALS-FTD genetics and genomics, aiming to shed light on overlapping pathogenic mechanisms that may unite disparate mutations under a common umbrella and direct the search for disease-modifying therapies. We have learned much since the discovery of C9-linked ALS-FTD. The next decade promises to illuminate many new aspects of these overlapping neurodegenerative diseases. Building on multi-disciplinary efforts of international consortia such as Project MinE (www.projectmine.com), GENFI (http://genfi.org.uk/) and RiMOD-FTD (https://www.neurodegenerationresearch.eu/initiatives/annual-calls-for-proposals/closed-calls/risk-factors-2012/risk-factor-call-results/rimod-ftd/), we may begin to fully resolve ALS-FTD genetic architecture and understand why individuals carrying a particular variant go on to develop ALS, FTD, or ALS-FTD.

AUTHOR CONTRIBUTIONS

FC wrote the first draft. AR and HH critically reviewed the final version of this editorial. All authors approved the final version of this editorial.

REFERENCES

Brenner, D., Yilmaz, R., Müller, 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

Chia, R., Chiò 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

Conforti, F. L., Spataro, R., Sproviero, W., Mazzei, R., Cavalcanti, F., Condino, F., et al. (2012). Ataxin-1 and ataxin-2 intermediate-length PolyQ expansions in amyotrophic lateral sclerosis. *Neurology* 79, 2315–2320. doi: 10.1212/WNL.0b013e318278b618

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

Elden, A. C., Kim, H. J., Hart, M. P., Chen-Plotkin, A. S., Johnson, B. S., Fang, X., et al. (2010). Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. *Nature* 466, 1069–1075. doi: 10.1038/nature09320

Guerreiro, R., Gibbons, E., Tábuas-Pereira, M., Kun-Rodrigues, C., Santo, G. C., and Bras, J. (2020). Genetic architecture of common non-Alzheimer's disease dementias. *Neurobiol. Dis.* 142:104946. doi: 10.1016/j.nbd.2020.104946

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, 1268–1283.e6. doi: 10.1016/j.neuron.2018.02.027

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

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* 36, 259–262. doi: 10.1038/362059a0

Tazelaar, G. H. P., Boeynaems, S., De Decker, M., van Vugt, J. J. F. A., Kool, L., Goedee, H. S., et al. (2020). ATXN1 repeat expansions confer risk for amyotrophic lateral sclerosis and contribute to TDP-43 mislocalization. *Brain Commun.* 2:fcaa064. doi: 10.1093/braincomms/fcaa064

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ORIGINAL RESEARCH
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Comparative Analysis of *C9orf72* and Sporadic Disease in a Large Multicenter ALS Population: The Effect of Male Sex on Survival of *C9orf72* Positive Patients

Francesca Trojsi[1†], Mattia Siciliano[1,2†], Cinzia Femiano[1], Gabriella Santangelo[2], Christian Lunetta[3,4], Andrea Calvo[5], Cristina Moglia[5], Kalliopi Marinou[6], Nicola Ticozzi[7,8], Christian Ferro[9], Carlo Scialò[10], Gianni Sorarù[11], Amelia Conte[12], Yuri M. Falzone[13], Rosanna Tortelli[14], Massimo Russo[4,15], Valeria Ada Sansone[3,16], Adriano Chiò[5], Gabriele Mora[6], Vincenzo Silani[7,8], Paolo Volanti[9], Claudia Caponnetto[10], Giorgia Querin[11], Mario Sabatelli[12,17], Nilo Riva[13], Giancarlo Logroscino[14], Sonia Messina[4,15], Antonio Fasano[18], Maria Rosaria Monsurrò[1], Gioacchino Tedeschi[1] and Jessica Mandrioli[18]*

[1] Department of Advanced Medical and Surgical Sciences, MRI Research Center SUN-FISM, University of Campania "Luigi Vanvitelli", Naples, Italy, [2] Department of Psychology, Università degli Studi della Campania "L. Vanvitelli", Naples, Italy, [3] NEuroMuscular Omnicentre (NEMO), Serena Onlus Foundation, Milan, Italy, [4] NEMO Sud Clinical Center for Neuromuscular Diseases, Aurora Onlus Foundation, Messina, Italy, [5] ALS Center, "Rita Levi Montalcini" Department of Neuroscience, University of Torino, Turin, Italy, [6] Department of Neurorehabilitation–ALS Center, IRCCS Scientific Clinical Institute Maugeri, Milan, Italy, [7] Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy, [8] Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, University of Milan, Milan, Italy, [9] Neurorehabilitation Unit/ALS Center, Scientific Clinical Institutes (ICS) Maugeri, IRCCS, Messina, Italy, [10] Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal, and Child Health (DINOGMI), University of Genova, IRCCS AOU San Martino-IST, Genova, Italy, [11] Department of Neurosciences, Neuromuscular Center, University of Padova, Padua, Italy, [12] NEuroMuscular Omnicentre (NEMO), Serena Onlus Foundation–Pol. A. Gemelli Foundation, Rome, Italy, [13] Department of Neurology, Institute of Experimental Neurology (INSPE), Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy, [14] Department of Clinical Research in Neurology, University of Bari "A. Moro", at Pia Fondazione "Card. G. Panico", Lecce, Italy, [15] Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy, [16] Department of Biomedical Sciences for Health, University of Milan, Milan, Italy, [17] Department of Geriatrics, Neurosciences and Orthopedics, Institute of Neurology, Catholic University of Sacred Heart, Rome, Italy, [18] Department of Neuroscience, S. Agostino-Estense Hospital and University of Modena and Reggio Emilia, Modena, Italy

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Edited by:
Henry Houlden,
University College London,
United Kingdom

Reviewed by:
Sulev Kõks,
University of Tartu, Estonia
Amber L. Southwell,
University of Central Florida,
United States

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

[†]These authors have contributed equally to this work

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We investigated whether the *C9orf72* repeat expansion is associated with specific clinical features, comorbidities, and prognosis in patients with amyotrophic lateral sclerosis (ALS). A cohort of 1417 ALS patients, diagnosed between January 1, 2009 and December 31, 2013 by 13 Italian ALS Referral Centers, was screened for the *C9orf72* repeat expansion, and the analyses were performed comparing patients carrying this expansion (ALS-C9Pos) to those negative for this and other explored ALS-related mutations (ALS without genetic mutations, ALSwoGM). Compared to the ALSwoGM group, ALS-C9Pos patients ($n = 84$) were younger at disease onset, at the first clinical observation and at diagnosis ($p < 0.001$). After correcting for these differences, we found that ALS-C9Pos patients had higher odds of bulbar onset, diagnosis of frontotemporal dementia (FTD) and family history of ALS, FTD, and Alzheimer's disease and had lower odds of spinal onset, non-invasive ventilation, hypertension and psychiatric diseases than

ALSwoGM patients. Among these variables, those related to shorter survival time were: bulbar onset, presence of FTD, hypertension, psychiatric disease, and family history of ALS ($p < 0.05$). Cox proportional hazards regression multivariate analysis suggested that carrying the *C9orf72* repeat expansion was an independent factor negatively impacting on survival time in men (HR 1.58, 95% CI 1.07–2.33, $p = 0.021$), but not in women ($p > 0.05$) as well as in the whole sample ($p > 0.05$). When compared to ALSwoGM, ALS-C9Pos showed an earlier disease onset, no significant diagnostic delay and a higher odds of bulbar onset, FTD and family history of ALS and dementia. Moreover, male sex drove the negative effect of expanded variant on survival, confirming the hypothesis that sex is likely to be a crucial factor in the biology of *C9orf72*-related disease.

Keywords: amyotrophic lateral sclerosis, *C9orf72* expansion, gender, comorbidity, survival

INTRODUCTION

The pathological expansion of a hexanucleotide repeat in the *C9orf72* gene is the most common genetic mutation identified in patients with amyotrophic lateral sclerosis (ALS), reported in 40–50% of patients with familial ALS and 5–10% of patients with sporadic ALS (DeJesus-Hernandez et al., 2011; Renton et al., 2011; Majounie et al., 2012). Patients carrying the expansion (ALS-C9Pos) have been described as phenotypically different from non-mutated patients in that most cohorts of ALS-C9Pos patients exhibited, at variable extent, higher prevalence of bulbar onset, earlier age at onset and reduced survival with higher incidence of comorbid frontotemporal dementia (FTD) and/or family history of dementia or ALS (Byrne et al., 2012, 2013; Chiò et al., 2012; Cooper-Knock et al., 2012; Majounie et al., 2012; Sabatelli et al., 2012; Irwin et al., 2013; Umoh et al., 2016; Hardiman et al., 2017). Although the *C9orf72* repeat expansion has been revealed a relevant negative prognostic factor in survival analyses (Byrne et al., 2012, 2013; Chiò et al., 2012; Sabatelli et al., 2012; Irwin et al., 2013; Umoh et al., 2016), the potential associations between this variant and demographic and clinical features have not still completely elucidated. Among the most robust evidence on prognostic char (Rooney et al., 2017) a previously unrecognized interaction between the *C9orf72* repeat expansion and sex (Rooney et al., 2017). Nevertheless, the same authors suggested to further evaluate the role of other clinical variables, such as the presence of cognitive changes, in the abovementioned interaction effect. Additionally, Miltenberger-Miltenyi et al. (2018) found that the *C9orf72* expansion was associated with FTD, shorter survival and faster % Forced Vital Capacity (FVC) decline in ALS, but not with a faster rate of functional decay.

In this large multicenter cohort we aimed at: (i) examining the potential associations between *C9orf72* repeat expansion and phenotype, site of onset, family history, therapy, and others comorbidities; (ii) exploring if ALS patients carrying *C9orf72* repeat expansion differed from patients without genetic mutation in the survival profile, both in the whole sample and stratified by sex. We expected to identify potential novel associations between *C9orf72* repeat expansion and a number of demographic and clinical features, focusing on the effect of sex on the survival profile.

MATERIALS AND METHODS

Patient Data Collection

This study has been designed and performed in 13 ALS Italian referral centers, located in 10 Italian Regions: ALS Centers of Turin, Padua, Genoa, Naples, Modena, Lecce, Rome, NEMO Clinical Centers in Milan, and Messina, ALS Centers of ICS Maugeri in Milan and Mistretta, ALS Centers at San Raffaele Institute, and IRCCS Istituto Auxologico Italiano in Milan (Calvo et al., 2017; Trojsi et al., 2017b; Mandrioli et al., 2018a). We included 1417 patients, diagnosed with definite or clinical and laboratory-supported probable ALS, according to the Revised El Escorial Criteria (Brooks et al., 2000), from January 1st, 2009 to December 31st, 2013, in whom genetic data were available (**Figure 1**).

Data have been recorded into an electronic database available to all involved centers. According to previously used selection methods (Umoh et al., 2016), all patients followed at the involved ALS referral centers were consecutively asked to donate DNA for research purposes, and the only criteria for inclusion were the diagnosis of ALS and the consent to donate blood for genetic screening. Caring neurologists collected a detailed phenotypic profile for each ALS patient, including the following information: among demographic data, sex, age at onset, at clinical observation and at diagnosis; among clinical data, site, and time of onset, clinical phenotype [classic, bulbar, predominant upper motor neuron (UMN-p), flail arm, flail leg, and respiratory ALS] (Chiò et al., 2011), presence of concomitant dementia and family history of ALS, FTD or other neurodegenerative diseases (i.e., Parkinson's and Alzheimer's disease), metabolic (i.e., diabetes), oncologic, cardiovascular (i.e., hypertension, atrial fibrillation, and heart failure), auto-immune, hematological, gastroenteric, and psychiatric diseases. The genetic analysis included screening for *SOD1*, *FUS*, *TARDBP*, and *C9orf72* status (normal or expanded), four genes accounting for up to 70% of all cases of familial ALS (Hardiman et al., 2017). When mutations of these genes or *C9orf72* expansion were not revealed and in presence of family history of ALS and/or FTD, mutations of *ALS2*, *ANG*, *DYT11*, *OPTN*, and *PGRN* were also explored. *C9orf72* status was determined by repeat primed PCR as described previously (with individual laboratory-based validation and quality control

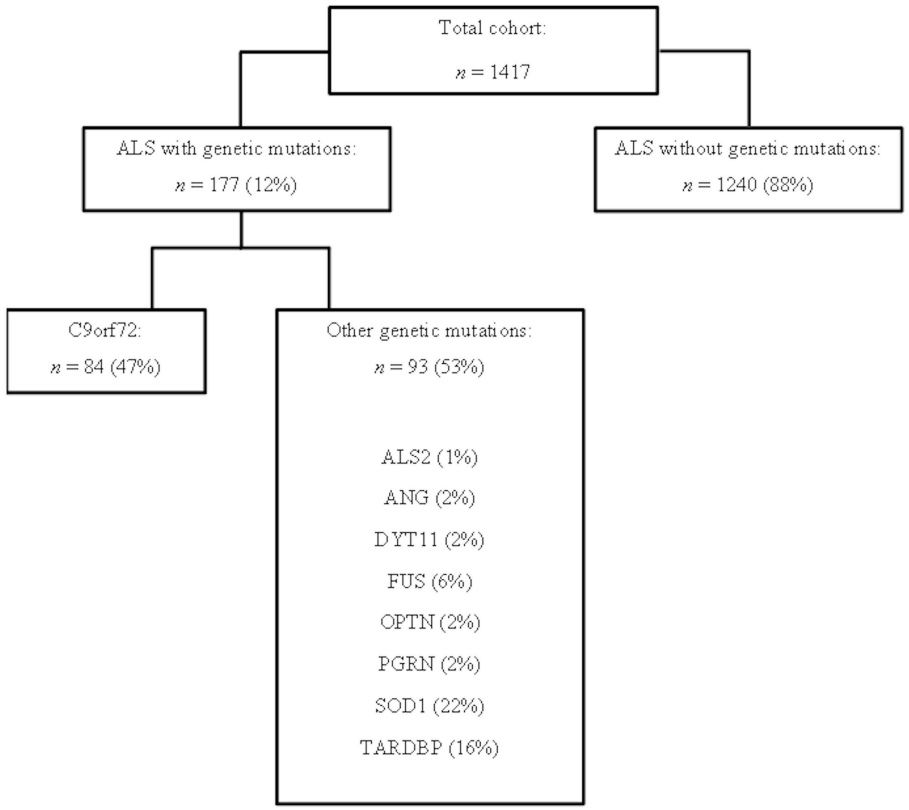

FIGURE 1 | Flow chart representing the studied cohort from 13 ALS Italian referral centers, in which genetic data were available.

by Southern blot analyses) (DeJesus-Hernandez et al., 2011; Renton et al., 2011; Byrne et al., 2012, 2013; Chiò et al., 2012; Cooper-Knock et al., 2012; Majounie et al., 2012; Sabatelli et al., 2012; Irwin et al., 2013; Umoh et al., 2016). Among outcome information, dates of percutaneous endoscopic gastrostomy (PEG), non-invasive ventilation (NIV) and tracheotomy/death were also collected.

This study was approved by the Ethical Committees of the participating ALS centers and conducted according to the principles expressed in the Declaration of Helsinki. Patient or family written consent was obtained from each participant.

Statistical Analysis

Descriptive statistics are reported as count and percentage for categorical variables (i.e., sex) or mean and standard deviation for continuous variables (i.e., age at onset, age at clinical observation, age at diagnosis and diagnostic delay).

The comparisons between ALS-C9Pos and ALS patients without genetic mutations (ALSwoGM) were performed using one-way analysis of variance (ANOVA), and Pearson chi-square test (χ^2), when appropriate.

Logistic regression analyses (hierarchical method) were used for measuring the association between the presence of *C9orf72* expansion and clinical phenotype (i.e., classic, bulbar, flail arm, flail leg, and UMN-p), site of onset (i.e., spinal and bulbar), therapeutic interventions (i.e., NIV, PEG, tracheostomy, and riluzole), other diseases (i.e., FTD, diabetes, hypertension, heart disease, cancer, autoimmune, hematological, gastroenteric, and psychiatric), and family history (i.e., ALS, FTD, and Parkinson's and Alzheimer's disease). All logistic regression models were adjusted for baseline demographic and clinical differences between ALS-C9Pos and ALSwoGM, and the results were presented as adjusted odds ratio (OR). Kaplan-Meier univariate analysis was used to determine the effect of *C9orf72* repeat expansion on the survival time for the whole sample as well as for the men and the women. Moreover, the univariate effect on survival time of the variables associated to the presence of *C9orf72* repeat expansion was explored by Kaplan-Meier analysis for categorical variables (using the log-rank test) and by Cox proportional hazards analysis for continuous variables (using hazard ratios or HR, and 95% confidence interval or 95% CI).

Finally, Cox proportional hazards regression multivariate analysis (Forward Conditional method) was performed entering the variables associated with the survival time in univariate analyses in order to explore the effect of the *C9orf72* repeat expansion on survival time in a multivariate model. These analyses were performed in the whole sample and, then, stratifying by sex. Survival time was defined as time from symptom onset to time of death/tracheotomy. Patients who were alive at time of analysis were censored.

Statistical analyses were performed using IBM Statistical Package for Social Science (SPSS) version 20, with p-value < 0.05.

TABLE 1 | Descriptive statistics of amyotrophic lateral sclerosis patients with pathogenic *C9orf72* expansion (ALS-C9Pos) and without genetic mutations (ALSwoGM).

	ALS-C9Pos ($n = 84$)	ALSwoGM ($n = 1240$)	F/χ^2	p
Sex, *n* (%)				
Male	47 (54%)	681 (55%)	0.03	0.854
Female	37 (46%)	559 (45%)		
Mean age at onset, years (SD)	58.49 (9.55)	63.41 (11.6)	14.44	**<0.001**
Mean age at clinical observation, years (SD)	64.36 (9.78)	69.82 (11.47)	18.10	**<0.001**
Mean age at diagnosis, years (SD)	59.47 (9.55)	64.69 (11.44)	16.69	**<0.001**
Diagnostic delay, months (SD)	11.73 (8.58)	15.35 (21.85)	2.28	0.131

ALS-C9Pos, amyotrophic lateral sclerosis with pathogenic C9orf72 expansion; ALSwoGM, amyotrophic lateral sclerosis without genetic mutations; significant differences are signed in ***bold****; SD, standard deviation.*

RESULTS

The *C9orf72* repeat expansion was identified in 84 cases (**Figure 1**). ALSwoGM were 1240, excluding 93 ALS patients carrying other genetic mutations (**Figure 1**).

Comparing ALS-C9Pos to ALSwoGM patients, there were significant differences in age at onset, age at clinical observation, and age at diagnosis (**Table 1**).

After having adjusted for demographic and clinical differences, logistic regression analyses showed that pathogenic *C9orf72* expansion was associated with higher OR of bulbar onset, FTD diagnosis, and family history of ALS, FTD, and Alzheimer's disease. In addition, the *C9orf72* expansion was associated with lower OR of spinal onset, NIV, hypertension, and psychiatric diseases (**Table 2**). Noteworthy, no ALS patients with pathogenic *C9orf72* expansion had respiratory phenotype or respiratory onset.

Kaplan-Meier analysis showed that ALS-C9Pos patients had shorter survival time than ALSwoGM patients (**Figure 2**). When the overall sample was stratified according to sex, this pattern of results was confirmed only for men (**Figure 3**). However, a preliminary Kaplan-Meier analysis did not show significant differences in the survival time comparing men to women for the overall sample, regardless of the stratification due to the presence of *C9orf72* repeat expansion (**Supplemental Figure 1**). Among the variables associated with the presence of *C9orf72* expansion, those related to shorter survival time on univariate analyses included the following: (1) higher age at onset (HR 1.04, 95% CI 1.03–1.04, $p < 0.0001$); (2) higher age at clinical observation (HR 1.03, 95% CI 1.02–1.04, $p < 0.0001$); (3) higher age at diagnosis (HR 1.03, 95% CI 1.02–1.04, $p < 0.0001$); (4) site of onset, with worse outcome for bulbar onset (log-rank test, $p < 0.0001$); (5) the presence of FTD (log-rank test, $p < 0.0001$); (6) hypertension (log-rank test, $p < 0.0001$); (7) psychiatric diseases (log-rank test, $p = 0.047$); (8) family history of ALS (log-rank test, $p = 0.015$).

TABLE 2 | Relationship between pathogenic *C9orf72* expansion and phenotype, site onset, family history, therapy, and others comorbidities in patients with amyotrophic lateral sclerosis.

	ALS-C9Pos *n (%)*	ALSwoGM *n (%)*	p	OR	95%CI
Classic phenotype					
Yes	53 (63%)	633 (51%)	0.132	1.42	[0.89, 2.26]
No	31 (37%)	607 (49%)			
Bulbar phenotype					
Yes	16 (19%)	224 (18%)	0.434	1.25	[0.71, 2.24]
No	68 (81%)	1016 (82%)			
Flail arm phenotype					
Yes	1 (1%)	79 (6%)	0.102	0.19	[0.02, 1.39]
No	83 (99%)	1161 (94%)			
Flail leg phenotype					
Yes	1 (1%)	76 (6%)	0.113	0.20	[0.02, 1.46]
No	83 (99%)	1164 (94%)			
Umn phenotype					
Yes	5 (6%)	108 (9%)	0.375	0.65	[0.25, 1.66]
No	79 (94%)	1132 (91%)			
Spinal onset					
Yes	51 (61%)	881 (71%)	**0.009**	0.53	[0.33, 0.85]
No	33 (39%)	358 (29%)			
Bulbar onset					
Yes	32 (38%)	346 (28%)	**0.012**	1.83	[1.14, 2.93]
No	52 (62%)	893 (72%)			
NIV					
Yes	26 (31%)	580 (47%)	**0.015**	0.55	[0.33, 0.89]
No	58 (69%)	660 (53%)			
PEG					
Yes	31 (37%)	415 (34%)	0.377	1.23	[0.77, 1.98]
No	53 (63%)	825 (66%)			
Tracheostomy					
Yes	17 (20%)	203 (16%)	0.386	1.28	[0.73, 2.24]
No	67 (80%)	1037 (84%)			
Riluzole					
Yes	74 (88%)	1043 (84%)	0.591	1.20	[0.61, 2.4]
No	10 (12%)	197 (16%)			
FTD					
Yes	22 (26%)	93 (7%)	**<0.001**	5.41	[3.10, 9.45]
No	62 (74%)	1147 (93%)			
Diabetes					
Yes	3 (4%)	116 (9%)	0.143	0.41	[0.13, 1.34]
No	81 (96%)	1124 (91%)			
Hypertension					
Yes	19 (23%)	561 (45%)	**0.004**	0.45	[0.26, 0.78]
No	65 (77%)	679 (55%)			
Heart Diseases					
Yes	5 (6%)	203 (16%)	0.080	0.43	[0.17, 1.10]
No	79 (94%)	1037 (84%)			
Cancer					
Yes	4 (5%)	141 (11%)	0.119	0.44	[0.15, 1.23]
No	80 (95%)	1099 (89%)			

(Continued)

TABLE 2 | Continued

	ALS-C9Pos *n (%)*	ALSwoGM *n (%)*	*p*	OR	95%CI
Autoimmune diseases					
Yes	6 (7%)	90 (7%)	0.979	0.99	[0.41, 2.35]
No	78 (93%)	1150 (93%)			
Hematological diseases					
Yes	1 (1%)	48 (4%)	0.332	0.37	[0.05, 2.74]
No	83 (99%)	1192 (96%)			
Gastroenteric diseases					
Yes	7 (8%)	197 (16%)	0.070	0.48	[0.21, 1.06]
No	77 (92%)	1043 (84%)			
Psychiatric diseases					
Yes	4 (5%)	143 (12%)	**0.043**	0.35	[0.12, 0.96]
No	80 (95%)	1097 (88%)			
Family history of ALS					
Yes	38 (45%)	51 (4%)	**<0.001**	17.26	[10.25, 29.05]
No	46 (55%)	1189 (96%)			
Family history of PD					
Yes	3 (4%)	49 (4%)	0.640	0.75	[0.23, 2.49]
No	81 (96%)	1191 (96%)			
Family history of FTD					
Yes	15 (18%)	14 (1%)	**<0.001**	16.23	[7.39, 35.62]
No	69 (82%)	1226 (99%)			
Family history of AD					
Yes	18 (21%)	100 (8%)	**0.001**	2.70	[1.52, 4.79]
No	66 (79%)	1140 (92%)			

ALS, amyotrophic lateral sclerosis; ALS-C9Pos, amyotrophic lateral sclerosis with pathogenic C9orf72 expansion; ALSwoGM, amyotrophic lateral sclerosis without genetic mutations; adjOR, adjusted odds ratio; AD, Alzheimer's disease; FTD, frontotemporal dementia; NIV, non-invasive ventilation; PD, Parkinson's disease; PEG, percutaneous endoscopic gastrostomy; significant associations are signed in ***bold****.*

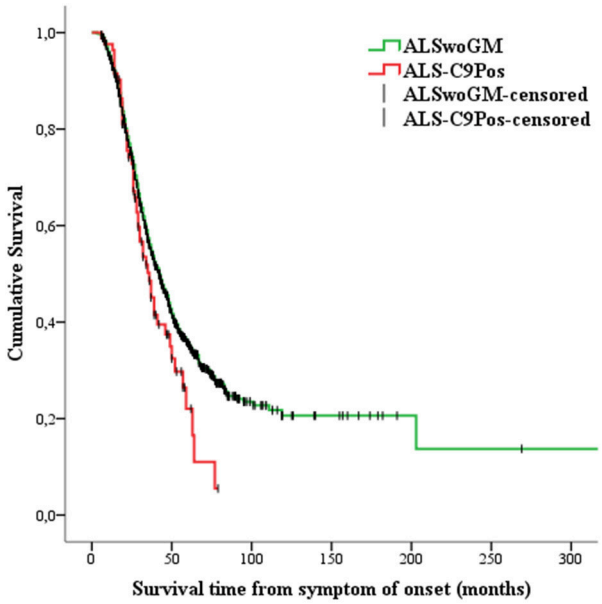

FIGURE 2 | Kaplan-Meier plots of survival probabilities: the patients carrying the pathogenic *C9orf72* repeat expansion (or ALS-C9Pos, red line) display shorter survival time [median survival of 36 months (95% CI 30–43)] than the patients without genetic mutations (ALSwoGM, green line) [median survival of 42 months (95% CI 39–45)]. Log-rank $\chi^2 = 3.88$, $p = .049$; +: censored cases.

Cox proportional hazards regression multivariate analysis showed that shorter survival time was associated with the presence of *C9orf72* expansion in men (HR 1.58, 95% CI 1.07–2.33, $p = 0.021$), but not in women ($p > 0.05$) as well as in the whole sample ($p > 0.05$) (**Table 3**; **Figure 4**; see also the **Supplemental Table 1** for variables that were not included in the equation for each step of the Cox proportional hazards regression multivariate analysis).

DISCUSSION

Patients with ALS carrying the *C9orf72* repeat expansion have been shown to exhibit remarkable clinical and pathological features suggesting that this hexanucleotide expansion identifies a distinct population of patients, with significant implications on therapeutic interventions design and screening for inclusion in clinical trials. In our multicenter analysis, performed on data from 1417 patients, we compared demographic and clinical features of a population of ALS-C9Pos patients to those of a cohort of ALSwoGM patients to determine whether these two groups were phenotypically distinct. Our results partly confirmed previous evidence in that ALS-C9Pos patients were younger at onset, at first clinical observation and at diagnosis and exhibited higher odds of bulbar onset, FTD diagnosis, and family history of ALS, FTD, and Alzheimer's disease. Remarkably, we revealed that ALS-C9Pos patients, especially males, had shorter survival than ALSwoGM patients, thereby enhancing the emerging hypothesis that sex may represent a crucial variable in the pathobiology of *C9orf72*-mediated disease.

Our findings derived from between-groups comparisons mirrored previous results regarding lower age at disease onset (Byrne et al., 2012; Cooper-Knock et al., 2012; Irwin et al., 2013) and diagnosis (Byrne et al., 2012) in ALS-C9Pos populations compared to cohorts of non-expanded ALS patients. Interestingly, the lower age at first observation and diagnosis described in our cohort of ALS-C9Pos patients was likely due to the higher attention to appearance of ALS symptoms revealed in subjects with known family history of ALS, as previously described in ALS-C9Pos patients (Umoh et al., 2016; Turner et al., 2017). Additionally, we reported an increased odds of bulbar onset in ALS-C9Pos patients, as also described in other cohorts of expanded patients (Chiò et al., 2012; Irwin et al., 2013; Cooper-Knock et al., 2014). This clinical onset has been used to identify a more aggressive ALS phenotype, since patients with bulbar disease may carry a worse prognosis (Magnus et al., 2002; Chiò et al., 2011) and peculiar neuropsychological and neuroimaging profiles (Cistaro et al., 2012; Trojsi et al., 2017a), as also confirmed by our results in which bulbar onset arises as an independent feature associated to *C9orf72* expansion and negatively impacting on prognostic outcome.

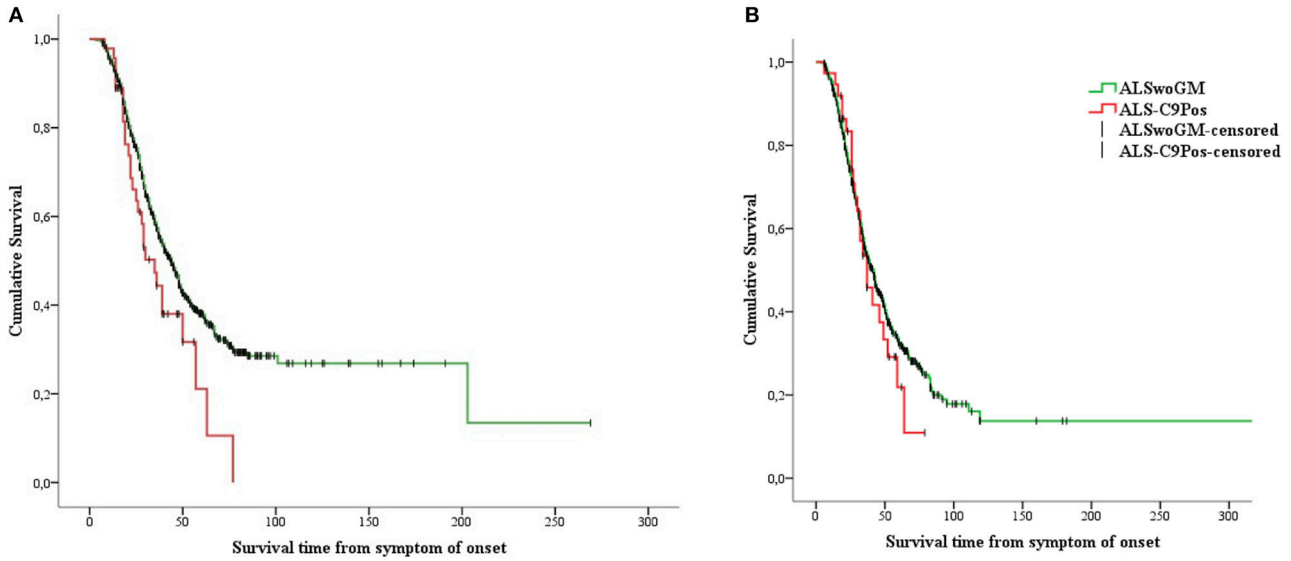

FIGURE 3 | Kaplan-Meier plots of survival probabilities, stratifying the overall sample by sex: shorter survival time is displayed in ALS-C9Pos patients (red line) compared to ALSwoGM patients (green line) only for males. **(A)** (male): Log-rank $\chi^2 = 4.33$, $p = 0.037$; median survival was 35 months (95% CI 26–44) for ALS-C9Pos ($n = 47$), and 44 months (95% CI 40–48) for ALSwoGM ($n = 681$). **(B)** (female): Log-rank $\chi^2 = 0.43$, $p = 0.510$; median survival was 37 months (95% CI 26–47) for ALS-C9Pos ($n = 37$), and 42 months (95% CI 37–46) for ALSwoGM ($n = 559$). +: censored cases.

As expected, we revealed that ALS-C9Pos patients were more likely to exhibit FTD diagnosis and to report family history of ALS, FTD, and Alzheimer's disease, thereby confirming the previously identified clinical profile of ALS-C9Pos patients (Byrne et al., 2012, 2013; Chiò et al., 2012; Cooper-Knock et al., 2012, 2014; Sabatelli et al., 2012; Umoh et al., 2016; Hardiman et al., 2017). However, we established diagnosis of dementia in patients' relatives retrospectively from medical records or from patients' reports and this may have underestimated significant cognitive changes in their pedigrees. Moreover, in the studied ALS population the formal neuropsychological testing was performed using heterogeneous protocols among the different referral centers, without collecting cognitive and behavioral scores for each patient (Trojsi et al., 2017a).

Of note is also that in our population the *C9orf72* expansion was associated with lower odds of spinal onset, NIV, hypertension, and psychiatric diseases. This evidence was not unexpected with regard to the lower odds of association between the *C9orf72* expansion and spinal onset and NIV, in consideration of the above discussed higher odds of association between the *C9orf72* expansion and bulbar onset, among possible onsets, and given the higher association between the *C9orf72* expansion and dementia, proven to induce less adherence and compliance to treatments, including NIV (Govaarts et al., 2016; Mandrioli et al., 2018b).

The association between arterial hypertension and ALS has been previously investigated, resulting in conflicting evidence regarding the prognostic role of this comorbidity (Moreau et al., 2012; Körner et al., 2013; Moglia et al., 2017; Mandrioli et al., 2018a). In particular, some studies on large ALS clinic-based cohorts reported conflicting results on the association between premorbid or comorbid arterial hypertension and shorter survival in ALS patients (Körner et al., 2013; Mandrioli et al., 2018a). Probably, our result of a lower odds of association between *C9orf72* expansion and hypertension may be due to the lack of this comorbidity among clinical features potentially related to the *C9orf72*-mediated pathology, although hypertension was proven associated to shorter survival time in ALS-C9Pos patients on univariate analysis. To note, among the potential prognostic factors for survival in ALS, although we have no information regarding comorbid familial hypercholesterolemia (FH), 3.14% of ALSwoGM patients and 3.57% of ALS-C9Pos patients had hypercholesterolemia. In the light of the recent polygenic evidence that low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) are causally associated with ALS (Chen et al., 2018), large-scale genome-wide association studies (GWASs) and deep sequencing for rare variants of LDL-C and TC risk alleles will be needed.

Additionally, the lower odds of association between the *C9orf72* expansion and psychiatric diseases found in our population, although apparently unexpected, may be explained on the basis of previous literature referring that behavioral abnormalities, rather than psychosis or other psychiatric syndromes *per se*, within clinical profile of FTD, were frequently observed among *C9orf72* expansion carriers (Watson et al., 2016; Devenney et al., 2017; Ducharme et al., 2017). Furthermore, as to the prevalence of neuropsychiatric conditions in relatives of ALS patients, although psychiatric symptoms have been described more frequently in ALS kindreds than in non-ALS pedigrees, the presence of *C9orf72* expansion was demonstrated not to fully account for this association (Byrne et al., 2013), suggesting that only some subphenotypes of ALS may share pleiotropic genetic

TABLE 3 | Cox proportional hazards regression multivariate analysis (Forward Conditional method) performed both in the whole sample and stratified by sex.

Variable	B (SE)	*p*-Value	HR	95% CI
Whole sample				
Step 1				
Age at onset	0.04 (0.00)	<0.001	1.04	1.03–1.05
Step 2				
Age at onset	0.04 (.00)	<0.001	1.04	1.03–1.05
Diagnostic delay	−0.05 (0.00)	<0.001	0.95	0.94–0.96
Step 3				
Age at onset	0.04 (0.00)	<0.001	1.04	1.03–1.05
Diagnostic delay	−0.05 (0.00)	<0.001	0.95	0.94–0.96
FTD	0.46 (0.12)	<0.001	1.58	1.25–1.99
Step 4				
Age at onset	0.03 (0.00)	<0.001	1.03	1.03–1.04
Diagnostic delay	−0.05 (0.00)	<0.001	0.95	0.94–0.96
Site of onset (Bulbar = 0; Spinal = 1)	−0.25 (0.08)	0.001	0.77	0.66–0.90
FTD	0.42 (0.12)	<0.001	1.53	1.21–1.94
Step 5[a]				
Age at onset	0.04 (0.00)	<0.001	1.04	1.03–1.05
Diagnostic delay	−0.05 (0.00)	<0.001	0.95	0.94–0.96
Site of onset (Bulbar = 0; Spinal = 1)	−0.25 (0.08)	0.002	0.78	0.66–0.91
FTD	0.38 (0.12)	0.002	1.47	1.16–1.86
Family history of ALS	0.31 (0.14)	0.037	1.35	1.01–1.79
Males				
Step 1				
Diagnostic delay	−0.05 (0.00)	<0.001	0.95	0.94–0.96
Step 2				
Diagnostic delay	−0.06 (0.00)	<0.001	0.95	0.93–0.96
Age at clinical observation	0.04 (0.00)	<0.001	1.04	1.03–1.05
Step 3				
Diagnostic delay	−0.06 (0.00)	<0.001	0.95	0.93–0.96
Age at clinical observation	0.04 (0.00)	<0.001	1.03	1.02–1.05
Site of onset (Bulbar = 0; Spinal = 1)	−0.32 (0.12)	0.005	0.72	0.58–0.91
Step 4[b]				
Diagnostic delay	−0.06 (0.00)	<0.001	0.95	0.93–0.96
Age at clinical observation	0.04 (0.00)	<0.001	1.04	1.03–1.05
Site of onset (Bulbar = 0; Spinal = 1)	−0.31 (0.12)	0.008	0.73	0.58–0.92
C9orf72 expansion status (No = 0; SI = 1)	0.46 (0.20)	0.021	1.58	1.07–2.33
Females				
Step 1				
Age at onset	0.04 (0.00)	<0.001	1.04	1.03–1.05
Step 2				
Age at onset	0.04 (0.00)	<0.001	1.04	1.03–1.05
Diagnostic delay	−0.05 (0.00)	<0.001	0.95	0.94–0.96
Step 3[c]				
Age at onset	0.04 (0.00)	<0.001	1.04	1.03–1.05
Diagnostic delay	−05 (0.00)	<0.001	0.95	0.94–0.96
FTD	0.65 (0.18)	<0.001	1.91	1.33–2.73

SE, Standard Error; HR, Hazard Ratio; CI, Confidence Interval; FTD, frontotemporal dementia; [a]*Variables not in equation at last step: age at diagnosis, age at clinical observation, hypertension, psychiatric diseases, and C9orf72 expansion status;* [b]*Variables not in equation at last step: age at diagnosis, age at onset, FTD, hypertension, psychiatric diseases, and family history of ALS;* [c]*Variables not in equation at last step: age at diagnosis, age at clinical observation, site of onset, hypertension, psychiatric diseases, family history of ALS, and C9orf72 expansion status.*

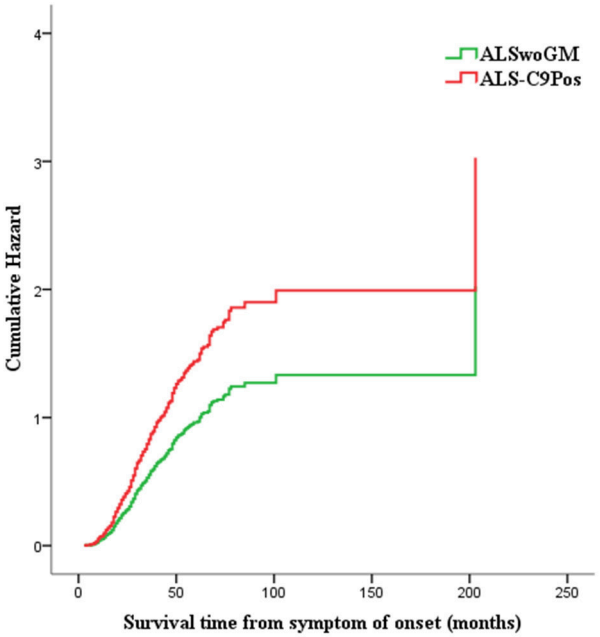

FIGURE 4 | Hazard plot representing the risk of death/tracheotomy for males carrying the pathogenic *C9orf72* repeat expansion (or ALS-C9Pos, red line) compared to males without genetic mutations (or ALSwoGM, green line): shorter survival time is associated with the presence of *C9orf72* repeat expansion (HR 1.58, 95% CI 1.07–2.33, $p = 0.021$).

risk with neuropsychiatric illnesses (O'Brien et al., 2017). Finally, there is some evidence that intermediate *C9orf72* repeat lengths are associated with personal or family history of FTD and/or psychiatric illness, although the "critical" *C9orf72* repeat size required for initiation of neurodegeneration remains unknown (Ng and Tan, 2017).

Our findings regarding the reduced overall survival in the ALS-C9Pos population compared to ALSwoGM patients and the significant associations between some clinical variables and shorter survival time in ALS-C9Pos patients are consistent, respectively, with previous evidence from other ALS-C9Pos cohorts compared to non-expanded ALS cohorts (Byrne et al., 2012; Chiò et al., 2012; Cooper-Knock et al., 2012; Irwin et al., 2013; Umoh et al., 2016; Hardiman et al., 2017; Rooney et al., 2017; Trojsi et al., 2017b) and from sporadic patients (Chiò et al., 2009; Watanabe et al., 2015). Interestingly, the multivariate analysis showed that shorter survival time was associated with the presence of *C9orf72* expansion in men when stratifying our ALS population by sex, thereby pointing toward the hypothesis that the male sex may drive the effect of the *C9orf72* repeat expansion rather than other prognostic factors, such as dementia and the clinical phenotype *per se*. To note, considering that the male sex could be more prone to certain clinical pathologies that decrease survival than the female one, to explore the differences in survival time between men and women, we performed a preliminary Kaplan-Meier analysis that did not show significant differences in the survival time between men and women for the overall sample,

regardless of the stratification due to the presence of *C9orf72* repeat expansion (**Supplemental Figure 1**).

The result of our multivariate analysis, showing a shorter survival time in men carrying the *C9orf72* expansion, resembles what recently revealed in a combined analysis of the prognostic characteristics of the *C9orf72* repeat expansion, performed in 4925 ALS cases from Dutch, Irish, and Italian population-based national registers and from two (Belgian and UK) clinical research center cohorts (Rooney et al., 2017). In this study, Rooney et al. revealed a previously unrecognized interaction between the expanded variant and male patients with spinal onset disease who exhibited a shorter survival (Rooney et al., 2017). In comparison to our results on survival, the findings by Rooney et al. (2017) were derived from a more powered analysis, adequately sound to highlight interactions between the presence of *C9orf72* expansion and more demographic features, such as sex and site of onset. However, male sex emerges from both studies as a crucial interacting factor in the biology of *C9orf72*-mediated disease, in contrast to previous analyses performed in non-expanded ALS cohorts, that reported that female sex was an independent predictor of faster functional decline (Chiò et al., 2012; Watanabe et al., 2015). Nevertheless, the pathobiology of the observed interactions between both *C9orf72*- positive and -negative variants and sex remains still unclear. In this regard, the role of environmental risk factors should be emphasized, as underlined by a recent long-term population-based analysis from the Piemonte and Valle d'Aosta Register for ALS (PARALS), that showed that incidence of ALS increased in the last two decades, mostly in women. A probable explanation for this was derived from a birth cohort effect in women, who profoundly modified their lifestyle mainly from 1920, thereby being more exposed to possible environmental risk factors for ALS (i.e., physical activity and cigarette smoking) (Chiò et al., 2017). These results all together pointed to the potential pathogenic role of exogenous factors with different gender-effect, probably derived from interaction with specific genetic backgrounds. In case of *C9orf72* repeat expansion, several pathogenic mechanisms have been described, such as haploinsufficiency, toxic RNA interfering with the function of RNA-binding proteins or other cellular factors, presence of toxic dipeptide repeat proteins, and alterations of nucleocytoplasmic transport (Freibaum and Taylor, 2017). However, the potential interactions between these mechanisms and environmental risk factors for ALS have not been elucidated. Finally, the *C9orf72* repeat expansion is known to have an incomplete and age-dependent penetrance and, in this regard, a recent penetrance model analysis of a large cohort, drawn from the published literature, reported an older age of onset among female carriers in general, and among females with bulbar onset in particular (Murphy et al., 2017), thereby inducing to hypothesize potential hormonal or X-linked factors influencing disease type and site of onset.

Limitations of our study were the retrospective nature of the analysis, causing the unavailability of some data (such as the neuropsychological scores collected, the specific psychiatric manifestations, and the *C9orf72* repeat size) and the multicenter design of the study. Moreover, the sample size was relatively small, thereby implying that our study was not sufficiently powered to use regression models to explore the effects of known important survival covariates, including age of onset, site of onset, diagnostic delay, and *C9orf72*, and whether the expanded variant differentially affects outcome in ALS subgroups. In addition, the genetic panel shared by all participating centers was initially limited to screening *SOD1*, *C9orf72*, *TARDBP,* and *FUS* mutations, and was extended to other ALS- or FTD-related mutations only in familial cases. Specifically, in the absence of a family history of ALS, a case-control design, in which patient samples are compared with samples from people without ALS, has been recognized as the simplest approach for gene discovery, although requiring the ability to sequence the whole genome to identify the rare variation. As low-frequency variation seems to have a large role in the genetic architecture of ALS, including apparently sporadic ALS, looking for such variants has been shown crucial (Al-Chalabi et al., 2017). Increasing efforts have been made and will be further needed to understand the genetic component of ALS risk, especially carrying out large-scale international collaborations for exome analysis or more detailed genotyping of apparently sporadic patients.

Overall, we underlined that patients with ALS carrying the *C9orf72* repeat expansion exhibit remarkable clinical and pathological features suggesting that this hexanucleotide expansion identifies a distinct population of patients. In particular, the interaction between the *C9orf72* repeat expansion and gender should be further investigated to identify future disease-related prognostic models with potential, significant implications for screening of patients, and incorporation into clinical trials.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

This study was approved by the Ethical Committees of the participating ALS centers and conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from all the participants of this study.

AUTHOR CONTRIBUTIONS

FT, MSi, CiF, GaS, CL, AnC, CM, KM, NT, ChF, CS, GiS, AmC, YF, RT, MR, VAS, AdC, GM, ViS, PV, CC, GQ, MSa, NR, GL, SM, AF, MM, GT, and JM conceived and designed the experiments. FT, MSi, CiF, GaS, CL, AnC, CM, KM, NT, ChF, CS, GiS, AmC, YF, RT, MR, VAS, AdC, GM, ViS, PV, CC, GQ, MSa, NR, GL, SM, AF, MM, and JM performed the experiments. FT, MSi, and GaS analyzed the data. FT, CiF, GaS, CL, AnC, CM, KM, NT, ChF, CS, GiS, AmC, YF, RT, MR, VAS, AdC, GM, ViS, PV, CC, GQ, MSa, NR, GL, SM, AF, MM, GT, and JM contributed reagents, materials and analysis tools. FT, MSi, GaS, CL, AnC, CM, KM, NT, ChF, CS, GiS, AmC, YF, RT, MR, VAS, AdC, GM, ViS, PV , GT, and JM wrote and/or revised the paper.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2019.00485/full#supplementary-material

Supplemental Figure 1 | Kaplan-Meier plots of survival probabilities, stratifying the whole sample by sex: no difference is displayed for cumulative survival in males [green line; median survival of 43 months (95% CI 39–47)] compared to females [blue line; median survival of 41 months (95% CI 37–45)]. Log-rank $\chi^2 = 2.11$, $p = 0.149$; +: censored cases.

REFERENCES

Al-Chalabi, A., van den Berg, L. H., and Veldink, J. (2017). Gene discovery in amyotrophic lateral sclerosis: implications for clinical management. *Nat. Rev. Neurol.* 13:96–104. doi: 10.1038/nrneurol.2016.182

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. doi: 10.1080/146608200300079536

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

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

Calvo, A., Moglia, C., Lunetta, C., Marinou, K., Ticozzi, N., Ferrante, G. D., et al. (2017). Factors predicting survival in ALS: a multicenter Italian study. *J. Neurol.* 264, 54–63. doi: 10.1007/s00415-016-8313-y

Chen, X., Yazdani, S., Piehl, F., Magnusson, P. K. E., and Fang, F. (2018). Polygenic link between blood lipids and amyotrophic lateral sclerosis. Neurobiol. *Aging* 67, 202.e1–202.e6. doi: 10.1016/j.neurobiolaging.2018.03.022

Chiò, A., Borghero, G., Restagno, G., Mora, G., Drepper, C., Traynor, B. J., et al. (2012). Clinical characteristics of patients with familial amyotrophic lateral sclerosis carrying the pathogenic GGGGCC hexanucleotide repeat expansion of *C9ORF72*. *Brain* 135, 784–793. doi: 10.1093/brain/awr366

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

Chiò, A., Logroscino, G., Hardiman, O., Swingler, R., Mitchell, D., Beghi, E., et al. (2009). Prognostic factors in ALS: a critical review. *Amyotroph. Lateral Scler.*10, 310–323. doi: 10.3109/17482960802566824

Chiò, A., Mora, G., Moglia, C., Manera, U., Canosa, A., Cammarosano, S., et al. (2017). Secular trends of amyotrophic lateral sclerosis: the Piemonte and Valle d'Aosta Register. *JAMA Neurol.* 74, 1097–1104. doi: 10.1001/jamaneurol.2017.1387

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. Imaging* 39, 251–259. doi: 10.1007/s00259-011-1979-6

Cooper-Knock, J., Hewitt, C., Highley, J. R., Brockington, A., Milano, A., Man, S., et al. (2012). Clinico-pathological features in amyotrophic lateral sclerosis with expansions in *C9ORF72*. *Brain* 135, 751–764. doi: 10.1093/brain/awr365

Cooper-Knock, J., Shaw, P. J., and Kirby, J. (2014). The widening spectrum of *C9ORF72*-related disease; genotype/phenotype correlations and potential modifiers of clinical phenotype. *Acta Neuropathol.* 127, 333–335. doi: 10.1007/s00401-014-1251-9

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. M., Landin-Romero, R., Irisha, M., Hornberger, M., Mioshi, E., Halliday, G. M., et al. (2017). The neural correlates and clinical characteristics of psychosis in the frontotemporal dementia continuum and the *C9orf72* expansion. *NeuroImage Clin.* 13, 439–445. doi: 10.1016/j.nicl.2016.11.028

Ducharme, S., Bajestan, S., Dickerson, B. C., and Voon, V. (2017). Psychiatric presentations of *C9orf72* mutation: what are the diagnostic implications for clinicians? *J. Neuropsychiatry Clin. Neurosci.* 29, 195–205. doi: 10.1176/appi.neuropsych.16090168

Freibaum, B. D., and Taylor, J. P. (2017). The role of dipeptide repeats in *C9ORF72*-related ALS-FTD. *Front. Mol. Neurosci.*10:35. doi: 10.3389/fnmol.2017.00035

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

Hardiman, O., Al-Chalabi, A., Chiò, A., Corr, E. M., Logroscino, G., Robberecht, W., et al. (2017). Amyotrophic lateral sclerosis. *Nat. Rev. Dis. Primers* 3:17085. doi: 10.1038/nrdp.2017.85

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. Psychiatr.* 84, 163–169. doi: 10.1136/jnnp-2012-303507

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

Magnus, T., Beck, M., Giess, R., Puls, I., Naumann, M., and Toyka, K. V. (2002). Disease progression in amyotrophic lateral sclerosis: predictors of survival. *Muscle Nerve* 25, 709–714. doi: 10.1002/mus.10090

Majounie, E., Renton, A. E., Mok, K., Dopper, E. G., Waite, A., Rollinson, S., et al. (2012). Frequency of the *C9orf72* hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study. *Lancet Neurol.* 11, 323–330. doi: 10.1016/S1474-4422(12)70043-1

Mandrioli, J., Ferri, L., Fasano, A., Zucchi, E., Fini, N., Moglia, C., et al. (2018a). 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

Mandrioli, J., Malerba, S. A., Beghi, E., Fini, N., Fasano, A., Zucchi, E., et al., (2018b). Riluzole and other prognostic factors in ALS: a population-based registry study in Italy. *J. Neurol.* 265, 817–827. doi: 10.1007/s00415-018-8778-y

Miltenberger-Miltenyi, G., Conceição, V. A., Gromicho, M., Pronto-Laborinho, A. C., Pinto, S., and de Carvalho, M. (2018). *C9orf72* expansion is associated with accelerated decline of respiratory function and decreased survival in amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* 90, 118–120. doi: 10.1136/jnnp-2018-318032

Moglia, C., Calvo, A., Canosa, A., Bertuzzo, D., Cugnasco, P., Solero, L., et al. (2017). Influence of arterial hypertension, type 2 diabetes and cardiovascular risk factors on ALS outcome: a population-based study. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 18, 590–597. doi: 10.1080/21678421.2017.1336560

Moreau, C., Brunaud-Danel, V., Dallongeville, J., Duhamel, A., Laurier-Grymonprez, L., de Reuck, J., et al. (2012). Modifying effect of arterial hypertension on amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* 13, 194–201. doi: 10.3109/17482968.2011.610110

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

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

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 patients with amyotrophic lateral

sclerosis. *JAMA Neurol*. 74, 1425–1430. doi: 10.1001/jamaneurol.2017.2699

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

Rooney, J., Fogh, I., Westeneng, H.-J., Vajda, A., McLaughlin, R., Heverin, M., et al. (2017). *C9orf72* expansion differentially affects males with spinal onset amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* 88, 295–300. doi: 10.1136/jnnp-2016-314093

Sabatelli, M., Conforti, F. L., Zollino, M., Mora, G., Monsurrò, M. R., Volanti, P., et al. (2012). *C9ORF72* hexanucleotide repeat expansions in the Italian sporadic ALS population. *Neurobiol. Aging* 33, 1848.e15–1848.e20. doi: 10.1016/j.neurobiolaging.2012.02.011

Trojsi, F., Di Nardo, F., Santangelo, G., Siciliano, M., Femiano, C., Passaniti, C., et al., (2017a). 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., Femiano, C., Santangelo, G., Lunetta, C., Calvo, A., et al. (2017b). Comorbidity of dementia with amyotrophic lateral sclerosis (ALS): insights from a large multicenter Italian cohort. *J. Neurol.* 264, 2224–2231. doi: 10.1007/s00415-017-8619-4

Turner, M. R., Al-Chalabi, A., Chiò, A., Hardiman, O., Kiernan, M. C., Rohrer, J. D., et al. (2017). Genetic screening in sporadic ALS and FTD. *J. Neurol. Neurosurg. Psychiatry* 88, 1042–1044. doi: 10.1136/jnnp-2017-315995

Umoh, M. E., Fournier, C., Li, Y., Polak, M., Shaw, L., Landers, J. E., et al. (2016). Comparative analysis of *C9orf72* and sporadic disease in an ALSclinic population. *Neurology* 87, 1024–1030. doi: 10.1212/WNL.0000000000003067

Watanabe, H., Atsuta, N., Nakamura, R., Hirakawa, A., Watanabe, H., Ito, M., et al. (2015). Factors affecting longitudinal functional decline and survival in amyotrophic lateral sclerosis patients. *Amyotroph. Lateral Scler. Front. Degener*. 16, 230–236. doi: 10.3109/21678421.2014.990036

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

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Comprehensive Genetic Analysis of a Hungarian Amyotrophic Lateral Sclerosis Cohort

Kornélia Tripolszki[1], Piyush Gampawar[2], Helena Schmidt[2], Zsófia F. Nagy[1], Dóra Nagy[1], Péter Klivényi[3], József I. Engelhardt[3] and Márta Széll[1]*

[1] Department of Medical Genetics, University of Szeged, Szeged, Hungary, [2] Research Unit for Genetic Epidemiology, Gottfried Schatz Research Center, Molecular Biology and Biochemistry, Medical University of Graz, Graz, Austria, [3] Department of Neurology, University of Szeged, Szeged, Hungary

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the degeneration of motor neurons. Genetic factors play a key role in ALS, and identifying variants that contribute to ALS susceptibility is an important step toward understanding the etiology of the disease. The frequency of protein altering variants in ALS patients has been extensively investigated in populations of different ethnic origin. To further delineate the genetic architecture of the Hungarian ALS patients, we aimed to detect potentially damaging variants in major and minor ALS genes and in genes related to other neurogenetic disorders. A combination of repeat-sizing of *C9orf72* and next-generation sequencing (NGS) was used to comprehensively assess genetic variations in 107 Hungarian patients with ALS. Variants in major ALS genes were detected in 36.45% of patients. As a result of repeat sizing, pathogenic repeat expansions in the *C9orf72* gene were detected in 10 patients (9.3%). According to the NGS results, the most frequently mutated genes were *NEK1* (5.6%), *NEFH*, *SQSTM1* (3.7%), *KIF5A*, *SPG11* (2.8%), *ALS2*, *CCNF*, *FUS*, *MATR3*, *TBK1*, and *UBQLN2* (1.9%). Furthermore, potentially pathogenic variants were found in *GRN* and *SIGMAR1* genes in single patients. Additional 33 novel or rare known variants were detected in minor ALS genes, as well as 48 variants in genes previously linked to other neurogenetic disorders. The latter finding supports the hypothesis that common pathways in different neurodegenerative diseases may contribute to the development of ALS. While the disease-causing role of several variants identified in this study has previously been established, other variants may show reduced penetrance or may be rare benign variants. Our findings highlight the necessity for large-scale multicenter studies on ALS patients to gain a more accurate view of the genetic pattern of ALS.

Keywords: amyotrophic lateral sclerosis, oligogenic inheritance, next-generation sequencing, mutation screening, *C9orf72* repeat expansion, genetic heterogeneity

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****Correspondence:***
Kornélia Tripolszki
tripolszkikornelia@gmail.com

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the degeneration of upper and lower motor neurons (UMN and LMN, respectively) in the motor cortex, brain stem, and spinal cord, with a life expectancy of 3–5 years from symptom onset (Peters et al., 2015). Approximately 5–10% of all cases show a family history of ALS (fALS), whereas the

remaining 90–95% seem to occur sporadically (sALS, Renton et al., 2014); nevertheless, fALS and sALS cases are indistinguishable regarding their clinical features. The genetic background of ALS is complex: more than 30 major genes have been associated with the disease, and more than 100 additional genes have been associated with disease risk (Amyotrophic Lateral Sclerosis Online Database, Abel et al., 2012). Variants of these genes have been implicated in several pathological mechanisms of ALS, including protein homeostasis, RNA metabolism, endosomal and vesicular transport, DNA repair, excitotoxicity, mitochondrial dysfunction, autophagy, nucleocytoplasmic transport, oligodendrocyte degeneration, axonal transport, and neuroinflammation (Hardiman et al., 2017; van Damme et al., 2017). The significance of trans-ethnic study design for human genetics is broadly documented (Morris, 2011; Asimit et al., 2016). Variants in the same genes are thought to contribute to the genetic etiology of both fALS and sALS (Renton et al., 2014). According to twin studies, the estimated heritability of sALS is about 60% (Al-Chalabi et al., 2010). Pathogenic variants have been described in 40–80% of fALS cases and in 5–15% of sALS patients (van Damme, 2018).

We have previously reported the prevalence and clinical characteristics of Hungarian patients with variants in the *SOD1, SETX*, and *C9orf72* genes (Tripolszki et al., 2017a; Tripolszki et al., 2017b). The aim of the present study was to investigate the variants in the set of genes that have been associated with ALS so far, using next-generation sequencing (NGS) and repeat sizing of the *C9orf72* gene in a cohort of 107 clinically well-characterized Hungarian patients.

PATIENTS AND METHODS

Patients

Patient recruitment was performed by senior neurologists at the Department of Neurology, University of Szeged. One hundred four patients were of Hungarian and three patients were of Romanian origin (total n = 107). Patients were unrelated to other enrolled patients and met the revised El Escorial and Awaji-shima criteria for ALS (de Carvalho and Swash, 2009; Ludolph et al., 2015). Regarding the family history of the patients, only one patient (#122u) showed a positive family history, and two patients (#99u and #93u) had relatives with uncertain diagnosis of ALS. Four patients (#91u, #90u, #87u, and #56r) had first- or second-degree relatives with other neurodegenerative diseases (Parkinson's or Alzheimer's disease). The samples of 37 patients (without pathogenic variants) from the previously studied cohort (Tripolszki et al., 2017a) were also used in this analysis. The whole cohort was prescreened by Sanger sequencing for two major ALS genes (*SOD1* and *TARDBP*), and no disease-causing variants were detected.

Because Hungarian population-specific databases have not been established yet, variants originating from whole-exome sequencing (WES) data of other studies were used as control in-house database. This in-house database included variants of 184 individuals (without any neurological diseases) of Hungarian (n = 133, mean age: 51 years) or Austrian (n = 51, mean age: 67.5 years) origin. To assess the frequency of the detected R261H *NEK1* variant in the Hungarian general population, an additional 186 samples from healthy individuals (mean age: 67 years) were used.

Methods

DNA Extraction

Genomic DNA was isolated from whole EDTA-containing venous blood using the DNeasy® Blood & Tissue Kit (QIAGEN, Gödöllő, Hungary).

C9orf72 Repeat Expansion Detection

A two-step protocol was applied for the detection of the GGGGCC repeat expansion (RE) in the *C9orf72* gene. A sizing PCR was performed to determine the number of hexanucleotide repeats in the normal range as described previously (Akimoto et al., 2014). Normal repeat length was defined as ≤28 repeats. Samples revealing a single peak product were further analyzed by long-read PCR using the AmplideX PCR/CE *C9orf72* (RUO) Assay (Asuragen, Inc.) as previously described (Suh et al., 2018). Amplidex PCR technique uses gene-specific and repeat-specific primers and provides an accurate capillary electrophoresis sizing of alleles up to 145 GGGGCC repeats and identifies the presence of expanded alleles over 145 repeats.

Next-Generation Sequencing

Patient DNA was target-enriched using a custom design SureSelect panel containing 247 genes (see **Supplementary Table 1** for gene lists) in 86 patients and Human All exon V6 kit in 21 patients (Agilent Technologies, Santa Clara, CA, USA), according to the manufacturer's recommendations. Sequencing was performed on Illumina NextSeq 500 sequencer (Illumina Inc., San Diego, CA, USA). As a result of sequencing, the mean on-target coverage was 189× in case of the panel data and 71× per base in case of whole exome data with an average percentage of targets covered greater or equal to 10× of 96% and 90%, respectively. Data analysis was performed according to the best practices to identify single-nucleotide variants and small insertions/deletions. Paired-end reads were aligned to the Human Reference Genome (UCSC Genome Browser build hg19) using the Burrows–Wheeler Aligner (BWA). Duplicates were marked using the Picard software package. Genome Analysis Toolkit (GATK) was used for variant calling (BaseSpace BWA Enrichment Workflow v2.1.1. with BWA 0.7.7-isis-1.0.0, Picard: 1.79 and GATK v1.6-23-gf0210b3), and variants tagged "PASS" by GATK were used for downstream analysis and annotated using the ANNOVAR software tool (version 2017 July 17, Wang et al., 2010). In case of whole exome data, the variant files were parsed for genetic variants in genes of the custom design SureSelect panel (247 genes, **Supplementary Table 1**). Raw reads of potentially relevant variants were manually checked using the Integrative Genomics Viewer (Robinson et al., 2011; Thorvaldsdóttir et al., 2013).

Variant Filtering

Calls per sample with a read depth of <10 or an allele balance of <0.3, intronic and synonymous variants, and variants with a population frequency higher than 0.1% in either the ExAC

Browser V0.3.1 (http://exac.broadinstitute.org) or 1000 Genomes Database (www.1000genomes.org/) were excluded from further analysis. Because the *SOD1* D91A variant is the most common known pathogenic variant seen in the Amyotrophic Lateral Sclerosis Online Database (ALSoD) with minor allele frequency (MAF) of 0.001, we used it as a criterion for filtering out variants with higher MAF. Other population databases were also used for additional variant information: Kaviar (version 2015 09 23; Glusman et al., 2011), dbSNP 138 (Sherry et al., 2002), and gnomAD (Lek et al., 2016). The combination of eight variant prioritization tools available from the dbNSFP database v3.0 (MetaSVM, M*etaL*R, DANN, PROVEAN, SIFT, Polyphen2, MutationTaster, MutationAssessor) was used to predict the effect of each variant on the corresponding protein (Liu et al., 2016). Variants identified in our patients were cross-checked in ALSoD (Abel et al., 2012), ALS Data Browser (ALSdb, http://alsdb.org) containing variants from 3,239 ALS cases and 11,808 controls (version v3 updated on Dec 03 2018), and ClinVar (Jan 30, 2017) databases, as well as in case reports in the literature. Variants that were found in our control in-house database—except for the R261H *NEK1* variant that was characterized by reduced penetrance—were excluded from further analysis. The detected variants were classified in accordance with the guideline of the American College of Medical Genetics and Genomics (ACMG, Richards et al., 2015). All genetic changes with a read depth <25 were validated by Sanger sequencing.

Gene Sets of Custom Design Panel

Based on the ALSoD and literature (Abel et al., 2012; Garton et al., 2017; Krüger et al., 2016), two gene sets containing all major and minor genes involved in ALS-associated pathways were generated: Set 1, categorized as major ALS genes, contained 30 genes that fulfilled the criteria for causation, and Set 2, contained 101 risk or candidate genes (ALSoD). A third extended gene set contained 116 genes related to other neurodegenerative and neuromuscular disorders (such as hereditary spastic paraplegia, spinal muscular atrophy, distal hereditary motor neuropathy, variants of Charcot–Marie–Tooth disease, distal myopathy, etc.) that may have genetic or symptomatic link to ALS (based on GeneReviews, Online Mendelian Inheritance in Man; Abel et al., 2012; Krüger et al., 2016; Garton et al., 2017; **Supplementary Table 1**).

Statistical Analysis

Statistical analysis of the R261H *NEK1* variant in patients and healthy individuals was carried out according to the guidelines of case–control allelic association study design (Lewis, 2002). All statistical analyses were performed using RStudio version 1.0.153 (RStudio Team, 2015). Frequencies were compared using X^2 statistics ($p < 0.05$).

RESULTS

A total of 107 ALS patients (62 females, 45 males; mean age at disease onset: 60 years; age range: 30–79 years) were analyzed in this study. All patients presented UMN and LMN signs, and 69% also presented bulbar signs.

Pathogenic repeat expansions in the *C9orf72* gene were detected in 10 patients (9.3%, mean age of onset: 60.8 years, age range: 49–72 years). According to NGS data, 28 variants were detected in 14 major ALS genes that passed the filtering criteria and assessment in Integrative Genome Viewer. Furthermore, we identified 33 variants in 26 minor ALS genes (**Supplementary Table 2**) and 48 variants in 31 genes associated with other neurodegenerative or neuromuscular diseases (**Supplementary Table 3**). No patients were identified as being homozygous for any of the studied variants.

Genetic Variants Detected in Major ALS Genes

Combining NGS and repeat sizing, variants in major ALS genes were detected in 36.45% (39/107) of patients including patients with variants considered to be of uncertain significance (VUS). The detected variants have MAF below 0.001, with an exception for variants in NEK1, as these variants have a reduced penetrance (Nguyen et al., 2018). Based on ACMG variant classification, two of the 29 detected major ALS variants were categorized as pathogenic, four as likely pathogenic and the remaining 23 variants as VUS. The most common pathogenic genetic alteration was the *C9orf72* hexanucleotide RE, present in 9.3% of our patients, with all 10 patients carrying more than 145 GGGGCC repeats. Bulbar symptoms, primarily the alteration of the speech, was the initial sign in seven out of the 10 patients with *C9orf72* RE. None of these patients had dementia according to the information obtained from the relatives (in case the patients could not speak or move at the examination) or the results of the MMSE. Three patients had concomitant thyroid disease, and two had a disease of the cervical spine with myelopathy, which may have influenced the signs of ALS (**Table 1**).

Apart from the repeat expansion, a rare missense variant of uncertain significance (R431Q) was also detected in the *C9orf72* gene. According to the NGS results, the most frequently mutated genes were *NEK1* (6/107, 5.6%), *NEFH*, *SQSTM1* (4/107, 3.7%), *KIF5A, SPG11* (3/107, 2.8%), *ALS2, CCNF, FUS, MATR3, TBK1,* and *UBQLN2* (2/107, 1.9%). Furthermore, potentially relevant variants were found in the *GRN* and *SIGMAR1* genes in single patients (**Table 2**). Because of the relatively high prevalence of the *NEK1* R261H variant in our patient cohort (5/107), we further evaluated 186 additional healthy controls (total 370) for this variant. R261H was identified in 5/107 (4.67%) patients and 4/370 (1.08%) controls, showing an enrichment in patients (MAF: 0.0234 vs 0.0054; $p = 0.0162$).

Six (6/107, 5.61%) patients had two rare variants in different major ALS genes. Two of these patients carried the C9orf72 RE and additional variants in the SQSTM1 or NEK1 genes. Only one patient (#108u) was detected to carry a pathogenic and a likely pathogenic variant in two different major ALS genes. In case of the other five patients with multiple major ALS gene variants, at least one of the two variants was categorized as VUS (**Table 3**).

Additionally, a novel variant (c.-25C > T) in the 5′ untranslated region of the *FUS* gene was also detected. As the screening of untranslated regions was not in the scope of our research, we did not examine it further.

TABLE 1 | Patients carrying the C9orf72 repeat expansion.

Patient ID	Age at onset (age range group, years)	Duration before the 1st exam	Early signs and symptoms	Signs at the 1st exam	ALSFRS-R	MMSE	Other diseases	Other major ALS gene variant	Family history
#122u	70-75	1.4 y	dysarthria dysphagia	B, PB +++ LMN +++ UMN+	9/48	NA	stenosis of the cervical spinal canal and myelopathy	–	Younger sister and mother probably ALS
#108u	45-50	2 ys	dysarthria	B, PB +++ LMN +++ UMN ++	16/48	NA	–	SQSTM1, R393Q	no
#99u	55-60	8 months	dysarthria	B, PB +++ LMN ++ UMN +	36/48	30/30	Hypo-thyreoidism	NEK1, N250S	Mother questioned /no medical data/ Uncertain family history
#96u	70-75	3 months	Para-paresis	B, PB – LMN + UMN ++	37/48	28/30	–	–	no
#83r	50-55	1 y	dysarthria, dysphagia	B, PB +++ LMN + UMN++	37/48	28/30	Hashimoto thyroiditis	–	no
#85r	60-65	6 months	Peroneal palsy	B, PB – LMN + UMN+	41/48	30/30	Hashimoto thyroiditis CV-CVI-CVII disc herniation operated	–	no
#50u	55-60	1 y	dysarthria dysphagia	B, PB +++ LMN + UMN +	45/48	30/30	–	–	no
#63u	60-65	9 months	dysarthria dysphagia peroneal palsy	B, PB +++ LMN + UMN +	33/48	28/30	–	–	no
#88u	55-60	1 y	Dysarthria dysphagia	B, PB +++ LMN + UMN +	41/48	NA	CVI-VII Disc protrusion	–	no
#75r	65-70	5 months	Para-paresis	B, PB – LMN+++ UMN++	35/48	30/30	–	–	no

B, PB, Bulbar and pseudobulbar; UMN, upper motor neuron; LMN, lower motor neuron; ALSFRS-R, ALS Functional Rating Scale Revised; MMSE, Mini-Mental State Examination.

No SOD1 and TARDBP gene variants were found in this cohort. We would like to point out that 37 of the analyzed samples were overlapping samples from a previous study (Tripolszki et al., 2017a) and were known to be negative for SOD1 and TARDBP mutations. Still, based on earlier results, one would expect to detect SOD1 variants in the further 70 samples.

Variants Detected in Minor ALS Genes

By focusing on the analysis of minor ALS genes, 33 variants (31 missense and 2 splicing) were detected in 26 genes corresponding to 29 patients (27.1% of all patients, **Supplementary Table 2**). No patients were identified as being homozygous for any of the detected variants.

A patient was carrying two novel variants (T2583I and G4290R) in the *DYNC1H1* gene; both variants localized in the motor domain of the protein. Due to the limitation of short-read sequencing and the lack of parental DNA, we could not assess whether the two variants were present in a compound heterozygous state or as a complex allele. Several other candidate variants of uncertain significance were identified (**Supplementary Table 2**) in minor genes. Results of future replication studies will reveal which of these variants are truly causative.

Genetic Variants of Genes Related to Other Neurodegenerative and Neuromuscular Diseases

Additionally, an analysis of 116 genes of other neurodegenerative diseases was performed to reveal potentially disease-causing variants. A total of 48 missense variants in 31 different genes were identified in 41 of our patients. Two of these variants were classified as likely pathogenic and 46 as VUS (**Supplementary Table 3**).

Among others, a known missense variant was detected in the *GJB1* gene (R230C), which is associated with mitochondrial disorders and Charcot–Marie–Tooth disease, and two variants

TABLE 2 | Major ALS gene variants detected in the Hungarian ALS cohort.

Gene	Transcript	Nucleotide change	Amino acid change	PopMax MAF (ExAc)	dbSNP	ALSdb MAF ALS	ALSdb MAF Control	Pathogenicity (ACMG)	MetaSVM/ MetaLR/ PROVEAN/ SIFT/ PolyPhen2/ MutTast/ MutAs/ DANN	No patients	References	Patient ID
ALS2	NM_020919	c.G3529T	p.G1177X	0	rs386134180	0	0	Pathogenic	D/D/D/D/D/ A/H/0.993	1	Sztriha et al., 2008	#62r
ALS2	NM_020919	c.G4496A	p.R1499H	$2{,}49x10^{-5}$	rs566436589	0	0	VUS	T/T/D/D/P/ D/L/1	1	–	#59r
C9orf72		GGGGCC Repeat expansion		0	–	0	0	Pathogenic	–	10	Renton et al., 2011; DeJesus-Hernandez et al., 2011	#99u, #108u, #75r, #50u, #63u, #88u, #96u, #85r, #83r, #122u
C9orf72	NM_001256054	c.G1292A	p.R431Q	$5{,}83x10^{-5}$	–	0	0	VUS	T/T/N/T/D/ D/L/0.999	1	–	#56u
CCNF	NM_001761	c.C1714T	p.R572W	$4{,}14x10^{-5}$	rs199743115	0	$4{,}24x10^{-5}$	VUS	T/T/D/D/D/ D/M/0.999	1	–	#107u
CCNF	NM_001761	c.C316G	p.L106V	0	rs990719669	0	$4{,}24x10^{-5}$	VUS	T/T/N/D/D/ D/M/0.998	1	–	#85u
FUS	NM_001170937	c.A74G	p.Y25C	$8{,}24x10^{-6}$	rs141516414	0	0	VUS	D/D/D/D/D/ D/M/0.992	1	–	#87u
FUS	NM_001170937	c.C317T	p.P106L	$2{,}47X10^{-5}$	rs374191107	0	$4{,}24x10^{-5}$	VUS	T/T/N/T/B/ D/M/0.997	1	Huey et al., 2012	#110u
GRN	NM_002087	c.T1003C	p.C335R	0	–	0	0	VUS	D/D/D/D/D/ D/H/0.996	1	–	#106u
KIF5A	NM_004984	c.G2272A	p.E758K	$5{,}54x10^{-4}$	rs140281678	$1{,}5x10^{-3}$	$8{,}90x10^{-4}$	VUS	T/T/N/D/B/ D/N/0.998	2	–	#85u, #57r
KIF5A	NM_004984	c.G1735A	p.A579T	0	rs760135493	$1{,}544x10^{-4}$	$4{,}24x10^{-5}$	VUS	T/T/N/T/B/ D/M/0.995	1	–	#83u
MATR3	NM_001194956	c.C31T	p.P11S	0	rs995345187	0	0	VUS	T/T/N/D/D/ D/N/0.998	1	–	#58r
MATR3	NM_018834	c.G824A	p.S275N	0	–	0	0	VUS	T/T/N/T/B/ D/N/0.99	1	–	#105u
NEFH	NM_021076	c.C1013T	p.T338I	0	rs774252076	0	0	VUS	D/D/D/D/D/ D/M/0.997	2	–	#63r, #75u
NEFH	NM_021076	c.G443C	p.R148P	0	–	0	0	VUS	D/D/D/D/D/ D/L/0.882	1	–	#69u
NEFH	NM_021076	c.C1514T	p.P505L	0	rs1414968372	$1{,}609x10^{-4}$	0	VUS	T/T/D/D/B/ N/L/0.843	1	–	#106u
NEK1	NM_001199397	c.G782A	p.R261H	$3{,}73x10^{-3}$	rs200161705	$6{,}6x10^{-3}$	$3{,}30x10^{-3}$	VUS	T/T/D/D/P/ D/M/0.999	5	Kenna et al., 2016; Brenner et al., 2016; Nguyen et al., 2018	#56r, #48r, #51, #90u, #93u

(*Continued*)

TABLE 2 | Continued

Gene	Transcript	Nucleotide change	Amino acid change	PopMax MAF (ExAc)	dbSNP	ALSdb MAF ALS	ALSdb MAF Control	Pathogenicity (ACMG)	MetaSVM/ MetaLR/ PROVEAN/ SIFT/ PolyPhen2/ MutTast/ MutAs/ DANN	No patients	References	Patient ID
NEK1	NM_001199397	c.A749G	p.N250S	0	rs368762503	0	0	VUS	T/T/D/T/P/ D/N/0.988	1	–	#99u
SIGMAR1	NM_001282205	c.T125G	p.I42R	0	rs1206984068	0	0	VUS	T/T/D/D/D/ D/M/0.987	1	–	#73u
SPG11	NM_025137	c.G6101A	p.R2034Q	0	rs750101301	0	0	VUS	T/T/N/T/B/ D/L/0.998	1	–	#64r
SPG11	NM_025137	c.C6352G	p.L2118V	$8{,}72x10^{-6}$	rs766851227	0	$4{,}25x10^{-5}$	VUS	D/D/N/D/D/ D/M/0.998	1	–	#71u
SPG11	NM_025137	c.G6009T	p.E2003D	0	–	0	0	VUS	T/T/N/T/B/ D/M/0.969	1	–	#104u
SQSTM1	NM_003900	c.C1175T	p.P392L	$9{,}00x10^{-4}$	rs104893941	$1{,}5x10^{-4}$	$2{,}20x10^{-3}$	Likely pathogenic	D/D/D/D/B/ A/L/0.996	2	Fecto et al., 2011	#57u, #64u
SQSTM1	NM_003900	c.G1165C	p.E389Q	0	rs1391182750	0	0	VUS	D/D/N/T/B/ D/L/0.99	1	–	#73u
SQSTM1	NM_003900	c.G1178A	p.R393Q	$4{,}94x10^{-5}$	rs200551825	0	$4{,}24x10^{-5}$	Likely pathogenic	D/D/N/D/P/ D/M/0.999	1	Kwok et al., 2014	#108u
TBK1	NM_013254	c.1888_1890del	p.K631del	0	–	0	0	VUS	–	1	–	#90u
TBK1	NM_013254	c.T1190C	p.I397T	$1{,}00x10^{-4}$	rs755069538	0	0	Likely pathogenic	T/T/N/T/B/ D/L/0.908	1	Pozzi et al., 2017	#97u
UBQLN2	NM_013444	c.A1174G	p.M392V	0	rs1384003425	0	0	Likely pathogenic	T/T/N/T/B/ D/L/0.955	1	Huang et al., 2017	#91u
UBQLN2	NM_013444	c.A252T	p.Q84H	0	–	0	0	VUS	T/T/N/T/D/ D/L/0.871	1	–	#111u

PopMax MAF (ExAc), Maximal general minor allele frequency of the variant in the ExAc database; ACMG, guideline of the American College of Medical Genetics and Genomics; dbSNP, Single Nucleotide Polymorphism Database reference SNP ID number for the variant; ALSdb MAF ALS, Minor allele frequency in ALS Data Browser (ALSdb) variants from 3,239 ALS cases and 11,808 con; ALSdb MAF Control, Minor allele frequency in ALS Data Browser (ALSdb) containing variants from 11,808 controls; No patients, Number of patients with this variant in this study; VUS, variant of uncertain significance; MetaSVM and MetaLR prediction: D, Damaging, T, Tolerated; PROVEAN: D, Deleterious, N, Neutral; SIFT: D, Deleterious, T, Tolerated; PolyPhen2: D, Damaging, B, Benign; MutTast (Mutation Taster): D, Disease causing, A, Disease causing automatic, MutAs (Mutation Assessor): N, Neutral; L, Low; M, Medium; H, High; DANN, The value range is 0 to 1, with 1 given to the variants predicted to be the most damaging.

TABLE 3 | Patients with two major ALS gene variants.

Patient ID	Age of onset (age range group, years)	Duration before the 1st exam	Early signs and symptoms	Symptoms	Gene	Variant	PopMax MAF (ExAc)	dbSNP	Pathogenicity (ACMG)	Other disease
#73u	65-70	1.5 y	Tetraparesis	B, PB ++, LMN ++, UMN +++	*SQSTM1*	p.E389Q	0	rs1391182750	VUS	Paget disease, Hyperparathyreodism, Hypothyreodism, Colon cancer (operated)
					SIGMAR1	p.I42R	0	rs1206984068	VUS	
#85u	60-65	6 months	Four extremity weakness with spasticity with muscle atrophy and fasciculations, especially in the interosseus muscles	B, PB +, LMN ++, UMN ++	*CCNF*	p.L106V	0	rs990719669	VUS	
					KIF5A	p.E758K	5.54×10^{-4}	rs140281678	VUS	
#90u	35-40	6 months	Psychomotor activity was slowing down, corticospinal tract lesion signs bilaterally	Memory loss, dementia.B, PB+, LMN ++, UMN +++	*TBK1*	p.K631del	3.73×10^{-3}	–	VUS	–
					NEK1	p.R261H		rs200161	VUS	
#99u	55-60	8 months	Dysarthria	B, PB +++, LMN ++, UMN+	*NEK1*	p.N250S	0	rs368762503	VUS	Hypothyreoidism
					C9orf72	Repeat expansion	0	–	Pathogenic	
#106u	50-55	9 months	Four extremity weakness with spasticity	B, PB –, UMN+++, LMN+++	*GRN*	p.C335R	0	–	VUS	Alcoholism, polyneuropathy, cervical myelopathy caused by CIII-IV-V. disc protrusions
					NEFH	p.P505L	0	rs1414968372	VUS	
#108u	45-50	2 years	Dysarthria	UMN, B, PB+++ LMN+++, UMN+++, B	*SQSTM1*	p.R393Q		rs200551825	VUS	–
					C9orf72	Repeat expansion	0	–	Pathogenic	

B, PB, Bulbar and pseudobulbar, UMN, upper motor neuron, LMN, lower motor neuron, PopMax MAF (ExAc), Maximal general minor allele frequency of the variant in the ExAc database; ACMG, guideline of the American College of Medical Genetics and Genomics; dbSNP, Single Nucleotide Polymorphism Database reference SNP ID number for the variant; VUS, variant of uncertain significance.

of conflicting significance in the *GBE1* gene (H398R and R166C), which is associated with autosomal recessive adult-type polyglucosan body disease (Online Mendelian Inheritance in Man). We identified sequence alterations in additional genes that are listed in **Supplementary Table 3**; however, most of these variants are unlikely to be implicated in our patients' phenotypes.

DISCUSSION

The frequency of causative variants in ALS patients has been extensively investigated in populations of different ethnic origins. Here, we used a combination of repeat-sizing of the *C9orf72* gene and next-generation sequencing to perform a comprehensive genetic analysis of 107 Hungarian ALS patients. Our genetic analysis included all known ALS-associated major and minor genes and an additional list of genes associated with other neurogenetic diseases.

Including the *C9orf72* RE, a total of 29 genetic variations have been detected in 14 different major ALS genes, leading to a positive result in 36.45% (39/107) of our ALS patients (**Table 2**). According to ACMG variant classification, 2 of the 29 detected major ALS variants were categorized as pathogenic, 4 as likely pathogenic and the remaining 23 variants as variants of uncertain significance. Furthermore, 33 variants in 26 minor ALS genes (**Supplementary Table 2**) and 48 variants in 31 genes associated with other neurodegenerative diseases (**Supplementary Table 3**) were detected. A major challenge of using NGS data is the critical evaluation of the significance of detected variants, especially those that are very rare or novel. While the disease-causing role of several variants identified in this study has previously been well established (ALSoD, Abel et al., 2012), other variants may show reduced penetrance or may be rare benign alterations.

In accordance with the previous cohort studies, the most frequent genetic alteration was the *C9orf72* repeat expansion, detected in 10 patients (9.3%) of this cohort. The initial signs of ALS in these patients were predominantly bulbar dysfunctions, especially altered speech (seven out of 10 patients). It is known that *C9orf72* repeat expansion has been found in ~7% of sporadic ALS cases of European ancestry (Renton et al., 2014). Several studies reported missense variants in the coding region of the *C9orf72* gene (Kenna et al., 2013; Koppers et al., 2013; Krüger et al., 2016), but the relevance of *C9orf72* variants detected in the coding region is not yet understood. Therefore, we cannot determine the importance of the R431Q missense variant detected in this study.

We identified two missense variants in the *NEK1* gene (both classified as VUS): the R261H variant in five and the N250S variant in a single patient. The *NEK1* gene has been recently recategorized as a major ALS-associated gene (Kenna et al., 2016), following two prior studies that identified *NEK1* as an ALS candidate gene (Cirulli et al., 2015; Brenner et al., 2016). R261H was earlier described to significantly increase ALS risk in both fALS and sALS in independent cohort studies (Brenner et al., 2016; Kenna et al., 2016; Gratten et al., 2017; Nguyen et al., 2018). In our evaluation, we observed an enrichment of the R261H variant in patients [5/107 (4.67%), MAF = 0.0234 patients vs controls 4/370 (1.08%), MAF = 0.0054]. According to the gnomAD and Kaviar databases, the maximum allele frequency of the R261H variant is 0.004 (Glusman et al., 2011; Lek et al., 2016). Earlier studies detected R261H in 1.8% of ALS patients and 0.66% of controls with minor allele frequencies 0.009 and 0.0033, respectively (Nguyen et al., 2017). Based on these results, we assume that the *NEK1* R261H variant is more frequent in the Hungarian population (both in patients and controls) than in other populations, although further large cohort studies are needed to confirm this conclusion. This study provides additional evidence that *NEK1* missense variants may contribute to the development of sALS.

Missense variants in the *NEFH* gene were detected in four patients: the T338I variant in two cases and the R148P and P505L variants in single cases. *NEFH* encodes the heavy neurofilament protein, and its variants have been associated with neuronal damage in ALS (Figlewicz et al., 1994). The T338I and R148P variants affect the conserved central coiled-coil rod domain of the protein mediating dimerization; therefore, we suggest their potential deleterious effect on the protein. In the individual carrying the P505L *NEFH* variant, an additional novel alteration (C335R) was detected in the *GRN* gene. Loss-of-function *GRN* variants are primarily considered to cause frontotemporal lobar degeneration (Mackenzie et al., 2006), but there is evidence that missense *GRN* variants are also linked to the pathogenesis of ALS (Sleegers et al., 2008). The novel *GRN* variant reported in this study results in a cysteine-to-arginine change in the cysteine-rich granulin A domain.

Four cases were identified to carry *SQSTM1* variants: the P392L in two cases and the E389Q and R393Q in single patients. All three alterations are located within the C-terminal ubiquitin-associated (UBA) end of the sequestome 1 protein. Variants of the *SQSTM1* gene were originally reported in Paget's disease of bone (Laurin et al., 2002). However, recent publications suggest a link between *SQSTM1* variants and ALS/FTD (Fecto et al., 2011). The P392L and R393Q variants are known variants reported by other study groups (Fecto et al., 2011; Kwok et al., 2014). Interestingly, the patient (#73u) carrying the novel E389Q variant was also diagnosed with Paget's disease of bone. In addition, this patient also carried a variant of unknown significance (I42R) in the *SIGMAR1* gene in heterozygous form. This case exemplifies the relevant observation of phenotypic pleiotropy and highlights the complexity of the phenotype–genotype correlation.

Variants in the *KIF5A* gene has been previously linked to autosomal dominant hereditary spastic paraparesis (SPG10) and to Charcot–Marie–Tooth disease type 2 (CMT2; Reid et al., 2002; Crimella et al., 2016; Liu et al., 2014; Jennings et al., 2017). Nonetheless, recent studies proved that *KIF5A* variants have a role in ALS (Brenner et al., 2018; Nicolas et al., 2018). According to earlier studies, *KIF5A* variants described in SPG10 or CMT2 patients occur in the kinesin motor domain (amino acid positions 9–327) and in the alpha-helical coiled-coil domain (amino acid positions 331–906) (Kaji et al., 2016; Guinto et al., 2017). In contrast, variants causing ALS are found in the C-terminal cargo-binding domain (amino acids 907–1032). In the present study, we found two variants: the E758K variant in two patients and the A579T variant in one case, with both variants located

within the coiled-coil domain (amino acid positions 331–906) of the protein, which is not in line with previous findings. Without additional functional evidence, the pathogenicity of these variants is uncertain.

Three rare missense variants (R2034Q, L2118V, and E2003D) of the *SPG11* gene were found. The high detection rate of missense variants of this gene is probably due to the large size of the coding region; therefore, we suggest that these SPG11 variants are unlikely to be deleterious. Variants in the *SPG11* gene are most commonly associated with autosomal recessive spastic paraplegia, although homozygous variants have been recently identified in juvenile ALS (Orlacchio et al., 2010; Daoud et al., 2012), and heterozygous missense variants in sALS (Kenna et al., 2013; Couthouis et al., 2014).

Variants in *UBQLN2* have been shown to be a cause of dominant X-linked ALS (Deng et al., 2011). A previously reported (M392V, Huang et al., 2017) and a novel variant (Q84H) were found in the *UBQLN2* gene. The novel Q84H variant affects the N-terminal ubiquitin-like domain of the ubiquilin-2 protein, which is involved in binding to proteasome subunits (Ko et al., 2004).

FUS variants have been mostly detected in familial ALS cases that are localized within the C-terminus of the FUS protein (Shang and Huang, 2016). However, the two rare *FUS* variants (Y25C and P106L) that were detected in this study were located in the N-terminal "prion-like" Q/G/S/Y domain (amino acids 1–165) of the protein. Although the majority of FUS mutations linked to ALS are located in the extreme C-terminus of the protein, several studies show that N-terminal variants may also be damaging (Nomura et al., 2014, Murakami et al., 2015).

In the TBK1 gene, a known missense variant (I397T) and a novel non-frameshift deletion (K631del) were identified in our patient cohort. The patient (#90u) carrying the novel K631del deletion was a 37-year-old patient who also showed symptoms of frontotemporal dementia (FTD). This is in line with the data from previous studies; according to which, *TBK1* is a causative gene of ALS–FTD (Cirulli et al., 2015, Freischmidt et al., 2015). The *NEK1* R261H variant was also present in this patient. A combined effect of the two major ALS gene variants may contribute to the early onset and fast progression of the disease in patient #90.

CCNF variants are a rare cause of ALS–FTD; in diverse geographic familial cohorts, variants in *CCNF* were present at frequencies ranging from 0.6 to 3.3% (Williams et al., 2016). In this Hungarian cohort, we identified two patients (1.9%) with *CCNF* variants (L106V and R572W). The detected R572W variant affects the nuclear localization signal 2 (amino acids 568–574) of the CCNF protein.

A previously characterized pathogenic nonsense variant (G1177X) and a rare missense alteration (R1499H) were detected in the *ALS2* gene, both in heterozygous form. The alsin protein encoded by the *ALS2* gene is involved in endosome/membrane trafficking and fusion, cytoskeletal organization, and neuronal development/maintenance (Hadano et al., 2007). Both homozygous and compound heterozygous variants in the *ALS2* gene have been described as causative for juvenile ALS (Yang et al., 2001). The G1177X nonsense variant was first detected in compound heterozygous form in a family with two affected siblings suffering from infantile ascending spastic paralysis with bulbar involvement (Sztriha et al., 2008). The ages of onset of the patients with the *ALS2* variants reported in this study were later than juvenile ALS onset, which generally manifests before 25 years of age (Orban et al., 2007). Previous studies suggested that heterozygous variants in the *ALS2* may be causative for adult-onset sALS (Kenna et al., 2013; Couthouis et al., 2014).

MATR3 encodes three protein isoforms that have been described as nuclear-matrix and DNA/RNA binding proteins involved in transcription and stabilization of mRNA (Belgrader et al., 1991; Salton et al., 2011; Coelho et al., 2015). In the present study, two novel heterozygous variants (P11S, S275N) were detected. The P11S variant affects the b isoform of the MATR3 protein (NM_001194956 and NP_001181885), contributing to splicing alteration of other isoforms. Further evidence is required to elucidate the mechanism of pathogenicity of these alterations.

We discovered several variants in ALS candidate and risk genes. In a patient with LMN-dominant ALS with slow progression, we found two novel variants (T2583I and G4290R) in the *DYNC1H1* gene. Variants in the *DYNC1H1* gene result in impairment of retrograde axonal transport leading to progressive motor neuron degeneration in mice (Hafezparast et al., 2003) and have been described in a range of neurogenetic diseases, including Charcot–Marie–Tooth type 2O, spinal muscular atrophy, and hereditary spastic paraplegia (Weedon et al., 2011; Harms et al., 2012; Poirier et al., 2013; Strickland et al., 2015; Beecroft et al., 2017). A few studies described heterozygous variants in the *DYNC1H1* gene in fALS and sALS patients, suggesting its role in ALS (Puls et al., 2003; Münch et al., 2004). Based on our findings, we strengthen the potential link between *DYNC1H1* variants and ALS.

Given that there are genetic and symptomatic overlaps among many neurodegenerative diseases, it has been suggested that causative variants might play roles in multiple disorders (Pang et al., 2017). Two heterozygous variants (H398R and R166C) were detected in the *GBE1* gene. This gene is associated with autosomal recessive adult polyglucosan body disease (APBD), which is characterized by UMN signs, cognitive impairment, and decreased activity of the glycogen branching enzyme (Lossos et al., 1998). *GBE1* variants have been recently detected in German ALS patients (Krüger et al., 2016). Although the majority of *GBE1* disease-causing variants were detected in homozygous or compound heterozygous form, a substantial percentage of individuals with APBD carry a single variant in one allele (Ubogu et al., 2005; Akman et al., 2015).

An oligogenic model of ALS has been proposed (van Blitterswijk et al., 2012), with several studies suggesting that ALS may be caused by a single highly penetrant variant or a combination of several less penetrant variants (Martin et al., 2017). In addition, environmental factors have also been implicated in disease development (Fang et al., 2009). In earlier studies, the frequency of patients with more than one major ALS gene variants was ranging from 1.6% to 3.8% (Kenna et al., 2013; Cady et al., 2015; Zhang et al., 2018). In this study, we describe six patients (6/107, 5.61%) with two variants in major ALS genes (**Table 3**). Only patient #108u was detected to carry a pathogenic and a likely pathogenic variant in two different

major ALS genes; in case of the other five patients with multiple major ALS gene variants, at least one of the two variants was categorized as VUS (**Table 3**). Co-occurrence of multiple variants is most frequently observed in patients who carry the C9orf72 RE (Nguyen et al., 2018). Two of the six patients with multiple variants in our cohort carried the *C9orf72* RE and additional variants in the *SQSTM1* or *NEK1* genes. In addition, it has been described that oligogenic inheritance is also associated with an earlier age of onset and rapid disease progression (Cady et al., 2015; Nguyen et al., 2018). In our cohort, most of the patients with two variants showed earlier onset, faster progression, or both, although a cohort of larger size is needed to confirm these observations. Additionally, many of our cases with major ALS gene variants also have several variants in other risk genes (**Supplementary Table 2**) or in genes associated with other diseases (**Supplementary Table 3**); still, the relevance of these results will only become clear when additional larger cohorts are studied.

Our results support the hypothesis that sALS has a complex model of inheritance, in which multiple variants and environmental factors contribute to disease susceptibility (van Blitterswijk et al., 2012; Martin et al., 2017). In general, this cohort of 107 ALS cases uncovers a heterogeneous genetic architecture with variants in numerous major and minor ALS genes. Several major ALS genes have been also linked to other diseases such as *SQSTM1*—Paget disease, and *KIF5A*—spastic paraplegia 10. In line with this, our results support the observation of phenotypic pleiotropy, where variants of a single gene contribute to different phenotypes. These findings further highlight the necessity for large-scale multicenter studies on ALS patients for a better understanding of the underlying genetic causes. Large-scale consortium approaches, such as Project MinE, will improve the separation of true causative genetic variants from irrelevant ones, which will help to gain a more accurate view of the genetic pattern of ALS. With this study, which represents the first comprehensive genetic study in the Hungarian ALS patients, we contribute to this approach.

DATA AVAILABILITY

The raw sequencing data of the 107 patients have been deposited in the NCBI Sequence Read Archive with BioProject accession no. PRJNA549957 (https://www.ncbi.nlm.nih.gov/sra/PRJNA549957).

ETHICS STATEMENT

The investigation was approved by the Hungarian Investigational Review Board at University of Szeged and the Ethics Committee at Medical University of Graz. Written informed consent was obtained from patients and healthy individuals, and the study was conducted according to the principles of the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

Conception, design, and coordination of the study were performed by KT, JE, DN, and MS. Acquisition of clinical data and sample collection were performed by JE, PK, PG, and HS. Analysis and interpretation of data were done by KT, JE, PG, ZN, and DN. Drafting of the manuscript was done by KT, JE, and ZN. Revision of the manuscript was done by KT, MS, PG, JE, PK, HS, and DN.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2019.00732/full#supplementary-material

REFERENCES

Abel, O., Powell, J. F., Andersen, P. M., and Al-Chalabi, A. (2012). ALSoD: a user-friendly online bioinformatics tool for amyotrophic lateral sclerosis genetics. *Hum. Mutat.* 33, 1345–1351. doi: 10.1002/humu.22157

Akimoto, C., Volk, A. E., van Blitterswijk, M., Van den Broeck, M., Leblond, C. S., Lumbroso, S., et al. (2014). A blinded international study on the reliability of genetic testing for GGGGCC-repeat expansions in C9orf72 reveals marked differences in results among 14 laboratories. *J. Med. Genet.* 51, 419–424. doi: 10.1136/jmedgenet-2014-102360

Akman, H. O., Kakhlon, O., Coku, J., Peverelli, L., Rosenmann, H., Rozenstein-Tsalkovich, L., et al. (2015). Deep intronic GBE1 mutation in manifesting heterozygous patients with adult polyglucosan body disease. *JAMA Neurol.* 72, 441–445. doi: 10.1001/jamaneurol.2014.4496

Al-Chalabi, A., Fang, F., Hanby, M. F., Leigh, P. N., Shaw, C. E., Ye, W., et al. (2010). An estimate of amyotrophic lateral sclerosis heritability using twin data. *J. Neurol. Neurosurg. Psychiatry* 81, 1324–1326. doi: 10.1136/jnnp.2010.207464

Asimit, J. L., Hatzikotoulas, K., McCarthy, M., Morris, A. P., and Zeggini, E. (2016). Trans-ethnic study design approaches for fine-mapping. *Eur. J. Hum. Genet.* 24, 1330–1336. doi: 10.1038/ejhg.2016.1

Beecroft, S. J., McLean, C. A., Delatycki, M. B., Koshy, K., Yiu, E., Haliloglu, G., et al. (2017). Expanding the phenotypic spectrum associated with mutations of DYNC1H1. *Neuromuscul–Disord.* 27, 607-615. doi: 10.1016/j.nmd.2017.04.011

Belgrader, P., Dey, R., and Berezney, R. (1991). Molecular cloning of matrin 3.A 125-kilodalton protein of the nuclear matrix contains an extensive acidic domain. *J. Biol. Chem.* 266, 9893–9899.

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

Brenner, D., Müller, K., Wieland, T., Weydt, P., Böhm, S., Lulé, D., et al. (2016). NEK1 mutations in familial amyotrophic lateral sclerosis. *Brain* 139, e28. doi: 10.1093/brain/aww033

Brenner, D., Yilmaz, R., Müller, 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

Cady, J., Allred, P., Bali, T., Pestronk, A., Goate, A., Miller, T. M., et al. (2015). Amyotrophic lateral sclerosis onset is influenced by the burden of rare variants in known amyotrophic lateral sclerosis genes. *Ann. Neurol.* 77, 100–113. doi: 10.1002/ana.24306

de Carvalho, M. D., and Swash, M. (2009). Awaji diagnostic algorithm increases sensitivity of El Escorial criteria for ALS diagnosis. *Amyotroph. Lateral Scler.* 10, 53–57. doi: 10.1080/17482960802521126

Cirulli, E. T., Lasseigne, B. N., Petrovski, S., Sapp, P. C., Dion, P. A., Leblond, C. S., et al. (2015). Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. *Science* 347, 1436–1441. doi: 10.1126/science.aaa3650

Coelho, M. B., Attig, J., Bellora, N., König, J., Hallegger, M., Kayikci, M., et al. (2015). Nuclear matrix protein Matrin3 regulates alternative splicing and forms overlapping regulatory networks with PTB. *EMBO J.* 34, 653–668. doi: 10.15252/embj.201489852

Couthouis, J., Raphael, A. R., Daneshjou, R., and Gitler, A. D. (2014). Targeted exon capture and sequencing in sporadic amyotrophic lateral sclerosis. *PLoS Genet.* 10, e1004704. doi: 10.1371/journal.pgen.1004704

Crimella, C., Baschirotto, C., Arnoldi, A., Tonelli, A., Tenderini, E., Airoldi, G., et al. (2016). 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

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.e5–839.e9. doi: 10.1016/j.neurobiolaging.2011.11.012

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-ons*et ALS* and ALS/dementia. *Nature* 477, 211–215. doi: 10.1038/nature10353

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

Fang, F., Quinlan, P., Ye, W., Barber, M. K., Umbach, D. M., Sandler, D. P., et al. (2009). Workplace exposures and the risk of amyotrophic lateral sclerosis. *Environ. Health Perspect.* 117, 1387–1392. doi: 10.1289/ehp.0900580

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

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

Freischmidt, A., Wieland, T., Richter, B., Ruf, W., Schaeffer, V., Müller, K., et al. (2015). Haploinsufficiency of TBK1 causes familial ALS and fronto-temporal dementia. *Nat. Neurosci.* 18, 631–636. doi: 10.1038/nn.4000

Garton, F. C., Benyamin, B., Zhao, Q., Liu, Z., Gratten, J., Henders, A. K., et al. (2017). Whole exome sequencing and DNA methylation analysis in a clinical amyotrophic lateral sclerosis cohort. *Mol. Genet. Genomic Med.* 5, 418–428. doi: 10.1002/mgg3.302

Glusman, G., Caballero, J., Mauldin, D. E., Hood, L., and Roach, J. C. (2011). Kaviar: an accessible system for testing SNV novelty. *Bioinformatics* 27, 3216–3217. doi: 10.1093/bioinformatics/btr540

Gratten, J., Zhao, Q., Benyamin, B., Garton, F., He, J., Leo, P. J., et al. (2017). Whole-exome sequencing in amyotrophic lateral sclerosis suggests NEK1 is a risk gene in Chinese. *Genome Med.* 9, 97. doi: 10.1186/s13073-017-0487-0

Guinto, C. O., Diarra, S., Diallo, S., Cissé, L., Coulibaly, T., Diallo, S. H., et al. (2017). mutation in KIF5A in a Malian family with spastic paraplegia and sensory loss. *Ann. Clin. Transl. Neurol.* 4, 272–275. doi: 10.1002/acn3.402

Hadano, S., Kunita, R., Otomo, A., Suzuki-Utsunomiya, K., and Ikeda, J. E. (2007). Molecular and cellular function of ALS2/alsin: implication of membrane dynamics in neuronal development and degeneration. *Neurochem. Int.* 51, 74–84. doi: 10.1016/j.neuint.2007.04.010

Hafezparast, M., Klocke, R., Ruhrberg, C., Marquardt, A., Ahmad-Annuar, A., Bowen, S., et al. (2003). Mutations in dynein link motor neuron degeneration to defects in retrograde transport. *Science* 300, 808–812. doi: 10.1126/science.1083129

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, 17085. doi: 10.1038/nrdp.2017.85

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

Huey, E. D., Ferrari, R., Moreno, J. H., Jensen, C., Morris, C. M., Potocnik, F., et al. (2012). FUS and TDP43 genetic variability in FTD and CBS. *Neurobiol. Aging* 33, 1016.e9-17. doi: 10.1016/j.neurobiolaging.2011.08.004

Huang, X., Shen, S., and Fan, D. (2017). No Evidence for pathogenic role of UBQLN2 mutations in sporadic amyotrophic lateral sclerosis in the mainland chinese population. *PLoS One* 12 (1), e0170943. doi: 10.1371/journal.pone.0170943

Jennings, S., Chenevert, M., Liu, L., Mottamal, M., Wojcik, E. J., and Huckaba, T. M. (2017). Characterization of kinesin switch I mutations that cause hereditary spastic paraplegia. *PLoS One* 12, e0180353. doi: 10.1371/journal.pone.0180353

Kaji, S., Kawarai, T., Miyamoto, R., Nodera, H., Pedace, L., Orlacchio, A., et al. (2016). Late-onset spastic paraplegia type 10 (SPG10) family presenting with bulbar symptoms and fasciculations mimicking amyotrophic lateral sclerosis. *J. Neurol. Sci.* 364, 45–49. doi: 10.1016/j.jns.2016.03.001

Kenna, K. P., McLaughlin, R. L., Byrne, S., Elamin, M., Heverin, M., Kenny, E. M., et al. (2013). Delineating the genetic heterogeneity of ALS using targeted high-throughput sequencing. *J. Med. Genet.* 50, 776–783. doi: 10.1136/jmedgenet-2013-101795

Kenna, K. P., van Doormaal, P. T., Dekker, A. M., Ticozzi, N., Kenna, B. J., Diekstra, F. P., et al. (2016). NEK1 variants confer susceptibility to amyotrophic lateral sclerosis. *Nat. Genet.* 48, 1037–1042. doi: 10.1038/ng.3626

Ko, H. S., Uehara, T., Tsuruma, K., and Nomura, Y. (2004). Ubiquilin interacts with ubiquitylated proteins and proteasome through its ubiquitin-associated and ubiquitin-like domains. *FEBS Lett.* 566, 110–114. doi: 10.1016/j.febslet.2004.04.031

Koppers, M., Groen, E. J., van Vught, P. W., van Rheenen, W., Witteveen, E., van Es, M. A., et al. (2013). Screening for rare variants in the coding region of ALS-associated genes at 9p21.2 and 19p13.3. *Neurobiol. Aging* 34, 1518.e5–1518.e7. doi: 10.1016/j.neurobiolaging.2012.09.018

Krüger, S., Battke, F., Sprecher, A., Munz, M., Synofzik, M., Schöls, 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

Kwok, C. T., Morris, A., and de Belleroche, J. S. (2014). Sequestosome-1 (SQSTM1) sequence variants in ALS cases in the UK: prevalence and coexistence of SQSTM1 mutations in ALS kindred with PDB. *Eur. J. Hum. Genet.* 22, 492–496. doi: 10.1038/ejhg.2013.184

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

Lek, M., Karczewski, K. J., Minikel, E. V., Samocha, K. E., Banks, E., Fennell, T., et al. (2016). Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 536, 285–291. doi: 10.1038/nature19057

Lewis, C. M. (2002). Genetic association studies: design, analysis and interpretation. *Brief Bioinf.* 3, 146–153. doi: 10.1093/bib/3.2.146

Liu, X., Wu, C., Li, C., and Boerwinkle, E. (2016). dbNSFP v3.0: A one-stop database of functional predictions and annotations for human nonsynonymous and splice-site SNVs. *Hum. Mutat.* 37, 235–241. doi: 10.1002/humu.22932

Liu, Y. T., Laurá, M., Hersheson, J., Horga, A., Jaunmuktane, Z., Brandner, S., et al. (2014). Extended phenotypic spectrum of KIF5A mutations: from spastic paraplegia to axonal neuropathy. *Neurology* 83, 612–619. doi: 10.1212/WNL.0000000000000691

Lossos, A., Meiner, Z., Barash, V., Soffer, D., Schlesinger, I., Abramsky, O., et al. (1998). Adult polyglucosan body disease in Ashkenazi Jewish patients carrying the Tyr329Ser mutation in the glycogen-branching enzyme gene. *Ann. Neurol.* 44, 867–872. doi: 10.1002/ana.410440604

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

Mackenzie, I. R., Baker, M., Pickering-Brown, S., Hsiung, G. Y., Lindholm, C., Dwosh, E., et al. (2006). The neuropathology of frontotemporal lobar degeneration caused by mutations in the progranulin gene. *Brain* 129, 3081–3090. doi: 10.1093/brain/awl271

Martin, S., Al Khleifat, A., and Al-Chalabi, A. (2017). What causes amyotrophic lateral sclerosis? *F1000Res.* 6, 371. doi: 10.12688/f1000research.10476.1

Morris, A. P. (2011). Transethnic meta-analysis of genomewide association studies. *Genet. Epidemiol.* 35, 809–822. doi: 10.1002/gepi.20630

Münch, 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

Murakami, T., Qamar, S., Lin, J. Q., Schierle, G. S., Rees, E., Miyashita, A., et al. (2015). ALS/FTD Mutation-induced phase transition of FUS liquid droplets and reversible hydrogels into irreversible hydrogels impairs RNP granule function. *Neuron* 88, 678–690. doi: 10.1016/j.neuron.2015.10.030

Nguyen, H. P., Van Mossevelde, S., Dillen, L., De Bleecker, J. L., Moisse, M., Van Damme, P., et al. (2017). NEK1 genetic variability in a Belgian cohort of ALS and ALS-FTD patients. *Neurobiol. Aging* 61, 255.e1–255.e7. doi: 10.1016/j.neurobiolaging.2017.08.021

Nguyen, H. P., Van Broeckhoven, C., and van der Zee, J. (2018). ALS genes in the genomic era and their implications for FTD. *Trends Genet.* 34, 404–423. doi: 10.1016/j.tig.2018.03.001

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, 1268–1283.e6. doi: 10.1016/j.neuron.2018.02.027

Nomura, T., Watanabe, S., Kaneko, K., Yamanaka, K., Nukina, N., and Furukawa, Y. (2014). Intranuclear aggregation of mutant FUS/TLS as a molecular pathomechanism of amyotrophic lateral sclerosis. *J. Biol. Chem.* 289, 1192–1202. doi: 10.1074/jbc.M113.516492

Orban, P., Devon, R. S., Hayden, M. R., and Leavitt, B. R., (2007). "Juvenile amyotrophic lateral sclerosis," in *Handbook of Clinical Neurology, Motor Neuron Disorders and Related Diseases*, vol. 82. Eds. A. A. Eisen, P. J. Shaw (Amsterdam, The Netherlands: Elsevier B.V.) 301–312. doi: 10.1016/S0072-9752(07)80018-2

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

Pang, S. Y., Teo, K. C., Hsu, J. S., Chang, R. S., Li, M., Sham, P. C., et al. (2017). The role of gene variants in the pathogenesis of neurodegenerative disorders as revealed by next generation sequencing studies: a review. *Transl. Neurodegener.* 6, 27. doi: 10.1186/s40035-017-0098-0

Peters, O. M., Ghasemi, M., and Brown, R. H. Jr. (2015). Emerging mechanisms of molecular pathology in ALS. *J. Clin. Invest.* 125, 1767–1779. doi: 10.1172/JCI71601

Poirier, K., Lebrun, N., Broix, L., Tian, G., Saillour, Y., Boscheron, C., et al. (2013). Mutations in TUBG1, DYNC1H1, KIF5C and KIF2A cause malformations of cortical development and microcephaly. *Nat. Genet.* 45, 639–647. doi: 10.1038/ng.2613

Pozzi, L., Valenza, F., Mosca, L., Dal Mas, A., Domi, T., Romano, A., et al. (2017). TBK1 mutations in Italian patients with amyotrophic lateral sclerosis: genetic and functional characterisation. *J. Neurol. Neurosurg. Psychiatry.* 88, 869-875. doi: 10.1136/jnnp-2017-316174

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. doi: 10.1038/ng1123

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., 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

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

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., et al. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* 17, 405–424. doi: 10.1038/gim.2015.30

Robinson, J. T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E. S., Getz, G., et al. (2011). Integrative genomics viewer. *Nat. Biotechnol.* 29, 24–26. doi: 10.1038/nbt.1754

Salton, M., Elkon, R., Borodina, T., Davydov, A., Yaspo, M. L., Halperin, E., et al. (2011). Matrin 3 binds and stabilizes mRNA. *PLoS One.* 6, e23882. doi: 10.1371/journal.pone.0023882

Shang, Y., and Huang, E. J. (2016). Mechanisms of FUS mutations in familial amyotrophic lateral sclerosis. *Brain Res.* 1647, 65–78. doi: 10.1016/j.brainres.2016.03.036

Sherry, S. T., Ward, M. H., Kholodov, M., Baker, J., Phan, L., Smigielski, E. M., et al. (2002). dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res.* 29, 308–311. doi: 10.1093/nar/29.1.308

Sleegers, K., Brouwers, N., Maurer-Stroh, S., van Es, M. A., Van Damme, P., van Vught, P. W., et al. (2008). Progranulin genetic variability contributes to amyotrophic lateral sclerosis. *Neurology* 71, 253–259. doi: 10.1212/01.wnl.0000289191.54852.75

Strickland, A. V., Schabhüttl, M., Offenbacher, H., Synofzik, M., Hauser, N. S., Brunner-Krainz, M., et al. (2015). Mutation screen reveals novel variants and expands the phenotypes associated with DYNC1H1. *J. Neurol.* 262, 2124–2134. doi: 10.1007/s00415-015-7727-2

Suh, E., Grando, K., and Van Deerlin, V. M. (2018). Validation of a long-read pcr assay for sensitive detection and sizing of C9orf72 hexanucleotide repeat expansions. *J. Mol. Diagn.* 20, 871–882. doi: 10.1016/j.jmoldx.2018.07.001

Sztriha, L., Panzeri, C., Kálmánchey, R., Szabó, N., Endreffy, E., Túri, S., et al. (2008). First case of compound heterozygosity in ALS2 gene in infantile-onset ascending spastic paralysis with bulbar involvement. *Clin. Genet.* 73, 591–593. doi: 10.1111/j.1399-0004.2008.00993.x

Thorvaldsdóttir, H., Robinson, J. T., and Mesirov, J. P. (2013). Integrative genomics viewer (IGV): high-performance genomics data visualization and exploration. *Brief Bioinf.* 14, 178–192. doi: 10.1093/bib/bbs017

Tripolszki, K., Csányi, B., Nagy, D., Ratti, A., Tiloca, C., Silani, V., et al. (2017a). Genetic analysis of the SOD1 and C9ORF72 genes in Hungarian patients with amyotrophic lateral sclerosis. *Neurobiol. Aging* 53, 195.e1–195.e5. doi: 10.1016/j.neurobiolaging.2017.01.016

Tripolszki, K., Török, D., Goudenège, D., Farkas, K., Sulák, A., Török, N., et al. (2017b). 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

Ubogu, E. E., Hong, S. T., Akman, H. O., Dimauro, S., Katirji, B., Preston, D. C., et al. (2005). Adult polyglucosan body disease: a case report of a manifesting heterozygote. *Muscle Nerve* 32, 675–681. doi: 10.1002/mus.20384

van Damme, P. (2018). How much of the missing heritability of ALS is hidden in known ALS genes? *J. Neurol. Neurosurg. Psychiatry* 89, 794. doi: 10.1136/jnnp-2018-318354

van Damme, P., Robberecht, W., and Van Den Bosch, L. (2017). Modelling amyotrophic lateral sclerosis: progress and possibilities. *Dis. Model Mech.* 10, 537-549. doi: 10.1242/dmm.029058

Wang, K., Li, M., and Hakonarson, H. (2010). ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res.* 38, e164. doi: 10.1093/nar/gkq603

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

Williams, K. L., Topp, S., Yang, S., Smith, B., Fifita, J. A., Warraich, S. T., et al. (2016). CCNF mutations in amyotrophic lateral sclerosis and frontotemporal dementia. *Nat. Commun.* 7, 11253. doi: 10.1038/ncomms11253

Yang, Y., Hentati, A., Deng, H. X., Dabbagh, O., Sasaki, T., Hirano, M., et al. (2001). The gene encoding alsin, a protein with three guanine-nucleotide exchange

factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. *Nat. Genet.* 29, 160–165. doi: 10.1038/ng1001-160

Zhang, H., Cai, W., Chen, S., Liang, J., Wang, Z., Ren, Y., et al. (2018). Screening for possible oligogenic pathogenesis in chinese sporadic ALS patients. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 19, 419–425. doi: 10.1080/21678421.2018.1432659

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REVIEW
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Mechanisms of Immune Activation by *c9orf72*-Expansions in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia

*Kyle J. Trageser[1], Chad Smith[1], Francis J. Herman[1], Kenjiro Ono[2] and Giulio Maria Pasinetti[1,3]**

[1] Department of Neurology, Icahn School of Medicine at Mount Sinai, New York, NY, United States, [2] Division of Neurology, Department of Medicine, Showa University School of Medicine, Tokyo, Japan, [3] Geriatrics Research, Education and Clinical Center, JJ Peters VA Medical Center, Bronx, NY, United States

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are neurodegenerative disorders with overlapping pathomechanisms, neurobehavioral features, and genetic etiologies. Individuals diagnosed with either disorder exhibit symptoms within a clinical spectrum. Symptoms of ALS involve neuromusculature deficits, reflecting upper and lower motor neurodegeneration, while the primary clinical features of FTD are behavioral and cognitive impairments, reflecting frontotemporal lobar degeneration. An intronic G_4C_2 hexanucleotide repeat expansion (HRE) within the promoter region of chromosome 9 open reading frame 72 (C9orf72) is the predominant monogenic cause of both ALS and FTD. While the heightened risk to develop ALS/FTD in response to C9orf72 expansions is well-established, studies continue to define the precise mechanisms by which this mutation elicits neurodegeneration. Studies show that G_4C_2 expansions undergo repeat-associated non-ATG dependent (RAN) translation, producing dipeptide repeat proteins (DRPs) with varying toxicities. Accumulation of DRPs in neurons, in particular arginine containing DRPs, have neurotoxic effects by potently impairing nucleocytoplasmic transport, nucleotide metabolism, lysosomal processes, and cellular metabolic pathways. How these pathophysiological effects of C9orf72 expansions engage and elicit immune activity with additional neurobiological consequences is an important line of future investigations. Immunoreactive microglia and elevated levels of peripheral inflammatory cytokines noted in individuals with C9orf72 ALS/FTD provide evidence that persistent immune activation has a causative role in the progression of each disorder. This review highlights the current understanding of the cellular, proteomic and genetic substrates through which G_4C_2 HREs may elicit detrimental immune activity, facilitating region-specific neurodegeneration in C9orf72 mediated ALS/FTD. We in particular emphasize interactions between intracellular pathways induced by C9orf72 expansions and innate immune inflammasome complexes, intracellular receptors responsible for eliciting inflammation in response to cellular

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***Correspondence:**
Giulio Maria Pasinetti
Giulio.Pasinetti@mssm.edu

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stress. A further understanding of the intricate, reciprocal relationship between the cellular and molecular pathologies resulting from C9orf72 HREs and immune activation may yield novel therapeutics for ALS/FTD, which currently have limited treatment strategies.

Keywords: amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), C9orf72, innate immunity, TDP-43, reactive oxygen species (ROS), therapeutics, microglia

INTRODUCTION

Amyotrophic lateral sclerosis and FTD are neurodegenerative diseases with many shared pathologies and symptoms, leading to the belief that they are heterogeneous manifestations along a spectrum. ALS is defined as a motor neuron disease involving corticomotor neuron and corticospinal neuron loss, manifesting in musculature deficits and leading to paralysis and death within 3 to 5 years of diagnosis, often as a result of respiratory failure (Pasinelli and Brown, 2006). Meanwhile, FTD is primarily characterized by degeneration of frontal and temporal lobar regions, leading to impairments in response inhibition, personality, and attention shifting. Clinical overlap exists between the two disorders as the frequency of FTD symptoms can be detected in up to 50% of ALS patients (Lomen-Hoerth et al., 2002; Strong et al., 2003). The prevalence of ALS and FTD symptomatic overlap is far greater in patients with repeat expansions in the G_4C_2 promoter of C9orf72 than in patients with sporadic forms ALS; the rate of disease progression in C9orf72 positive patients is also more rapid (Prado et al., 2015). Such overlap in clinical symptoms suggest C9orf72 mutations recruit related pathophysiological pathways responsible for overlapping neuropathological manifestations in ALS and FTD.

Approximately 10% of ALS cases are familial or hereditary. Of the various genetic causes of fALS, repeat expansions of the G_4C_2 promoter of C9orf72 account for ∼40% of cases, while the same mutation accounts for 18% of familial FTD cases (Renton et al., 2014; Takada, 2015). The *c9orf72* gene serves active physiological functions in a cell-specific manner. Wild-type *c9orf72* is translated into a guanine nucleotide exchange factor, involved in regulating vesicular trafficking and autophagy in neurons and immune cells (Iyer et al., 2018). In neurons, the proteins generated by *c9orf72* play a passive role in cellular functioning as the selective knockout of *c9orf72* from nestin expressing glia and neurons did not result in motor neuron degeneration, decreased survival, or other pathological hallmarks of ALS suggesting gain of function effects drive c9orf72 toxicity in neurons (Koppers et al., 2015). There is no precise quantity of G_4C_2 repeats that can be attributed to a definite diagnosis of ALS. Individuals with c9ALS/FTD have non-coding G4C2 repeats ranging from 66 to over 4400 units. Individuals without ALS typically carry between 2 and 30 repeats in the C9orf72 expansion, suggesting pathology results from excessive repeats (Gijselinck et al., 2016; Balendra and Isaacs, 2018). It is important to emphasize somatic heterogeneity of C9orf72 G_4C_2 repeats; the number of repeats quantified in circulating blood cells does not necessarily reflect the number of repeats in microglia or neurons. Pathogenic effects are clearly evident for larger expansions, however, as a linear relationship has been found between the length of the expansion and the rate of disease progression (Byrne et al., 2014). Large repeat pathologic expansions lead to cell-specific deleterious effects on the homeostatic function, including impaired nucleocytoplasmic transport, aberrant RAN translation, production of toxic dipeptide aggregates, and increased oxidative stress. A number of intrinsic and extrinsic cellular mechanisms responsible for recognizing such impairments in cellular activity involve components of the innate immune system.

Toll-like and nod-like receptors are innate immune sensors equipped to recognize moieties of pathogenic materials or imbalances in cellular molecular concentrations or electrical potential, such as those observed in cells from C9orf positive ALS model systems and patients. In particular, the intracellular NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome is unique among pattern recognition inflammasome complexes by its ability to recognize both chemical or electrical disequilibria and toxic protein aggregates, resulting in response the release of pro-inflammatory cytokines including IL-1β and IL-18 from a number of innate immunity cells (Herman and Pasinetti, 2018). A number of studies have described a pathogenic effect of persistent innate immune activation –notably microglia and leukocyte dysfunction– in the development and progression of ALS (Beers and Appel, 2019), and recently studies show activation of innate immune inflammasome complexes may play a contributing role in the pathogenesis in other genetic forms of ALS (McCombe and Henderson, 2011; Lall and Baloh, 2017). As these studies in C9orf72 positive ALS subjects also show that the extent of innate immune activation predicts development and progression of symptoms, it is imperative to define biological and cellular substrates through which C9orf72 expansions promote immune activation.

Abbreviations: 4-HNE, 4-hydroxy-2-non-enal; 8-oxo-dG, 8-oxo-2′-desoxyguanosine; ALS, amyotrophic lateral sclerosis; ASC, apoptosis-associated speck like protein containing a caspase recruitment domain; c9ALS/FTD, C9orf72 amyotrophic lateral sclerosis/frontotemporal dementia; C9orf72, chromosome 9 open reading frame 72; COX, cyclooxygenase; DPR, dipeptide repeat protein; fALS, familial ALS; FTD, frontotemporal dementia; GFAP, glial fibrillary acidic protein; HNRNPA1, heterogeneous nuclear ribonucleoprotein A1; HRE, hexanucleotide repeat expansion; IgG, immunoglobulin G; LPS, lipopolysaccharide; NfL, neurofilament light chain; NMJ, neuromuscular junction; PBMC, peripheral blood mononuclear cell; poly-GA, poly(Glycine-Alanine); poly-GP, Poly(Glycine-Proline); poly-GR, poly(Glycine-Arginine); poly-PA, poly(Proline-Alanine); poly-PR, poly(Proline-Arginine); RAN translation, repeat associated non-ATG dependent translation; RBP, ribosomal binding protein; RNP, ribonucleoprotein; RNS, reactive nitrogen species; ROS, reactive oxygen species; SGs, stress granules; SOD1, superoxide dismutase 1; TBPH, TAR DNA-binding protein-43 homolog; TDP-43, TAR DNA binding protein of 43 kDa.

Large C9orf72 HREs have a pleiotropic effect on normal cellular function; our review will discuss the various molecular pathologies associated with C9orf72 HREs and the subsequent interplay of these effects with the immune system. We further propose that recent evidence showing the role of oxidative stress-mediated innate immune signaling in the pathogenesis of the disease may provide a novel target for therapeutic interventions.

NEURONAL EFFECTS OF THE C9orf72 EXPANSION

Neuropathological Features

Individuals with C9orf72 positive ALS and FTD exhibit distinct region-specific neuropathological features and brain atrophy as assessed by post-mortem analysis. In individuals with ALS who display motor function impairments, C9orf72 repeat expansions preferentially affect the motor neurons of the ventral horn of the spinal cord and pyramidal cells of the corticospinal tract. Neuropathological assessments also find C9orf72 positive individuals with ALS stain positively for TDP-43 exclusively in motor regions (Murray et al., 2011). Meanwhile, cases presenting more on the FTD end of the spectrum show greater pathology and atrophy of neurons located in the frontal and temporal lobes. C9orf72 positive FTD patients can have ALS-like pathology in motor neurons with TDP-43 inclusions, but exhibit more extensive extra-motor pathology (Lee and Huang, 2017; McCauley and Baloh, 2019). Another study found that in six C9orf72 positive cases of FTD, moderate compact neuronal cytoplasmic inclusions were present in the granule cell layer of the hippocampal dentate gyrus, as well as in cerebellar granule cells (Mahoney et al., 2012).

Nucleotide Secondary and Tertiary Effects of the C9orf72 Expansion

Secondary and tertiary structural polymorphisms have been shown to occur in both RNA and DNA containing C9orf72 HREs (Kumar et al., 2016). In addition to regulation via proteins, RNA regulation is also mediated by intrinsic mechanisms of translation based upon RNA secondary structures. Guanine-rich intronic regions form highly stable four stranded quadruplex helices that can exist in equilibrium with hairpin structures due to non-covalent hydrogen bond interactions between guanine bases (Zhou et al., 2018). The multiple guanines present in the HRE results in the formation of structural polymorphisms based on G-quadruplexes and hairpins, which previous studies show results in the accumulation of aborted transcripts (Liu et al., 2019). In individuals with C9orf72 expansions, it has been shown that decreased levels of C9orf72 mRNA are present with an abundance of abortive transcripts (van Blitterswijk et al., 2015). Putatively, one may conclude that these structural polymorphisms contribute to the presence of abortive transcripts (**Figure 1C**).

Structural polymorphisms due to the presence of HREs occurring in DNA predisposes the cell to various pathologic effects, including increased transcription of the antisense strand (Bochman et al., 2012; Fratta et al., 2012). In a study with human cells transfected with G_4C_2 repeats, it was shown that DNA replication was impaired due to the presence of G-quadruplexes, with impairment being positively correlated with repeat length (Thys and Wang, 2015). G-quadruplexes also form in the RNA transcribed from the HRE. Consequently, RNA–DNA R-loops form and terminate transcription, causing an accumulation of aborted transcripts which pathologically bind ribonucleoproteins (RNP). RNA G-quadruplexes also bind RNA binding proteins forming an RNA granule or RNA foci (Fay et al., 2017). One of the major ribonucleoproteins bound, nucleolin, has been found to be bound and aberrantly localized due to binding with RNA–DNA R-loops in individuals with C9orf72 mediated ALS, negatively affecting the nucleolus of the cell by causing an aberrant distribution of nucleolin and ultimately nucleolar stress due to the processing of ribosomal RNA and accumulation of untranslated mRNA (Haeusler et al., 2014).

Transcriptional Consequences of the C9orf72 Expansion

Expanded regions of the HRE in intron 1 of C9orf72 undergo non-canonical repeat-associated non-ATG dependent (RAN) translation both in sense and antisense directions (Cleary et al., 2018). As the HRE sequence undergoes RAN translation, the resulting DPRs accumulate in the cell. With increasing repeat size, efficiency of RAN translation of DPR products also increases (Mori et al., 2013). Proteins are produced as a result of RAN translation in both the sense and antisense direction, with poly-(Glycine-Alanine) (poly-GA), poly-(Glycine-Arginine) (poly-GR), and poly-(Glycine-Proline) (poly-GP) in the sense direction and poly-(Glycine-Proline) (poly-GP), poly-(Proline-Arginine) (poly-PR), and poly-(Proline-Alanine) (poly-PA) in the antisense direction (**Figure 1A**) (Mori et al., 2013). These accumulating DPR form cytoplasmic inclusions and are located in common sites of neurodegeneration in ALS including the hippocampus, cerebellum, frontal cortex, and motor cortex (Freibaum and Taylor, 2017; Balendra and Isaacs, 2018). Cellular inclusions found in these areas increase in both quantity and size over time, correlating with disease progression (Chew et al., 2019).

The different DPRs have varying levels of toxicity within the cell and contribute to various pathologies (**Figure 1B**). Poly-GA has been found in the majority of cellular inclusions, putatively owing to the hydrophobic nature of the dipeptide (Kwon et al., 2014). In a study of human post-mortem cerebellar tissues in individuals with the C9orf72 HRE and non-expansion carrying individuals, strong poly-GA signals were only found in individuals with the HRE (Mori et al., 2013). Contributing to the theory of the pathogenicity of poly-GA, an *in vivo* study showed that poly-GA was necessary for the formation of inclusions, by comparing murine models of the full repeat expansion and a repeating

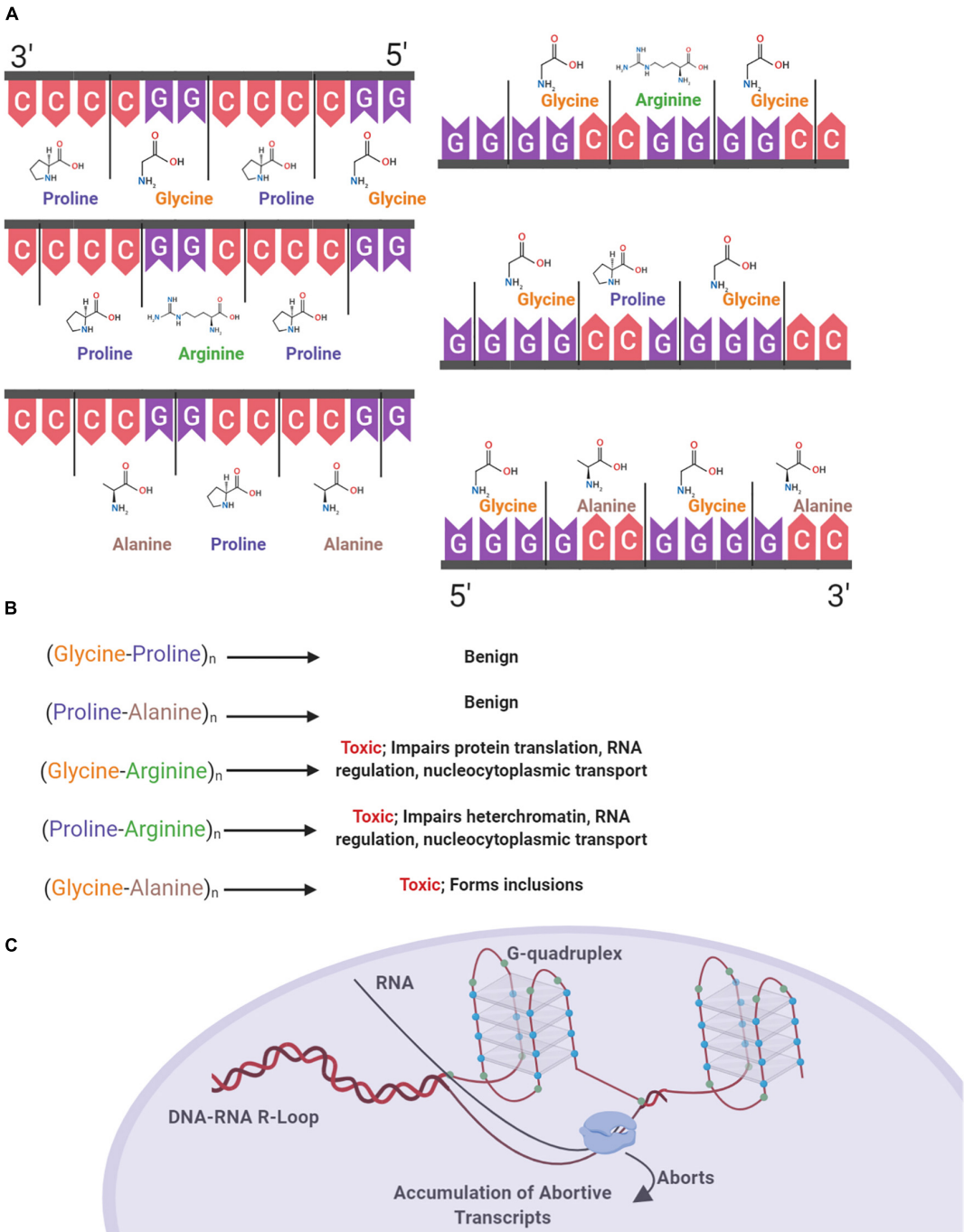

FIGURE 1 | Structural and proteinopathic effects of the C9orf72 hexanucleotide repeat expansion. The C9orf72 HRE consists of an expanded intronic sequence of GGGGCC in the open reading frame 72 of chromosome 9 and produces DRPs in one of three reading frames. DPRs are produced both in the sense (Glycine-Alanine, Glycine-Arginine, Glycine-Proline) and antisense direction (Glycine-Proline, Proline-Arginine, Proline-Alanine) **(A)**. These DPRs have varying degrees of toxicity within the cell, with the arginine containing DPR the most pathogenic **(B)**. The HRE forms a G-quadruplex structure due to hydrogen bonding between guanine bases (blue), and DNA–RNA R Loops form. RNA polymerase is unable to continuously transcribe mRNA, causing the accumulation of abortive transcripts **(C)**.

vector of Glycine-Arginine DPRs. Mice producing only poly-GR produced a diffuse distribution of DPR deposition, whereas the full HRE produced hallmark aggregates of poly-GR and poly-GA, representative of those found in cases of C9orf72 ALS (Zhang et al., 2018). These poly-GA containing inclusions are predominantly distributed throughout the cytoplasm, with a limited amount forming nuclear inclusions. Presence of inclusions leads to an increase

in stress in the endoplasmic reticulum, impairment of the ubiquitin-proteasome system, and ultimately leading to an increase in caspase-3, an indicator of cell death (Zhang Y.J. et al., 2014).

Arginine containing DPRs are the most pathological to the cell (Xu and Xu, 2018). *In vivo* models of fruit flies expressing a single variant of DPR demonstrate the relative toxicity of both poly-GR and Poly-PR when compared to alanine containing species. Fruit flies with expressing either 100 repeats of GR or 100 repeats of PR both had significantly decreased survival compared to alanine containing variants (Mizielinska et al., 2014). Poly-GR has been shown to have toxic effects on ribosomal proteins, by co-localizing with various ribosomal proteins and binding translation initiation factors, including eIF3η, in murine models as well as post-mortem C9orf72 ALS brain tissue (Lee et al., 2016; Zhang et al., 2018). Models solely comprised of repeating GR units were not found to form cellular inclusions perhaps due to the hydrophilic nature of the dipeptide (Kwon et al., 2014; Zhang et al., 2018). Like poly-GR, poly-PR does not aggregate due to being hydrophilic. However, the arginine residue may indicate that the protein is readily shuttled back to the nucleus due to the common post-translational methylation via arginine methyltransferases (Herrmann and Fackelmayer, 2009). Poly-PR has been shown to have deleterious effects on heterochromatin formation within the cell. *In vivo* models expressing only the proline-arginine dipeptide show severe mortality compared to control, with approximately 60% of mice dying by 4 weeks of age. Mechanisms of toxicity attributed to this dipeptide include gene silencing via histone modifications and the accrual of double stranded RNA (Zhang et al., 2019). Furthering the notion of the toxicity of the arginine containing DRPs, in human neurons cultured and transfected with different DPRs, it was shown that both poly-GR and poly-PR significantly impair translation (Moens et al., 2019).

Nucleocytoplasmic Transport Impairments in Response to C9orf72 Expansions

The mislocalization of nuclear proteins to the cytoplasm is one of the common findings in C9orf72 mediated ALS. Common proteins found to be aberrantly localized include RNA binding proteins RBPs serve as an important mediator in the process of post-transcriptional control of RNAs by participating in the transportation and splicing of mRNA, as well as RNA metabolism. RBPs are known to be impaired in C9orf72 ALS include TAR DNA Binding Protein of 43 kDa (TDP-43) and HNRNPA1 (Zhao et al., 2018; Prasad et al., 2019).

TDP-43 containing inclusions are found in approximately 90% of ALS cases, regardless of genetic or sporadic etiology (Mitchell et al., 2015). The basal function of the protein serves as a transcription regulator and a factor in post-transcriptional modifications such as alternative splicing (Winton et al., 2008). While location of TDP-43 typically constantly shifts between the nucleus and cytoplasm, it has been noted that in cases of ALS there is an increase in the cytoplasmic concentration of the protein due to impaired nucleocytoplasmic transport (Winton et al., 2008). In a study utilizing brain tissue from hexanucleotide repeat carrier individuals, it was shown that there was a strong correlation between the presence of TDP-43 inclusions and degree of neurodegeneration. DPR formation and accumulation are thought to precede the appearance of TDP-43 inclusions, as evidenced by the discovery of DRPs sometimes being found as a central component of TDP-43 inclusions (Mackenzie et al., 2013). *In vivo* Drosophila studies have interrogated the relationship between pathologic DPRs and TDP-43. In a fly model producing only HRE RNA and which does not undergo RAN translation, TAR DNA-binding protein-43 homolog (TBPH), the fly equivalent of TDP-43, was not found to be aberrantly mislocalized to the cytoplasm. However in fly models producing DPR, TBPH was found to be localized to the cytoplasm (Solomon et al., 2018). In a human study utilizing brain tissue from hexanucleotide repeat carrier individuals, it was shown that there was a strong correlation between the presence of TDP-43 inclusions and degree of neurodegeneration. DPR formation and accumulation are thought to precede the appearance of TDP-43 inclusions, as evidenced by the discovery of DRPs sometimes being found as a central component of TDP-43 inclusions (Mackenzie et al., 2013).

EFFECTS OF C9orf72 EXPANSIONS ON IMMUNE CELLS

In contrast to the gain of function effects of C9orf expansions in neurons, C9orf72 expansions in innate immune cells result in loss-of-function toxicity via impairment of cellular homeostatic processes including autophagy (Lall and Baloh, 2017). C9orf72 expression is particularly high in the dendritic immune cells and microglia, the resident innate immune cells of the brain, suggesting consequences of C9orf72 expansions differ based on cellular phenotypes (Zhang Y. et al., 2014; O'Rourke et al., 2016). C9orf72 mutations may have consequential effects on the regulation of synapses by microglia and may cause persistent microglial activation that has a pathogenic effect, exacerbating the progression and development of ALS. We will therefore review the evidence to support the claim that biological pathomechanisms induced by C9orf72 expansions alter immune activity to the consequence of neuronal health.

Activation and Distribution of Microglia

Microglia are resident innate immune cells of the brain. Microglia are derived from the initial primitive hematopoietic process in the extra embryonic yolk sac and migrate during fetal development to reach their final destination in the central nervous system. Evidence indicates their neuronal population is maintained through self-renewal throughout the lifespan (Dubbelaar et al., 2018; Li and Barres, 2018). Under homeostatic conditions microglia exist along an immunophenotypic spectrum between one of two overarching states: a surveillant phagocytic state and an activated pro-inflammatory state. While in a surveillant state microglia survey the neuronal environment, acting as resident brain sentinel cells, and monitor and prune synaptic connections (Shemer et al., 2015). Upon recognition of sterile

or pathogenic stress signals microglia adopt a proinflammatory profile defined by changes to their transcriptional profiles, by the upregulation of functional immune genes such as major histocompatility class complexes, Iba1 and CD86, and by the production and secretion of cytokines and free radicals that affect neuronal function (Lall and Baloh, 2017; Mammana et al., 2018; McCauley and Baloh, 2019). While microglia activation may serve a beneficial immediate role in clearing synaptic debris and pathogens, persistent microglia activation and inflammation have detrimental collateral effects on neuronal function. Numerous studies describe how microglia recognize and maintain an inflammatory immunophenotype in response in extracellular protein inclusions noted in other neurodegenerative disorders, including β-amyloid in Alzheimer's disease, and α-synuclein in Parkinson's disease (Kreutzberg, 1996).

Common among various etiologies of ALS, there is evidence that microglia adopt an inflammatory morphological state that predicts disease progression. Histological studies using post-mortem brain samples from ALS patients find that resident microglia increase in their population in proportion to the stage of disease progression (Geloso et al., 2017). Moreover, post-mortem brain samples from individuals with C9orf72 positive ALS find a positive correlation between the magnitude of expression of CD86 and Iba1, markers of microglia activation and proliferation, and the severity of ALS symptomology and magnitude of TDP-43 deposition (Brettschneider et al., 2012). Notably, this study showed that microglial pathology in the motor cortex was more severe in C9orf72 positive ALS than in cases of sporadic ALS. Additional *post-mortem* brain analysis of multiple white matter regions including the motor cortex confirm that microglia immunoreactivity is greater in individuals with C9orf72 mediated ALS compared to cases of sporadic ALS based on Iba1 and CD68 staining (Rostalski et al., 2019). Persistent region specific patterns of microglial activation in ALS is also demonstrated by the utilization of positron emission tomography (PET) scans in individuals with ALS. An injection of a radioactive tracer that labels the translocator (TSPO) protein in functionally immunoreactive microglia results in significantly higher signal intensity in primary motor, supplementary motor, and temporal areas of the brain (Corcia et al., 2012). These investigations indicate an immunoreactive microglia inflammatory phenotype that can have cytotoxic consequences is a component of ALS pathogenesis, and in particular for the C9orf72 phenotype. Whether activation of microglia is consequence of neurodegeneration or an instigator of it remains under investigation.

C9orf72 expression is higher in microglia than in any other cell type, including neurons and research suggests the wild type *c9orf72* gene plays a central role in maintaining immune homeostasis (O'Rourke et al., 2016). Meanwhile, in myeloid lineage cells, the wild type *c9orf72* gene serves an active role in maintaining immune homeostasis. In mice deficient for the *c9orf72* gene, it has been shown that there is upregulation of genes linked to inflammatory responses. Moreover, microglia isolated from these animals show increased levels of pro-inflammatory cytokines IL-6 and IL-1β. Further indicative of a pro-inflammatory state, hyperplasia of both the spleen and lymph nodes were observed in *c9orf72* knockout animals. Animals with this knockout did not directly display neurodegeneration, however it has been hypothesized that a lack of functional C9orf72 in microglia can cause defects in their ability to remove aberrantly folded proteins (O'Rourke et al., 2016).

Microglia isolated from a number of additional murine ALS model systems exhibit higher inflammatory potential as well. Microglia cultured from late stage mutant SOD1 mice were shown to have decreased mRNA levels of proteins associated with the anti-inflammatory end of the activation spectrum as well as increased levels of RNA for genes involved in the generation of ROS when compared to microglia cultured from the same mice early on in the progression of the disease. End disease stage microglia expressed an increase in levels of NOX2 mRNA, a component of NADPH oxidase responsible for the generation of superoxide, compared to microglia from mice in the initial stages of ALS. To further interrogate the contribution of microglia in neurodegeneration, microglia from both early and late stage ALS were co-cultured with motor neurons, and compared to motorneurons co-cultured with wild-type microglia. Motorneurons co-cultured with microglia from end stage animals exhibited increased cell death, and decreased neurite count when compared to both beginning stage microglia and wild type microglia co-cultures, indicative of the toxic role microglia may take on as the disease progresses (Liao et al., 2012; Geloso et al., 2017). Further *in vivo* studies of SOD1 mice have shown that there is upregulation of inflammatory genes such as *Apoe and Csf1* early on in the disease state, suggesting the role of neuroinflammation in the pathogenesis of ALS (Butovsky et al., 2015). Spinal cords from mutant mice additionally show microglial activation before symptoms of myasthenia are present which proceeds through the development of symptomology, indicating a temporal correlation (Volonté et al., 2019). Moreover, a pathological role for microglia dysfunction in ALS/FTD is further suggested in FTD by progranulin mutations and from variants in *TREM2*, a microglia expressed gene that increase susceptibility for ALS (Cruts et al., 2006).

Glial Reactivity

Excessive glial reactivity has been theorized to play a contributing role in the pathogenesis of ALS. In an *in vitro* study involving the culturing of primary motor neurons and microglia, when microglia were activated by human ALS immunoglobulin G (IgG), microglia transitioned to an activated state and damage to motor neurons occurred, leading to significant neuronal loss. Surviving motor neurons were found to have a smaller sized soma, fewer neurites, and a decrease in arborization (Zhao et al., 2004). Reinforcing this notion, the same results were replicated when microglia were incubated with LPS rather than ALS IgG, indicating an increased and pathological response originating from microglia. This same damage did not occur when a culture of only motor neurons was exposed to either LPS or ALS IgG, suggesting that the presence and activity of microglia is necessary for neurodegeneration to occur. Furthermore when incubated with an inhibitor of nitric oxide was added to culture prior to addition of either LPS or ALS IgG; motor neuron survival was greatly increased, strengthening the evidence supporting the

toxicity of ROS in the pathology of ALS (Zhao et al., 2004). To investigate the role of basal state microglia in SOD1 familial ALS, PU.1$^{-/-}$ mice, devoid of macrophages, neutrophils, T cells, B cells, and microglia were cross bred with SOD1^{G93A} mice. When transplanted with basal state wild-type microglia, disease progression was slowed and survival increased when compared to both SOD1^{G93A} mice with functioning, SOD1^{G93A} microglia, and SOD1^{G93A}/PU.1$^{-/-}$ mice, without microglia. *In vitro* studies were further carried out, comparing the effects of SOD1^{G93A} microglia on motor neurons. Wild type microglia produced less ROS, RNS, and neuronal death occurred when compared to SOD1^{G93A} mutation carrying microglia (Beers et al., 2006). In totality, these experiments further reinforce the notion of aberrant microglia cells contribution to the development of ALS pathology.

While resident microglia reside within the central nervous system, additional macrophages and monocytes reside outside of the CNS and are able to infiltrate and respond to disturbances (Mammana et al., 2018). Additional tissue resident cells of myeloid lineage include perivascular macrophages, meningeal macrophages, and macrophages of the choroid plexus. In addition to the increase in population of tissue resident microglia, infiltration of monocytes is also apparent, with cell populations identified by the expression of C-C chemokine receptor 2 (CCR2) in monocytes which is absent in microglia (Mammana et al., 2018). Chemokines produced by microglia, among other cells, include the C-C Motif Chemokine Ligand 2 (CCL2), which is produced during neuroinflammation and may serve as the cell population responsible for the attraction of monocytes expressing CCR2 (Shemer et al., 2015). Previous studies show that in disease states when the blood–brain barrier is compromised, monocytes with transcriptional profiles distinct from the tissue resident microglia penetrate the CNS and participate in response to damage (Li and Barres, 2018; McCauley and Baloh, 2019). Damage to the blood brain barrier has been previously demonstrated *in vivo* with SOD1 mice, as well as in *post-mortem* examination of sALS brain tissue (Garbuzova-Davis and Sanberg, 2014).

Astrocytes, the other dominant cell population in the brain, also exhibit susceptibility to C9orf72 expansions. Astrocytes are responsible for providing metabolic support to neurons, axon maintenance, protection against oxidative stress, and the regulation of neuroendothelial permeability (Bélanger et al., 2011; Garwood et al., 2017). In a murine model of a 149-repeat G_4C_2 expansion, it was shown that elevated levels of GFAP, a marker for astrocytes, preceded the cortical thinning as evidenced by NeuN + staining; the increase in GFAP immunoreactivity preceded the onset of cortical thinning at 6 months by a number of months (Chew et al., 2019). A number of putative mechanisms that link astrogliosis with the onset of ALS disease pathology have been investigated. *In vitro* evidence from human induced astrocytes from C9orf72 patients suggests that dysregulation of astrocyte miRNA involved in the regulation of axonal maintenance genes impairs extracellular trafficking between astrocytes and neurons, leading to motor neuron death (Varcianna et al., 2019). Other recent studies find that induced astrocytes from C9orf72 positive fALS and sporadic ALS individuals exhibit loss of metabolic flexibility, in particular in glucose and fructose metabolism (Allen et al., 2019). A major point of future interrogation lies in understanding how impaired astrocyte function activity may particularly increase susceptibility for motor neuron degeneration.

INTERPLAY OF c9orf72 EXPANSION PATHOPHYSIOLOGIES AND IMMUNE ACTIVATION

As described above c9orf72 HRE elicits a number of pathophysiological consequences, impairing both neuronal and immune cell function. We illustrate the gain-of-function effects of C9orf72 HREs in neurons and loss-of-function effects of C9orf72 HREs in immune cells. Presented below is evidence that effects of c9orf72 HREs in neurons have a complex, reciprocal, relationship with its effects in the immune system, with pathophysiologies generated in one cell type influencing and exacerbating effects of HREs in others. Dysfunction due to C9orf72 pathology may therefore create a self-perpetuating cycle in which expansion related effects trigger chronic immune action, causing further cellular dysfunction.

Reactive Oxygen Species

Other forms of familial ALS have directly implicated the pathologic nature of excessive ROS production or impaired breakdown. SOD1 normally functions to catalyze the reaction of superoxide into oxygen and hydrogen peroxide, which then is able to diffuse through lipid cell membranes and cause direct damage in cells (Ma et al., 2017). SOD1 mutations have been linked to a large number of fALS cases via various pathological mechanisms. Aberrant production and breakdown of ROS have been shown to occur in both sporadic and fALS, with these molecules targeting the NMJ in cases of both sporadic ALS and SOD1 mediated fALS. High levels of ROS have been shown to impair synaptic transmission in the NMJ via the depletion of presynaptic neurotransmitters available for release and upregulation of calcium levels within the terminal. Ultimately, later during disease progression, the nerve terminal shrinks and acetylcholine release is impaired (Pollari et al., 2014). Impairment within the NMJ initially presents as weakness in the muscles and ultimately results in paralysis (Campanari et al., 2016).

In addition to the release of cytokines, microglia produce ROS including hydrogen peroxide and superoxide, as well as RNS including nitric oxide. An overproduction of ROS or dysfunction in the breakdown of ROS leads to a state of oxidative stress in the cell (Kim et al., 2015). Excessive production of superoxide, one of the ROS implicated in the pathogenesis of ALS, can cause oxidative stress either directly or indirectly by creating secondary free radicals (Ma et al., 2017). Excess levels of ROS can catalyze the formation of other molecules into ROS, creating a cycle of ROS generation. These endogenous ROS can go on to act on the various macromolecules within the cell, including lipids and proteins. Reactions that involve hydroxyls include the addition of carbonyls to amino acids, making them susceptible to proteolysis

and reactions inducing the change of DNA bases making strands susceptible to breaks (Betteridge, 2000).

Reactive oxygen species biomarkers have additionally been suggested as a quantitative measure of disease progression. Human testing in individual with ALS has revealed NfL, 4-hydroxy-2-non-enal (4-HNE), and 8-oxo-2′-desoxyguanosine (8-oxo-dG), to demonstrate the ability to measure not only disease progression, but differentiate individuals with either a slow or fast disease progression. These markers indicate various downstream effects of oxidative stress including axonal health, DNA oxidation, and lipid peroxidation respectively (Devos et al., 2019). Post-mortem specimens from individuals with SOD1 linked fALS and individual with spontaneous ALS, elevated oxidative damage markers OH^8dG were found to occur in neurons from both sporadic ALS and familial ALS patients, suggesting ROS and microglia pathology as a commonality between forms of sporadic and fALS (Ferrante et al., 1997). Based upon longitudinal studies of serum cytokine levels in individuals with ALS, it appears that IL-6, TNF-α, and IFN-γ show the strongest correlation with ALS pathology (Lu et al., 2016). These heightened levels of inflammatory cytokines have various toxic effects on the cell. High levels of TNF-α have been shown to induce the formation of ROS via the activation of NADPH oxidase (Fischer and Maier, 2015). The resulting high levels of NADPH oxidase has been linked to neurodegeneration, putatively indicating a potential target for novel therapeutics (Gao et al., 2012).

Oxidative Stress and RAN Translation

During periods of increased stress, cells often rely upon atypical forms of translation. The integrated stress response, a process by which the cell responds to stressors including oxidative stress, has been implicated in cases of C9orf72 mediated ALS. When undergoing the integrated stress response, cells commonly reduce canonical translation. In response to the presence of oxidative stress, cells with the C9orf72 HRE increase levels of non-canonical RAN translation of DRPs (**Figure 2**) (Westergard et al., 2019). In order to do so, initiation factors including eIF2α are phosphorylated, which has the effect of reducing the initiation of canonical translation, ultimately increasing RAN translation, although the mechanism by which RAN translation efficiency is altered has not yet been determined (Cheng et al., 2018). This increased translation

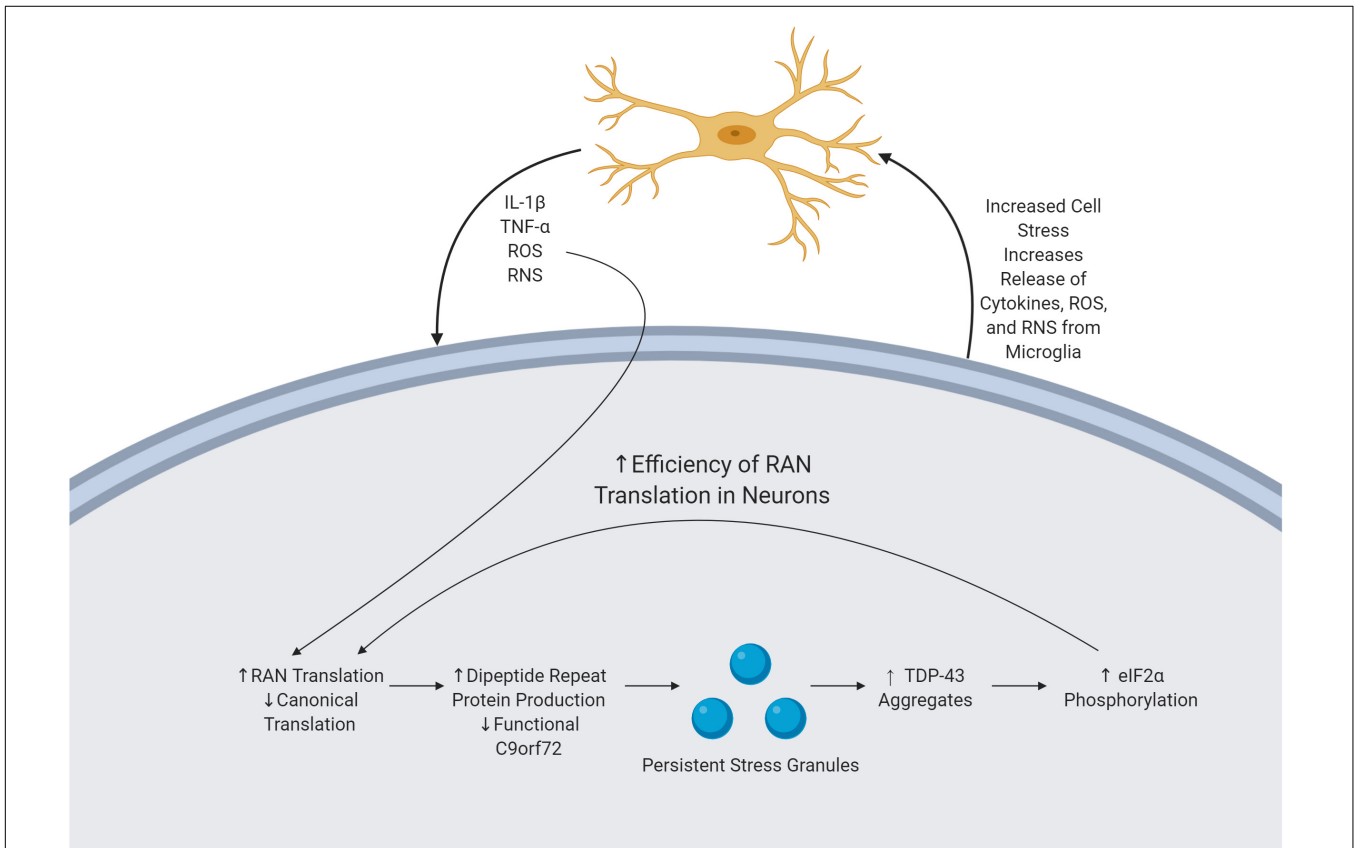

FIGURE 2 | Mechanism of immune activation by byproducts of the C9orf72 repeat expansion. C9orf72 pathology is driven by the presence of an expanded HRE, producing various cellular pathologies. DRPs are produced via RAN translation and subsequently accumulate, causing cellular stress. In response, the cell forms SGs which are unable to readily dissolve. TDP-43 is subsequently recruited to these SGs, and there is upregulation of eIF2α phosphorylation thereby increasing the efficiency of RAN translation, thereby perpetuating the cycle. Microglia sense neuronal stress and in response release various cytokines and ROS. ROS increase the efficiency of RAN translation, furthering the feedback loop of pathology and neurodegeneration.

of DPRs can lead to an increase in pathologies, ultimately accelerating neurodegeneration.

Persistent Stress Granules

Stress granules (SGs) are essential in mediating the response of the cell to environmental stress and subsequently stopping the buildup of aberrant, misfolded proteins (Chen and Liu, 2017). SGs are comprised of active mRNA and RBPs; the formation of the SG is believed to serve as a protective measure by the cell to prevent damage to these structures (Colombrita et al., 2009). Following the removal of the triggering stimuli, some SGs independently dissolve their structure, while others must undergo autophagy (Kedersha and Anderson, 2007; Chitiprolu et al., 2018). A major initiator in the formation of SGs is the presence of oxidative stress; SGs form within minutes of exposure to ROS (Kim et al., 2015; Chitiprolu et al., 2018). The presence of non-dissolving SGs in the cell has been linked to a persistent state of cellular stress. TDP-43 inclusions, pathognomonic for C9orf72 mediated ALS, are thought to exert a pathological effect through recruitment to SGs. TDP-43 pathology has been linked to the upregulation of eIF2α phosphorylation, thereby increasing the efficiency of RAN translation and the subsequent production of DPRs (**Figure 2**) (Kim et al., 2014).

In vitro experiments investigating the formation of SGs in response to oxidative stress have demonstrated that C9orf72 was routinely recruited into SGs. Low concentrations of the normal functioning C9orf72 gene product are commonly found in C9orf72 ALS. Reasons for this decrease include the production of abortive transcripts, DPRs, and dysregulated protein synthesis. Due to the low concentration of functional C9orf72, SGs less readily dissipate and there is an increase in the accumulation of TDP-43 aggregates (Sellier et al., 2016; Chitiprolu et al., 2018). Furthermore, decreased levels of normal C9orf72 gene product, as is found in individuals with C9orf72 ALS, increases cellular sensitivity to stressors via the impairment of assembly and dissolution of SGs (Maharjan et al., 2017). The persisting state of SGs in the cell impairs RNA metabolism and protein degradation, leading to the aggregation of aberrant proteins; a common finding in C9orf72 ALS.

Inflammasome Activation

As indicated above, the NLRP3 inflammasome is part of a family of intracellular innate immune sensors that are integral for cellular defense. Comprised of NLRP3, ASC, and Caspase-1, activation of the NLRP3 inflammasome complex involves a two-step paradigm: a priming signal is required to generate *nlrp3* transcription, while a second activation signal results in assembly of the oligomeric complex. Ultimately, the activation of the NLRP3 inflammasome leads to the activation of Caspase-1, which in turn activates pro-inflammatory cytokines such as IL-1β and IL-18. In certain cells, activation of inflammasome complexes results in pyroptosis, a form of inflammatory mediated cell death in which proinflammatory cytokines are released and the cell response is amplified and propagated (Swanson et al., 2019). It is critical to note that NLRP3 inflammasome activation can be achieved by a number of pathophysiological pathways generated by C9orf72 HREs previously described. These include lysosomal dysfunction, mitochondrial functional impairments, intracellular metabolic imbalances, and intracellular protein aggregates. TDP-43 inclusions, a pathomechanism noted in patients with genetic forms of TDP-43 ALS, readily activate the NLRP3 inflammasome in primary microglial cultures, resulting in increased production of IL-1β. Interestingly, motor neurons exposed directly to TDP-43 do not exhibit neurotoxicity; the presence of microglia exposed to extracellular TDP-43 protein and subsequent secretion of proinflammatory cytokines is necessary for neurodegeneration to occur (Zhao et al., 2015). Similar increases in expression of NLRP3 inflammasome components are also present in cases of post-mortem tissue from individuals with sporadic ALS, with significant upregulation of ASC and IL18 (Johann et al., 2015). Additionally, elevated serum levels of IL-18 have been

TABLE 1 | Overview of accepted therapeutics and trials targeting inflammation.

Drug	Target	Outcome	References
AAD-2004	COX inhibitor	Efficacy *in vivo*	Shin et al., 2012
Anakinra	IL-1 receptor antagonist	Efficacy *in vivo*, preclinical trials inconclusive	Maier et al., 2015
Aspirin	Non-selective COX inhibitor	Efficacy in case-control study	Tsai et al., 2015
Celecocoxib	Selective COX-2 inhibitor	Unsuccessful in clinical trials	Cudkowicz et al., 2006
Edaravone	ROS	FDA approval; first line treatment	Zhang et al., 2012; Cruz, 2018
Minocycline	Anti-inflammatory (mechanism unknown)	Efficacy *in vivo*, unsuccessful in phase III	Kriz et al., 2002; Keller et al., 2011
Niclosamide	S100A4 inhibitor	Efficacy *in vitro*	Serrano et al., 2019
Nimesulide	Selective COX-2 inhibitor	Efficacy *in vivo*	Pompl et al., 2003
NP001	NF-κB	Efficacy in subset of patients in phase II trials	Miller et al., 2014, 2015
Riluzole	Glutamatergic transmission	FDA approval; first line treatment	Mitchell et al., 2009

The nexus of neuroinflammation and the pathogenesis of ALS has been a target for the development of novel therapeutics. Currently, there are two FDA approved treatments for ALS: Riluzole, which targets glutamatergic transmission, and edaravone, which is believed to target ROS (Mitchell et al., 2009; Cruz, 2018). The reciprocal relationship of inflammation and neurodegeneration and a large case-control study showing an inverse relationship between individuals taking Aspirin and the incidence of ALS has yielded various preclinical and clinical trials into both selective and non-selective COX inhibitors (Pompl et al., 2003; Cudkowicz et al., 2006; Shin et al., 2012; Tsai et al., 2015). Furthermore, other components of the inflammasome have been targeted in recent times with some success both in clinical and preclinical trials (Maier et al., 2015; Miller et al., 2015; Serrano et al., 2019).

demonstrated in cases of sporadic ALS, thereby suggesting the possible upregulation of an upstream component of the inflammasome (Italiani et al., 2014).

Recently, the activation of the NLRP3 inflammasome was directly investigated in $SOD1^{G93A}$ mice. Spinal cord from animals with late stage disease showed significant upregulation of components of the NLRP3 inflammasome, including *nlrp3*, *pro-IL-1*β, *ASC*, and caspase-1. In this study, NLRP3 was also shown to be expressed in both microglia and astrocytes. To further determine the ubiquity of NLRP3 activation in models of ALS, the authors also analyzed spinal cord tissue for gene expression in TDP-43 mutant mice and similarly displayed a significant upregulation of NLRP3 inflammasome components NLRP3, Caspase-1, and ASC. In addition to these *in vivo* investigations, primary microglia cultures from both wild type mice were obtained and incubated in soluble $SOD1^{G93A}$. IL-1β release was found to occur in a dose-dependent manner in these cells, however when MCC950, a specific NLRP3 inhibitor, was added, cells significantly reduced their secretion of IL-1β. The same paradigm was conducted in wild-type cells incubated with mutant TDP-43 protein. Microglia were found to secrete IL-1β when stimulated with TDP-43 protein, and the effect was again abolished by the addition of MCC950 (Deora et al., 2019). This demonstration of the activation of the NLRP3 inflammasome in multiple *in vivo* and *in vitro* models of ALS, and the subsequent reduction in IL-1β secretion with an NLRP3 inhibitor suggests a commonality that may potentially be utilized as a novel target for therapeutics.

To maintain proper homeostasis, a fine balance must be struck between appropriate and disproportionate inflammatory responses to triggering stimuli. Excessive or chronic activation of the innate immune system has been linked to increased cellular stress and degeneration of motor neurons. This nexus of innate immune activation and neuronal health may serve as a potential target for novel therapeutics.

THERAPEUTIC APPROACHES

First Line Treatments

Currently, only two medications are on the market for the treatment of ALS: riluzole and edaravone (Zhang et al., 2012; Cruz, 2018) (**Table 1**). Riluzole was the first drug to be approved for the treatment of ALS by the United States Food and Drug Administration and acts via the modulation of glutamatergic transmission. Effects from treatment with riluzole are modest, with median survival time increasing from 2.25 years in non-treated patients to 3.07 years in treated patients (Mitchell et al., 2009). The most recently FDA approved treatment for ALS, edaravone, was previously approved as a treatment for ischemic stroke. In cases of ischemic stroke, large concentrations of inflammatory cytokines and reactive species are released, after which neuroinflammation and ultimately neurodegeneration occur (Yuan et al., 2014). Although the mechanism of action of edaravone has not been fully elucidated, edaravone is believed to act as a ROS scavenger and decrease the generation of ROS (Zhang et al., 2012; Cruz, 2018). In an *in vivo* study of rats with ischemic stroke treated with edaravone, it was shown that activated microglia produce less TNF-α, IL-1β, ROS, and iNOS compared to rats treated with vehicle (Yuan et al., 2014). Clinical trials of edaravone additionally proved to be successful in both slowing reduction in ALS functional rating scale (ALSFRS-R) score, as well as in the decrease in markers of oxidative stress in cerebrospinal fluid over the course of a 6 month course of treatment (Yoshino and Kimura, 2006; Luo et al., 2019).

Aspirin, Aspirin Derivatives, and Combating Inflammation

Mitigating neuroinflammatory sequelae as a therapeutic target is a promising strategy in developing novel therapeutics for ALS. Cyclooxygenase (COX) serves an essential role in inflammation pathways and has long been a target for medical treatments. COX exists as two separate isozymes, COX-1 and COX-2 which are responsible for the anabolism of integral molecules in inflammatory pathways such as prostaglandins (Vane and Botting, 2003; Fitzpatrick, 2004). Inhibition of the COX-2 isozyme has been demonstrated as an effective treatment strategy in *in vivo* models of ALS (Drachman et al., 2002; Pompl et al., 2003). The preferential COX-2 inhibitor, nimesulide, was shown to be efficacious in a SOD1 linked model of fALS both in terms of delaying motor impairment and decreasing levels of prostaglandin-E2 (Pompl et al., 2003). Unfortunately, selective COX-2 inhibitors such as celecoxib have so far not demonstrated efficacy over placebo treatment in clinical trials (Cudkowicz et al., 2006). This may however be related in part to the difficulty in establishing a diagnosis prior to the development of symptoms, during the period in which inflammation is present, but neurodegeneration has not yet begun.

One of the most commonly used non-selective COX inhibitors, Aspirin (acetylsalicylic acid), has been shown to have a potential use for various disorders and illnesses ranging from preventing myocardial infarction to treating psychiatric illness (Barbarawi et al., 2019; Müller, 2019). Aspirin salts previously have been investigated as potential therapeutics for ALS in transgenic SOD1 mice. When treatment was started early enough, motor pathologies were delayed, however end stage disease was ultimately not prevented (Barnéoud and Curet, 1999). In addition to the ability of aspirin to inhibit prostaglandin synthesis, it has also been proposed that aspirin can serve a role in the scavenging of ROS, specifically hydroxyl radicals (Aubin et al., 2002). In a total population based case-control study of individuals in Taiwan, aspirin use was found to have an inverse correlation to the incidence of ALS when controlling for confounders such as steroid use (Tsai et al., 2015). Other monophenolic acids, including structural isomers of the key active metabolite of aspirin, salicylic acid, have shown efficacy as anti-inflammatory and neuroprotective agents, with success specifically against hydrogen peroxide mediated damage (Winter et al., 2017).

Other derivatives of aspirin aimed at mitigating neuronal injury via neuroinflammation have shown promise in delaying the progressive neurodegeneration of ALS. One such novel compound, 2-hydroxy-5-[2-(4-trifluromethylphenyl)-ethylaminobenzoic acid] (AAD-2004) has been utilized in SOD1 transgenic mice to combat the inflammatory sequelae of ALS. When administered with AAD-2004, degeneration of motor neurons was decreased, as was production of free radicals, and activation of microglia in the spinal cord. Perhaps most promisingly, AAD-2004 was shown to be more efficacious than the currently approved treatment riluzole at delaying onset of disease symptoms, improving motor function, and increasing survival (Shin et al., 2012).

Additional attempts at targeting inflammatory effects of ALS include the use of minocycline, an antibiotic with anti-inflammatory effects. When administered in a murine model of SOD1G37R ALS, motor neuron degeneration was delayed and survival was increased. Additionally, less microglial activation in spinal cord motor neurons was observed both during initial states of the disease as well as in the final stages (Kriz et al., 2002). Although minocycline ultimately proved ineffective in stage III clinical trials, findings did suggest that the microgliosis persisting in the latter stages of the disease may be resistant to treatment: thereby underlying the importance of preventing the initial stages of microgliosis. To test this hypothesis, minocycline administration was initiated at varying time points of ALS disease state in a SOD1 mouse line. While treatment initiated after the onset of pathology did not have a positive effect on survival or motor symptoms, animals pretreated with minocycline were shown to have a greater mean survival time, highlighting the necessity of preventing the initial stages of inflammation before neurodegeneration can begin (Keller et al., 2011).

Targeting IL-1β, an end product of immune activation, has shown promise as a therapeutic target. IL-1 receptor antagonists such as Anakinra have previously been employed as a treatment strategy in SOD1 mice, yielding beneficial effects in survival and motor functions (Meissner et al., 2010). This *in vivo* success has led to at least one preclinical trial to determine the safety profile of the drug and to investigate its effects on inflammatory biomarkers. After treatment with Anakinra for 1 year, individuals did not show a statistically significant improvement in disease progression compared to a historical control cohort. Inflammatory markers including IL-6 and TNF-α both decreased at the 6 month time point; however statistical significance was not achieved, to which the authors attribute to the small sample size. Interestingly, participants in the trial generated antibodies against Anakinra after the 6 month time point, potentially preventing efficacy (Maier et al., 2015).

Additional investigations have been conducted on targeting NF-κB through the use of purified and pH adjusted sodium chlorite, NP001. In phase 1 testing of NP001, participants were monitored to determine the safety profile of the drug, as well as changes in markers of monocytic activation, CD16 and HLA-DR. Promisingly, NP001 was well-tolerated by participants at all tested doses and additionally lead to a decrease in HLA-DR, independent of dose, and a dose-dependent decrease in CD16 (Miller et al., 2014). When taken to phase 2 testing, the efficacy of NP001 was shown to be mixed among different subsets participants. Disease progression over the 6 month testing period was not significantly slowed across all individuals receiving NP001, however when classified by baseline inflammation, individual with a higher systemic level of inflammation before administration of NP001 exhibited a 41% reduction in disease progression (Miller et al., 2015).

Other routes of inhibiting inflammation in ALS have recently also been investigated. S100A4, a Ca^{2+} binding protein and DAMP, which has previously been demonstrated to play an essential role in multiple cellular processes, has been targeted as a treatment target. Similar to the temporal pattern of activation of microglia, in mutant SOD1 rats, S100A4 exhibits a significant increase in concentration in the pre-symptomatic phase ALS, and remains elevated throughout the disease course. In recent work in primary microglia derived from SOD1 mice, niclosamide, a transcriptional inhibitor of S100A4, was shown to have beneficial effects in the inhibition of NOX2, among other pro-inflammatory mechanisms in microglia (Serrano et al., 2019).

CONCLUSION

The intronic HRE of C9orf72 has a vast number of pathologies that ultimately lead to the development of ALS. A major, underexplored facet of the pathogenesis of the disease is the activation of the immune system by the various biochemical and molecular immediate effects of the expansion. This untapped direction may serve as a potential target for novel therapeutics targeting the immune effects of the expansion to either delay or ultimately prevent neurodegeneration. One major obstacle to exploring this route of treatment is early detection of ALS, increasing the mounting need for novel biomarkers of early disease progression. With inflammation being present in other forms of ALS, these therapeutic targets may additionally be extrapolated to other forms of the disease to improve outlook for individuals afflicted with the disorder.

AUTHOR CONTRIBUTIONS

KT wrote the manuscript. KT, CS, FH, KO, and GP conceptualized and edited the manuscript and approved this work for publication.

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REFERENCES

Allen, S. P., Hall, B., Woof, R., Francis, L., Gatto, N., et al. (2019). *C9orf72* expansion within astrocytes reduces metabolic flexibility in amyotrophic lateral sclerosis. *Brain* 1–20. doi: 10.1093/brain/awz302

Aubin, N., Curet, O., Deffois, A., and Carter, C. (2002). Aspirin and salicylate protect against MPTP-induced dopamine depletion in mice. *J. Neurochem.* 71, 1635–1642. doi: 10.1046/j.1471-4159.1998.71041635.x

Balendra, R., and Isaacs, A. M. (2018). C9orf72-mediated ALS and FTD: multiple pathways to disease. *Nat. Rev. Neurol.* 14, 544–558. doi: 10.1038/s41582-018-0047-2

Barbarawi, M., Kheiri, B., Zayed, Y., Gakhal, I., Al-Abdouh, A., Barbarawi, O., et al. (2019). Aspirin efficacy in primary prevention: a meta-analysis of randomized controlled trials. *High Blood Pressure Cardiovasc. Prevent.* 26, 283–291. doi: 10.1007/s40292-019-00325-5

Barnéoud, P., and Curet, O. (1999). Beneficial effects of lysine acetylsalicylate, a soluble salt of aspirin, on motor performance in a transgenic model of amyotrophic lateral sclerosis. *Exp. Neurol.* 155, 243–251. doi: 10.1006/EXNR.1998.6984

Beers, D. R., and Appel, S. H. (2019). Immune dysregulation in amyotrophic lateral sclerosis: mechanisms and emerging therapies. *Lancet Neurol.* 18, 211–220. doi: 10.1016/S1474-4422(18)30394-6

Beers, D. R., Henkel, J. S., Xiao, Q., Zhao, W., Wang, J., Yen, A. A., et al. (2006). Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 103, 16021–16026. doi: 10.1073/pnas.0607423103

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

Betteridge, D. J. (2000). What is oxidative stress? *Metab. Clin. Exp.* 49, 3–8. doi: 10.1016/S0026-0495(00)80077-3

Bochman, M. L., Paeschke, K., and Zakian, V. A. (2012). DNA secondary structures: stability and function of G-quadruplex structures. *Nat. Rev. Genet.* 13, 770–780. doi: 10.1038/nrg3296

Brettschneider, J., Toledo, J. B., Van Deerlin, V. M., Elman, L., McCluskey, L., Lee, V. M. Y., et al. (2012). Microglial activation correlates with disease progression and upper motor neuron clinical symptoms in amyotrophic lateral sclerosis. *PLoS One* 7:e39216. doi: 10.1371/journal.pone.0039216

Butovsky, O., Jedrychowski, M. P., Cialic, R., Krasemann, S., Murugaiyan, G., Fanek, Z., et al. (2015). Targeting miR-155 restores abnormal microglia and attenuates disease in SOD1 mice. *Ann. Neurol.* 77, 75–99. doi: 10.1002/ana.24304

Byrne, S., Heverin, M., Elamin, M., Walsh, C., and Hardiman, O. (2014). Intermediate repeat expansion length in C9orf72 may be pathological in amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 15, 148–150. doi: 10.3109/21678421.2013.838586

Campanari, M. L., García-Ayllón, M. S., Ciura, S., Sáez-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

Chen, L., and Liu, B. (2017). Relationships between stress granules, oxidative stress, and neurodegenerative diseases. *Oxid. Med. Cell. Long.* 2017:1809592. doi: 10.1155/2017/1809592

Cheng, W., Wang, S., Mestre, A. A., Fu, C., Makarem, A., Xian, F., et al. (2018). C9ORF72 GGGGCC repeat-associated non-AUG translation is upregulated by stress through eIF2α phosphorylation. *Nat. Commun.* 9:51. doi: 10.1038/s41467-017-02495-z

Chew, J., Cook, C., Gendron, T. F., Jansen-West, K., Del Rosso, G., Daughrity, L. M., et al. (2019). Aberrant deposition of stress granule-resident proteins linked to C9orf72-associated TDP-43 proteinopathy. *Mol. Neurodegen.* 14:9. doi: 10.1186/s13024-019-0310-z

Chitiprolu, M., Jagow, C., Tremblay, V., Bondy-Chorney, E., Paris, G., Savard, A., et al. (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

Cleary, J. D., Pattamatta, A., and Ranum, L. P. W. (2018). Repeat-associated non-ATG (RAN) translation. *J. Biol. Chem.* 293, 16127–16141. doi: 10.1074/jbc.R118.003237

Colombrita, C., Zennaro, E., Fallini, C., Weber, M., Sommacal, A., Buratti, E., et al. (2009). TDP-43 is recruited to stress granules in conditions of oxidative insult. *J. Neurochem.* 111, 1051–1061. doi: 10.1111/j.1471-4159.2009.06383.x

Corcia, P., Tauber, C., Vercoullie, J., Arlicot, N., Prunier, C., Praline, J., et al. (2012). Molecular imaging of microglial activation in amyotrophic lateral sclerosis. *PLoS One* 7:e52941. doi: 10.1371/JOURNAL.PONE.0052941

Cruts, M., Gijselinck, I., Van Der Zee, J., Engelborghs, S., Wils, H., Pirici, D., et al. (2006). Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature* 442, 920–924. doi: 10.1038/nature05017

Cruz, M. P. (2018). Edaravone (Radicava): a novel neuroprotective agent for the treatment of amyotrophic lateral sclerosis. *P T* 43, 25–28.

Cudkowicz, M. E., Shefner, J. M., Schoenfeld, D. A., Zhang, H., Andreasson, K. I., Rothstein, J. D., et al. (2006). Trial of celecoxib in amyotrophic lateral sclerosis. *Ann. Neurol.* 60, 22–31. doi: 10.1002/ana.20903

Deora, V., Lee, J. D., Albornoz, E. A., McAlary, L., Jagaraj, C. J., Robertson, A. A. B., et al. (2019). The microglial NLRP3 inflammasome is activated by amyotrophic lateral sclerosis proteins. *GLIA* [Epub ahead of print].

Devos, D., Moreau, C., Kyheng, M., Garçon, G., Rolland, A. S., Blasco, H., et al. (2019). A ferroptosis–based panel of prognostic biomarkers for Amyotrophic Lateral Sclerosis. *Sci. Rep.* 9:2918. doi: 10.1038/s41598-019-39739-5

Drachman, D. B., Frank, K., Dykes-Hoberg, M., Teismann, P., Almer, G., Przedborski, S., et al. (2002). Cyclooxygenase 2 inhibition protects motor neurons and prolongs survival in a transgenic mouse model of ALS. *Ann. Neurol.* 52, 771–778. doi: 10.1002/ana.10374

Dubbelaar, M. L., Kracht, L., Eggen, B., and Boddeke, E. W. G. M. (2018). The kaleidoscope of microglial phenotypes. *Front. Immunol.* 9:17531. doi: 10.3389/fimmu.2018.01753

Fay, M. M., Anderson, P. J., and Ivanov, P. (2017). ALS/FTD-associated C9ORF72 repeat RNA promotes phase transitions in vitro and in cells. *Cell Rep.* 21, 3573–3584. doi: 10.1016/j.celrep.2017.11.093

Ferrante, R. J., Browne, S. E., Shinobu, L. A., Bowling, A. C., Jay Baik, M., Macgarvey, U., et al. (1997). Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis. *J. Neurochem.* 69, 2064–2074. doi: 10.1046/j.1471-4159.1997.69052064.x

Fischer, R., and Maier, O. (2015). Interrelation of oxidative stress and inflammation in neurodegenerative disease: role of TNF. *Oxid. Med. Cell. Long.* 2015, 1–18. doi: 10.1155/2015/610813

Fitzpatrick, F. (2004). Cyclooxygenase enzymes: regulation and function. *Curr. Pharmaceut. Design* 10, 577–588. doi: 10.2174/1381612043453144

Fratta, P., Mizielinska, S., Nicoll, A. J., Zloh, M., Fisher, E. M. C., Parkinson, G., et al. (2012). C9orf72 hexanucleotide repeat associated with amyotrophic lateral sclerosis and frontotemporal dementia forms RNA G-quadruplexes. *Sci. Rep.* 2:1016. doi: 10.1038/srep01016

Freibaum, B. D., and Taylor, J. P. (2017). The role of dipeptide repeats in C9ORF72-Related ALS-FTD. *Front. Mol. Neurosci.* 10:35. doi: 10.3389/fnmol.2017.00035

Gao, H.-M., Zhou, H., and Hong, J. S. (2012). NADPH oxidases: novel therapeutic targets for neurodegenerative diseases. *Trends Pharmacol. Sci.* 33, 295–303. doi: 10.1016/j.tips.2012.03.008

Garbuzova-Davis, S., and Sanberg, P. R. (2014). Blood-CNS barrier impairment in ALS patients versus an animal model. *Front. Cell. Neurosci.* 8:21. doi: 10.3389/fncel.2014.00021

Garwood, C. J., Ratcliffe, L. E., Simpson, J. E., Heath, P. R., Ince, P. G., and Wharton, S. B. (2017). Review: astrocytes in Alzheimer's disease and other age-associated dementias: a supporting player with a central role. *Neuropathol. Appl. Neurobiol.* 43, 281–298. doi: 10.1111/nan.12338

Geloso, M. C., Corvino, V., Marchese, E., Serrano, A., Michetti, F., and D'Ambrosi, N. (2017). The dual role of microglia in ALS: mechanisms and therapeutic approaches. *Front. Aging Neurosci.* 9:242. doi: 10.3389/fnagi.2017.00242

Gijselinck, I., Van Mossevelde, S., Van Der Zee, J., Sieben, A., Engelborghs, S., De Bleecker, J., et al. (2016). The C9orf72 repeat size correlates with onset age of disease, DNA methylation and transcriptional downregulation of the promoter. *Mol. Psychiatry* 21, 1112–1124. doi: 10.1038/mp.2015.159

Haeusler, A. R., Donnelly, C. J., Periz, G., Simko, E. A. J., Shaw, P. G., Kim, M. S., et al. (2014). C9orf72 nucleotide repeat structures initiate molecular cascades of disease. *Nature* 507, 195–200. doi: 10.1038/nature13124

Herman, F. J., and Pasinetti, G. M. (2018). Principles of inflammasome priming and inhibition: implications for psychiatric disorders. *Brain Behav. Immun.* 73, 66–84. doi: 10.1016/j.bbi.2018.06.010

Herrmann, F., and Fackelmayer, F. O. (2009). Nucleo-cytoplasmic shuttling of protein arginine methyltransferase 1 (PRMT1) requires enzymatic activity. *Genes Cells* 14, 309–317. doi: 10.1111/j.1365-2443.2008.01266.x

Italiani, P., Carlesi, C., Giungato, P., Puxeddu, I., Borroni, B., Bossù, P., et al. (2014). Evaluating the levels of interleukin-1 family cytokines in sporadic amyotrophic lateral sclerosis. *J. Neuroinflamm.* 11:94. doi: 10.1186/1742-2094-11-94

Iyer, S., Subramanian, V., and Acharya, K. R. (2018). C9orf72, a protein associated with amyotrophic lateral sclerosis (ALS) is a guanine nucleotide exchange factor. *PeerJ* 6:e5815. doi: 10.7717/peerj.5815

Johann, S., Heitzer, M., Kanagaratnam, M., Goswami, A., Rizo, T., Weis, J., et al. (2015). NLRP3 inflammasome is expressed by astrocytes in the SOD1 mouse model of ALS and in human sporadic ALS patients. *Glia* 63, 2260–2273. doi: 10.1002/glia.22891

Kedersha, N., and Anderson, P. (2007). Mammalian stress granules and processing bodies. *Methods Enzymol.* 431, 61–81. doi: 10.1016/S0076-6879(07)31005-7

Keller, A. F., Gravel, M., and Kriz, J. (2011). Treatment with minocycline after disease onset alters astrocyte reactivity and increases microgliosis in SOD1 mutant mice. *Exp. Neurol.* 228, 69–79. doi: 10.1016/j.expneurol.2010.12.010

Kim, G. H., Kim, J. E., Rhie, S. J., and Yoon, S. (2015). The role of oxidative stress in neurodegenerative diseases. *Exp. Neurobiol.* 24, 325–340. doi: 10.5607/en.2015.24.4.325

Kim, H.-J., Raphael, A. R., LaDow, E. S., McGurk, L., Weber, R. A., Trojanowski, J. Q., et al. (2014). Therapeutic modulation of eIF2α phosphorylation rescues TDP-43 toxicity in amyotrophic lateral sclerosis disease models. *Nat. Genet.* 46, 152–160. doi: 10.1038/ng.2853

Koppers, M., Blokhuis, A. M., Westeneng, H.-J., Terpstra, M. L., Zundel, C. A. C., Vieira de Sá, R., et al. (2015). C9orf72 ablation in mice does not cause motor neuron degeneration or motor deficits. *Ann. Neurol.* 78, 426–438. doi: 10.1002/ana.24453

Kreutzberg, G. W. (1996). Microglia: a sensor for pathological events in the CNS. *Trends Neurosci.* 19, 312–318. doi: 10.1016/0166-2236(96)10049-7

Kriz, J., Nguyen, M. D., and Julien, J.-P. (2002). Minocycline slows disease progression in a mouse model of amyotrophic lateral sclerosis. *Neurobiol. Dis.* 10, 268–278. doi: 10.1006/NBDI.2002.0487

Kumar, V., Kashav, T., Islam, A., Ahmad, F., and Hassan, M. I. (2016). Structural insight into C9orf72 hexanucleotide repeat expansions: towards new therapeutic targets in FTD-ALS. *Neurochem. Int.* 100, 11–20. doi: 10.1016/J.NEUINT.2016.08.008

Kwon, I., Xiang, S., Kato, M., Wu, L., Theodoropoulos, P., Wang, T., et al. (2014). Poly-dipeptides encoded by the C9orf72 repeats bind nucleoli, impede RNA biogenesis, and kill cells. *Science* 345, 1139–1145. doi: 10.1126/SCIENCE.1254917

Lall, D., and Baloh, R. H. (2017). Microglia and C9orf72 in neuroinflammation and ALS and frontotemporal dementia. *J. Clin. Invest.* 127, 3250–3258. doi: 10.1172/JCI90607

Lee, K.-H., Zhang, P., Kim, H. J., Mitrea, D. M., Sarkar, M., Freibaum, B. D., et al. (2016). C9orf72 dipeptide repeats impair the assembly, dynamics, and function of membrane-less organelles. *Cell* 167, 774.e17–788.e17. doi: 10.1016/j.cell.2016.10.002

Lee, S., and Huang, E. J. (2017). Modeling ALS and FTD with iPSC-derived neurons. *Brain Res.* 1656, 88–97. doi: 10.1016/j.brainres.2015.10.003

Li, Q., and Barres, B. A. (2018). Microglia and macrophages in brain homeostasis and disease. *Nat. Rev. Immunol.* 18, 225–242. doi: 10.1038/nri.2017.125

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

Liu, C., Geng, Y., Miao, H., Shi, X., You, Y., Xu, N., et al. (2019). G-quadruplex structures formed by human telomeric DNA and C9orf72 hexanucleotide repeats. *Biophys. Rev.* 11, 389–393. doi: 10.1007/s12551-019-00545-y

Lomen-Hoerth, C., Anderson, T., and Miller, B. (2002). The overlap of amyotrophic lateral sclerosis and frontotemporal dementia. *Neurology* 59, 1077–1079. doi: 10.1212/WNL.59.7.1077

Lu, C.-H., Allen, K., Oei, F., Leoni, E., Kuhle, J., Tree, T., et al. (2016). Systemic inflammatory response and neuromuscular involvement in amyotrophic lateral sclerosis. *Neuroimmunol. Neuroinflamm.* 3:e244. doi: 10.1212/NXI.0000000000000244

Luo, L., Song, Z., Li, X., Huiwang, Zeng, Y., Qinwang, et al. (2019). Efficacy and safety of edaravone in treatment of amyotrophic lateral sclerosis—a systematic review and meta-analysis. *Neurol. Sci.* 40, 235–241. doi: 10.1007/s10072-018-3653-2

Ma, M. W., Wang, J., Zhang, Q., Wang, R., Dhandapani, K. M., Vadlamudi, R. K., et al. (2017). NADPH oxidase in brain injury and neurodegenerative disorders. *Mol. Neurodegen.* 12:7. doi: 10.1186/s13024-017-0150-7

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

Maharjan, N., Künzli, C., Buthey, K., and Saxena, S. (2017). C9ORF72 regulates stress granule formation and its deficiency impairs stress granule assembly, hypersensitizing cells to stress. *Mol. Neurobiol.* 54, 3062–3077. doi: 10.1007/s12035-016-9850-1

Mahoney, C. J., Beck, J., Rohrer, J. D., Lashley, T., Mok, K., Shakespeare, T., et al. (2012). Frontotemporal dementia with the *C9ORF72* hexanucleotide repeat expansion: clinical, neuroanatomical and neuropathological features. *Brain* 135, 736–750. doi: 10.1093/brain/awr361

Maier, A., Deigendesch, N., Müller, K., Weishaupt, J. H., Krannich, A., Röhle, R., et al. (2015). Interleukin-1 antagonist Anakinra in amyotrophic lateral sclerosis - A pilot study. *PLoS One* 10:e0139684. doi: 10.1371/journal.pone.0139684

Mammana, S., Fagone, P., Cavalli, E., Basile, M. S., Petralia, M. C., Nicoletti, F., et al. (2018). The role of macrophages in neuroinflammatory and neurodegenerative pathways of alzheimer's disease, amyotrophic lateral sclerosis, and multiple sclerosis: pathogenetic cellular effectors and potential therapeutic targets. *Int. J. Mol. Sci.* 19:E831. doi: 10.3390/ijms19030831

McCauley, M. E., and Baloh, R. H. (2019). Inflammation in ALS/FTD pathogenesis. *Acta Neuropathol.* 137, 715–730. doi: 10.1007/s00401-018-1933-9

McCombe, P. A., and Henderson, R. D. (2011). The role of immune and inflammatory mechanisms in ALS. *Curr. Mol. Med.* 11, 246–254. doi: 10.2174/156652411795243450

Meissner, F., Molawi, K., and Zychlinsky, A. (2010). Mutant superoxide dismutase 1-induced IL-1beta accelerates ALS pathogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 107, 13046–13050. doi: 10.1073/pnas.1002396107

Miller, R. G., Block, G., Katz, J. S., Barohn, R. J., Gopalakrishnan, V., Cudkowicz, M., et al. (2015). Randomized phase 2 trial of NP001-a novel immune regulator: safety and early efficacy in ALS. *Neurol. Neuroimmunol. NeuroInflamm.* 2:e100. doi: 10.1212/NXI.0000000000000100

Miller, R. G., Zhang, R., Block, G., Katz, J., Barohn, R., Kasarskis, E., et al. (2014). NP001 regulation of macrophage activation markers in ALS: a phase I clinical and biomarker study. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 15, 601–609. doi: 10.3109/21678421.2014.951940

Mitchell, D. J., O'brien, M. R., Joshi, M., and Mitchell, J. D. (2009). Audit of outcomes in motor neuron disease (MND) patients treated with riluzole Audit of outcomes in motor neuron disease (MND) patients treated with riluzole. *Amyotroph. Lateral Scler.* 7, 67–71. doi: 10.1080/14660820500396984

Mitchell, J. C., Constable, R., So, E., Vance, C., Scotter, E., Glover, L., et al. (2015). Wild type human TDP-43 potentiates ALS-linked mutant TDP-43 driven progressive motor and cortical neuron degeneration with pathological features of ALS. *Acta Neuropathol. Commun.* 3, 36. doi: 10.1186/s40478-015-0212-4

Mizielinska, S., Grönke, S., Niccoli, T., Ridler, C. E., Clayton, E. L., Devoy, A., et al. (2014). C9orf72 repeat expansions cause neurodegeneration in *Drosophila* through arginine-rich proteins. *Science* 345, 1192–1194. doi: 10.1126/science.1256800

Moens, T. G., Niccoli, T., Wilson, K. M., Atilano, M. L., Birsa, N., Gittings, L. M., et al. (2019). C9orf72 arginine-rich dipeptide proteins interact with ribosomal proteins in vivo to induce a toxic translational arrest that is rescued by eIF1A. *Acta Neuropathol.* 137, 487–500. doi: 10.1007/s00401-018-1946-4

Mori, K., Weng, S.-M., Arzberger, T., May, S., Rentzsch, K., Kremmer, E., et al. (2013). The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS. *Science* 339, 1335–1338. doi: 10.1126/science.1232927

Müller, N. (2019). COX-2 inhibitors, aspirin, and other potential anti-inflammatory treatments for psychiatric disorders. *Front. Psychiatry* 10:375. doi: 10.3389/fpsyt.2019.00375

Murray, M. E., Dejesus-Hernandez, M., Rutherford, N. J., Baker, M., Duara, R., Graff-Radford, N. R., et al. (2011). Clinical and neuropathologic heterogeneity of c9FTD/ALS associated with hexanucleotide repeat expansion in *C9ORF72*. *Acta Neuropathol.* 122, 673–690. doi: 10.1007/s00401-011-0907-y

O'Rourke, J. G., Bogdanik, L., Yáñez, A., Lall, D., Wolf, A. J., Muhammad, A. K. M. G., et al. (2016). C9orf72 is required for proper macrophage and microglial function in mice. *Science* 351, 1324–1329. doi: 10.1126/science.aaf1064

Pasinelli, P., and Brown, R. H. (2006). Molecular biology of amyotrophic lateral sclerosis: insights from genetics. *Nat. Rev. Neurosci.* 7, 710–723. doi: 10.1038/nrn1971

Pollari, E., Goldsteins, G., Bart, G., Koistinaho, J., and Giniatullin, R. (2014). The role of oxidative stress in degeneration of the neuromuscular junction in amyotrophic lateral sclerosis. *Front. Cell. Neurosci.* 8:131. doi: 10.3389/fncel.2014.00131

Pompl, P. N., Ho, L., Bianchi, M., Mcmanus, T., Qin, W., and PasinettI, G. M. (2003). A therapeutic role for cyclooxygenase-2 inhibitors in a transgenic mouse model of amyotrophic lateral sclerosis. *FASEB J.* 17, 725–727. doi: 10.1096/fj.02-0876fje

Prado, L. D. G. R., Bicalho, I. C. S., Magalhães, D., Caramelli, P., Teixeira, A. L., and de Souza, L. C. (2015). C9ORF72 and the FTD-ALS spectrum: a systematic review of neuroimaging studies. *Dementia Neuropsychol.* 9, 413–421. doi: 10.1590/1980-57642015DN94000413

Prasad, A., Bharathi, V., Sivalingam, V., Girdhar, A., and Patel, B. K. (2019). Molecular mechanisms of TDP-43 misfolding and pathology in amyotrophic lateral sclerosis. *Front. Mol. Neurosci.* 12:25. doi: 10.3389/fnmol.2019.00025

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

Rostalski, H., Leskelä, S., Huber, N., Katisko, K., Cajanus, A., Solje, E., et al. (2019). Astrocytes and microglia as potential contributors to the pathogenesis of C9orf72 repeat expansion-associated FTLD and ALS. *Front. Neurosci.* 13:486. doi: 10.3389/fnins.2019.00486

Sellier, C., Campanari, M., Julie Corbier, C., Gaucherot, A., Kolb-Cheynel, I., Oulad-Abdelghani, M., et al. (2016). Loss of C9ORF72 impairs autophagy and synergizes with polyQ Ataxin-2 to induce motor neuron dysfunction and cell death. *EMBO J.* 35, 1276–1297. doi: 10.15252/embj.201593350

Serrano, A., Apolloni, S., Rossi, S., Lattante, S., Sabatelli, M., Peric, M., et al. (2019). The S100A4 transcriptional inhibitor niclosamide reduces pro-inflammatory and migratory phenotypes of microglia: implications for amyotrophic lateral sclerosis. *Cells* 8:E1261. doi: 10.3390/cells8101261

Shemer, A., Erny, D., Jung, S., and Prinz, M. (2015). Microglia plasticity during health and disease: an immunological perspective. *Trends Immunol.* 36, 614–624. doi: 10.1016/j.it.2015.08.003

Shin, J. H., Lee, Y. A., Lee, J. K., Lee, Y. B., Cho, W., Im, D. S., et al. (2012). Concurrent blockade of free radical and microsomal prostaglandin E synthase-1-mediated PGE2 production improves safety and efficacy in a mouse model of amyotrophic lateral sclerosis. *J. Neurochem.* 122, 952–961. doi: 10.1111/j.1471-4159.2012.07771.x

Solomon, D. A., Stepto, A., Au, W. H., Adachi, Y., Diaper, D. C., Hall, R., et al. (2018). A feedback loop between dipeptide-repeat protein, TDP-43 and karyopherin-α mediates C9orf72-related neurodegeneration. *Brain* 141, 2908–2924. doi: 10.1093/brain/awy241

Strong, M. J., Lomen-Hoerth, C., Caselli, R. J., Bigio, E. H., and Yang, W. (2003). Cognitive impairment, frontotemporal dementia, and the motor neuron diseases. *Ann. Neurol.* 54, S20–S23. doi: 10.1002/ana.10574

Swanson, K. V., Deng, M., and Ting, J. P.-Y. (2019). The NLRP3 inflammasome: molecular activation and regulation to therapeutics. *Nat. Rev. Immunol.* 19, 477–489. doi: 10.1038/s41577-019-0165-0

Takada, L. T. (2015). The genetics of monogenic frontotemporal dementia. *Dementia Neuropsychol.* 9, 219–229. doi: 10.1590/1980-57642015DN93000003

Thys, R. G., and Wang, Y.-H. (2015). DNA replication dynamics of the GGGGCC repeat of the C9orf72 gene. *J. Biol. Chem.* 290, 28953–28962. doi: 10.1074/jbc.M115.660324

Tsai, C.-P., Lin, F.-C., Lee, J. K.-W., and Lee, C. T.-C. (2015). Aspirin use associated with amyotrophic lateral sclerosis: a total population-based case-control study. *J. Epidemiol.* 25, 172–177. doi: 10.2188/jea.JE20140070

van Blitterswijk, M., Gendron, T. F., Baker, M. C., DeJesus-Hernandez, M., Finch, N. A., Brown, P. H., et al. (2015). Novel clinical associations with specific C9ORF72 transcripts in patients with repeat expansions in C9ORF72. *Acta Neuropathol.* 130, 863–876. doi: 10.1007/s00401-015-1480-6

Vane, J. R., and Botting, R. M. (2003). The mechanism of action of aspirin. *Thromb. Res.* 110, 255–258. doi: 10.1016/s0049-3848(03)00379-7

Varcianna, A., Myszczynska, M. A., Castelli, L. M., O'Neill, B., Kim, Y., Talbot, J., et al. (2019). Micro-RNAs secreted through astrocyte-derived extracellular vesicles cause neuronal network degeneration in C9orf72 ALS. *EBioMedicine* 40, 626–635. doi: 10.1016/j.ebiom.2018.11.067

Volonté, C., Amadio, S., Fabbrizio, P., and Apolloni, S. (2019). Functional microglia neurotransmitters in amyotrophic lateral sclerosis. *Semin. Cell Dev. Biol.* 94, 121–128. doi: 10.1016/j.semcdb.2019.04.014

Westergard, T., McAvoy, K., Russell, K., Wen, X., Pang, Y., Morris, B., et al. (2019). Repeat-associated non-AUG translation in C9orf72-ALS/FTD is driven by neuronal excitation and stress. *EMBO Mol. Med.* 11:e9423. doi: 10.15252/emmm.201809423

Winter, A. N., Brenner, M. C., Punessen, N., Snodgrass, M., Byars, C., Arora, Y., et al. (2017). Comparison of the neuroprotective and anti-inflammatory effects of the anthocyanin metabolites, protocatechuic acid and 4-hydroxybenzoic acid. *Oxid. Med. Cell. Long.* 2017:6297080. doi: 10.1155/2017/6297080

Winton, M. J., Igaz, L. M., Wong, M. M., Kwong, L. K., Trojanowski, J. Q., and Lee, V. M.-Y. (2008). Disturbance of nuclear and cytoplasmic TAR DNA-binding protein (TDP-43) induces disease-like redistribution, sequestration, and aggregate formation. *J. Biol. Chem.* 283, 13302–13309. doi: 10.1074/jbc.M800342200

Xu, W., and Xu, J. (2018). C9orf72 dipeptide repeats cause selective neurodegeneration and cell-autonomous excitotoxicity in *Drosophila* glutamatergic neurons. *J. Neurosci.* 38, 7741–7752. doi: 10.1523/JNEUROSCI.0908-18.2018

Yoshino, H., and Kimura, A. (2006). Investigation of the therapeutic effects of edaravone, a free radical scavenger, on amyotrophic lateral sclerosis (Phase II study). *Amyotroph. Lateral Scler.* 7, 247–251. doi: 10.1080/17482960600881870

Yuan, Y., Zha, H., Rangarajan, P., Ling, E.-A., and Wu, C. (2014). Anti-inflammatory effects of Edaravone and Scutellarin in activated microglia in experimentally induced ischemia injury in rats and in BV-2 microglia. *BMC Neurosci.* 15:125. doi: 10.1186/s12868-014-0125-3

Zhang, P., Li, W., Li, L., Wang, N., Li, X., Gao, M., et al. (2012). Treatment with edaravone attenuates ischemic brain injury and inhibits neurogenesis in the subventricular zone of adult rats after focal cerebral ischemia and reperfusion injury. *Neuroscience* 201, 297–306. doi: 10.1016/j.neuroscience.2011.11.005

Zhang, Y., Chen, K., Sloan, S. A., Bennett, M. L., Scholze, A. R., O'Keeffe, S., et al. (2014). An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J. Neurosci.* 34, 11929–11947. doi: 10.1523/JNEUROSCI.1860-14.2014

Zhang, Y. J., Jansen-West, K., Xu, Y.-F., Gendron, T. F., Bieniek, K. F., Lin, W.-L., et al. (2014). Aggregation-prone c9FTD/ALS poly(GA) RAN-translated proteins cause neurotoxicity by inducing ER stress. *Acta Neuropathol.* 128, 505–524. doi: 10.1007/s00401-014-1336-5

Zhang, Y. J., Gendron, T. F., Ebbert, M. T. W., O'Raw, A. D., Yue, M., Jansen-West, K., et al. (2018). Poly(GR) impairs protein translation and stress granule dynamics in C9orf72-associated frontotemporal dementia and amyotrophic lateral sclerosis. *Nat. Med.* 24, 1136–1142. doi: 10.1038/s41591-018-0071-1

Zhang, Y.-J., Guo, L., Gonzales, P. K., Gendron, T. F., Wu, Y., Jansen-West, K., et al. (2019). Heterochromatin anomalies and double-stranded RNA accumulation

underlie C9orf 72 poly(PR) toxicity. *Science* 363:eaav2606. doi: 10.1126/science.aav2606

Zhao, M., Kim, J. R., van Bruggen, R., and Park, J. (2018). RNA-binding proteins in amyotrophic lateral sclerosis. *Mol. Cells* 41, 818–829. doi: 10.14348/molcells.2018.0243

Zhao, W., Beers, D. R., Bell, S., Wang, J., Wen, S., Baloh, R. H., et al. (2015). TDP-43 activates microglia through NF-κB and NLRP3 inflammasome. *Exp. Neurol.* 273, 24–35. doi: 10.1016/j.expneurol.2015.07.019

Zhao, W., Xie, W., Beers, D. R., Henkel, J. S., Simpson, E. P., Yen, A. A., et al. (2004). Activated microglia initiate motor neuron injury by a nitric oxide and glutamate-mediated mechanism. *J. Neuropathol. Exp. Neurol.* 63, 964–977. doi: 10.1093/jnen/63.9.964

Zhou, B., Geng, Y., Liu, C., Miao, H., Ren, Y., Xu, N., et al. (2018). Characterizations of distinct parallel and antiparallel G-quadruplexes formed by two-repeat ALS and FTD related GGGGCC sequence. *Sci. Rep.* 8:2366. doi: 10.1038/s41598-018-20852-w

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REVIEW
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The Overlapping Genetics of Amyotrophic Lateral Sclerosis and Frontotemporal Dementia

Yevgeniya A. Abramzon[1,2], Pietro Fratta[2], Bryan J. Traynor[1,3] and Ruth Chia[1]*

[1] Neuromuscular Diseases Research Section, Laboratory of Neurogenetics, National Institute on Aging, NIH, Bethesda, MD, United States, [2] Sobell Department of Motor Neuroscience and Movement Disorders, Institute of Neurology, University College London, London, United Kingdom, [3] Department of Neurology, Brain Science Institute, Johns Hopkins University, Baltimore, MD, United States

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are two diseases that form a broad neurodegenerative continuum. Considerable effort has been made to unravel the genetics of these disorders, and, based on this work, it is now clear that ALS and FTD have a significant genetic overlap. TARDBP, SQSTM1, VCP, FUS, TBK1, CHCHD10, and most importantly C9orf72, are the critical genetic players in these neurological disorders. Discoveries of these genes have implicated autophagy, RNA regulation, and vesicle and inclusion formation as the central pathways involved in neurodegeneration. Here we provide a summary of the significant genes identified in these two intrinsically linked neurodegenerative diseases and highlight the genetic and pathological overlaps.

Keywords: amyotrophic lateral sclerosis, frontotemporal dementia, neurological disorders, neurodegeneration, overlapping genetics

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****Correspondence:***
Yevgeniya A. Abramzon
yevgeniya.abramzon@nih.gov

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS, OMIM #105400) is a fatal neurological disorder affecting motor neurons located in the frontal cortex, brainstem, and spinal cord (Cleveland and Rothstein, 2001). The disease typically begins as muscle weakness in a limb, or occasionally with changes in voice or difficulty swallowing, which progresses to generalized weakness and paralysis of respiratory muscles leading to death due to respiratory failure. Approximately 10% of all ALS cases have a family history of the disease, while the remaining 90% are sporadic. The incidence of ALS is estimated to be 2.1 new cases per 100,000 population per year (Chio et al., 2013), and approximately 6,000 people are newly diagnosed with ALS each year in the United States alone. The number of ALS cases around the world is increasing due to the aging of the global population (Arthur et al., 2016). There are currently no effective treatments for ALS, except for edaravone, which reduces the decline in daily functioning, and riluzole, which prolongs patients' survival by a few months (Miller et al., 2012; Rothstein, 2017).

Frontotemporal degeneration (FTD) is one of the most common types of dementia in people under 65. FTD may be divided into three primary subtypes, namely behavioral variant, semantic dementia, and progressive non-fluent aphasia. Among these subtypes, behavioral variant FTD is the most commonly observed type of dementia associated with motor neuron disorders (Bird et al., 1999). The incidence of FTD is approximately 4.0 new cases per 100,000 population per year, with

40% of cases being familial (Ratnavalli et al., 2002). Similar to ALS and other neurological disorders, there is no effective treatment for FTD (Tsai and Boxer, 2014).

It is now recognized that ALS and FTD are two diseases that form a broad neurodegenerative continuum. One of the earliest hints of this overlap came from the clinical observation that both disorders can be present within the same family or even within the same individual. Cross-sectional studies performed over the last decade estimate that up to 50% of ALS patients develop cognitive impairment associated with FTD. Similarly, up to 30% of FTD patients develop motor dysfunction (Burrell et al., 2011).

Considerable progress has been made in unraveling the genetics of ALS and FTD, and it is now clear that the genetics of these two neurodegenerative conditions overlap significantly. TARDBP, SQSTM1, VCP, FUS, TBK1, CHCHD10, and most importantly C9orf72, are the critical genetic players, and their discoveries have implicated autophagy, RNA processing, and vesicle and inclusion formation as the central pathways involved in these forms of neurodegeneration.

Here we provide a summary of the significant genes identified in these two intrinsically linked neurodegenerative diseases and highlight where cross-talk exists. We describe the genes in the order of their relevance to ALS/FTD overlap, ranging from genes that have been demonstrated to cause both clinically and neuropathologically confirmed ALS and FTD to genes where the cognitive or motor symptoms are reported in the literature but pathological confirmation in not yet available.

The genes described in this review, clinical phenotypes and pathways associated with them are summarized in **Table 1**.

CHROMOSOME 9 OPEN READING FRAME 72 (C9ORF72)

In 2011, a hexanucleotide repeat expansion within the C9orf72 gene located on chromosome 9p21 was identified as a significant genetic cause of both ALS and FTD (DeJesus-Hernandez et al., 2011; Renton et al., 2011). This repeat expansion is the most common genetic cause of ALS, FTD, and ALS/FTD responsible for ∼11% of all ALS and ∼13% of all FTD cases. This discovery demonstrated that there is a more considerable genetic overlap between ALS and FTD than had been previously estimated. The majority of C9orf72-related FTD cases manifest behavioral symptoms with a much smaller percentage presenting with semantic dementia or with progressive non-fluent aphasia. C9orf72 repeat expansions have also been implicated as rare causes of other neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, progressive supranuclear palsy, ataxia, corticobasal syndrome, Huntington disease-like syndrome, and Creutzfeldt–Jakob disease (Beck et al., 2013; Hensman Moss et al., 2014; Devenney et al., 2018).

Several mechanisms have been proposed to explain how C9orf72 expansion causes neurological disease. These include (Cleveland and Rothstein, 2001) haploinsufficiency of C9orf72 protein (DeJesus-Hernandez et al., 2011; Renton et al., 2011; Chio et al., 2013; Shi et al., 2018; Shao et al., 2019) RNA toxicity due to accumulation of RNA containing the GGGGCC repeat in the brain and spinal cord (DeJesus-Hernandez et al., 2011; Renton et al., 2011; Arthur et al., 2016; Arzberger et al., 2018) dipeptide repeat (DPR) protein toxicity arising from repeat-associated non-AUG translation occurring off the expansion (Miller et al., 2012; May et al., 2014; Freibaum and Taylor, 2017) disruption of the nucleocytoplasmic transport (Freibaum et al., 2015; Jovicic et al., 2015; Zhang et al., 2015). Although the data for each of these mechanisms are compelling, it is not yet clear which of them plays the dominant role in determining neurodegeneration. The possibility of multiple mechanisms, operating in either unison or sequentially to bring about neuronal death, cannot be discounted.

Various mouse models have been created to elucidate the pathogenic mechanism underlying C9orf72 neurodegeneration. Though informative, these models have failed to resolve the exact mechanism, as the available information bolsters all four modes of neurodegeneration. For example, mice lacking C9orf72 in neurons and glial cells did not display motor neuron degeneration or defects in motor function associated with ALS (Koppers et al., 2015). BAC transgenic mice with expanded human C9orf72 hexanucleotide repeat that ranged between 100 and 1000 repeats developed RNA foci and dipeptide repeat proteins throughout the nervous system. However, there was no evidence of neurodegeneration or functional deficits (O'Rourke et al., 2015; Peters et al., 2015). Mice with more than 450 GGGGCC repeats have mild hippocampal neuronal loss and display signs of age-dependent anxiety and impaired cognitive functioning (Jiang et al., 2016).

More recent mouse models showed that that loss of C9orf72 in a gain-of-function C9ALS/FTD mouse model aggravates motor behavior deficits in a dose-dependent manner (Shao et al., 2019). Transgenic GFP-PR28 mice expressing arginine-rich poly(PR), the most toxic type of DPRs in neurons, did partially develop neuropathological features of C9FTD/ALS (Hao et al., 2019). Two other transgenic C9FTD/ALS mouse models demonstrated that poly(GR) affects translation and stress granule dynamics (Zhang et al., 2018) and compromises mitochondrial function by binding Atp5a1 (Choi et al., 2019).

TAR DNA-BINDING PROTEIN 43 (TARDBP)

Mutations in the TAR DNA-binding protein 43 (the gene that encodes the TDP-43 protein) were linked to ALS in 2008 (Sreedharan et al., 2008). Before that, it was recognized that TDP-43 cytoplasmic and nuclear inclusions are characteristic of both ALS and FTD. In ALS, the cytoplasmic accumulation of TDP-43 is found in neurons and glia of the primary motor cortex, brainstem motor nuclei, and spinal cord (Bodansky et al., 2010; Mackenzie et al., 2010). In FTD, the inclusions are observed in the neocortex and dentate granule cells of the hippocampus (Neumann et al., 2006; Davidson et al., 2007). TDP-43 mutations are the cause of ∼ 1% of all ALS cases. In contrast, an even smaller number of FTD cases arising from mutations in this gene have been described, despite the widespread presence of TDP-43 in FTD brains (Tan et al., 2017).

TABLE 1 | Key genes identified in ALS and FTD.

Gene	Locus	Neurological phenotypes	Pathway	Main localization
FUS	16p11.2	ALS, FTD, ALS (juvenile with BIs) ET, MND (lower), bvFTD?, PD?	Nucleocytoplasmic transport/splicing	Nucleus
TDP-43	1p36.22	ALS, FTD, ALS (flail arm variant), SNGP and chorea, MND	Nucleocytoplasmic transport/splicing	Nucleus
CHCHD10	22q11.23	ALS, ALS/FTD, Mitochondrial myopathy (autosomal dominant)	Mitochondrial dysfunction/ Synaptic integrity	Mitochondrion, nucleus
C9orf72	9p21.2	AD, ALS, FTD, ALS/FTD, BD, PD, Schizophrenia	Nucleocytoplasmic transport/splicing	Extracellular, nucleus, endosome, lysosome
UBQLN2	Xp11.21	ALS, FTD, Neurodegeneration, X-linked	Autophagy/Proteasome	Cytosol, plasma membrane, nucleus
TBK1	12q14.1	ALS, ALS/FTD, AD	Autophagy/inflammation	Nucleus, cytosol, endosome, mitochondrion
VCP	9p13.3	IBMPFD, ALS, IBMPFD and ALS, CMT2, HSP DMRV, Scapuloperoneal muscular dystrophy and dropped head fibers, AD?, Autism?	Autophagy/Mitochondrial function	Nucleus, endoplasmic reticulum, cytosol, extracellular, lysosome
SQSTM1	5q35	PDB, ALS, FTD, AD, early onset ALS/FTD, NADGP	Autophagy	Nucleus, cytosol, lysosome, endoplasmic reticulum, endosome

IBMPFD, inclusion body myopathy with Paget disease and frontotemporal dementia; AD, Alzheimer disease; ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia; HSP, hereditary spastic paraplegia; CMT2, charcot-marie-tooth disease, type 2; PDB, paget's disease of bone; PD, Parkinson disease; BD, bipolar disorder; MND, motor neuron disease; ET, essential tremor; Bis, basophilic inclusions; DMRV-Myopathy, rimmed vacuolar; NADGP, neurodegeneration, childhood onset with ataxia, dystonia and gaze palsy; SNGP, supranuclear gaze palsy.

TDP-43 is a DNA and RNA binding protein involved in many aspects of RNA metabolism, including splicing, microRNA biogenesis, transcription, and stabilization of messenger RNA (Buratti et al., 2001; Strong et al., 2007; Buratti and Baralle, 2008; Fiesel et al., 2010; Lagier-Tourenne et al., 2010). Two contrasting mechanisms have been proposed to explain TDP-43 related neurodegeneration, namely (Cleveland and Rothstein, 2001) loss of function arising from sequestration of critical TDP-43 protein within cytoplasmic aggregates leading to nuclear depletion of TDP-43 (Chio et al., 2013; Mitra et al., 2019; Roczniak-Ferguson and Ferguson, 2019) gain of function effect due to some inherent toxic property of the aggregates (Buratti and Baralle, 2012; Hergesheimer et al., 2019). However, the toxic role of aggregated TDP-43 in neurodegeneration is still under debate. Recent research has focused on the role of stress granules in the pathogenesis of TDP43-related ALS (Khalfallah et al., 2018). TDP-43 mutations have also been reported to alter liquid drop formation, though the pathophysiological role of this *in vitro* epiphenomena remains unclear (Conicella et al., 2016).

More than fifteen mouse models have been created in the last 2 years in an attempt to decipher the pathogenic roles of TDP-43 in autophagy, protein homeostasis, and clearance pathways involved in ALS and FTD. These rodent models showed that suppression of conditional TDP-43 transgene expression differentially affects early cognitive and social phenotypes in TDP-43 mice (Silva et al., 2019). In a TDP-43Q331K/Q331K knock-in mouse model of ALS-FTD, TDP-43 gains function due to impaired autoregulation (White et al., 2018b). In TDP-43M337V and TDP-43G298S knock-in mice, mutant TDP-43 causes early stage dose-dependent motor neuron degeneration (Ebstein et al., 2019). Mice with endogenous TDP-43 mutations exhibit gain of splicing function and characteristics of motor neuron degeneration (Fratta et al., 2018). Mouse models have also provided insight into how mutations in this gene may be underlying frontotemporal dementia. A recent TDP-43Q331K mouse model manifested cognitive dysfunction in the absence of motor dysfunction. Pathological examination showed that normal localization of TDP-43 within the cell, but there was evidence of perturbed regulation of TDP-43 (White et al., 2018a,b).

SEQUESTOSOME-1 (SQSTM1)

Mutations in Sequestosome-1 (SQSTM1) was initially discovered in patients with Paget's disease of bone (Laurin et al., 2002) and linked to ALS and behavioral FTD in 2011 (Fecto et al., 2011). SQSTM1 encodes p62, a multifunctional protein involved in a wide range of cellular functions, including apoptosis (Jung and Oh, 2019), NFKB1 signaling (Foster et al., 2019), ubiquitin-mediated autophagy (Zaffagnini et al., 2018; Gao et al., 2019; Park et al., 2019), and transcription regulation (Rea et al., 2013). p62 is also a standard component of ubiquitin-containing inclusions in several neurological disorders, including ALS and FTD. More than 100 variants have been identified in SQSTM1, and cumulatively they account for ∼1% of all ALS and up to 3% of all FTD cases. Defective p62 is prone to forming aggregates. Individuals with SQSTM1 variants have p62-positive inclusions in the motor neurons if presenting with ALS, and in the hippocampus and cerebral neocortex if presenting with FTD (Arai et al., 2003; Teyssou et al., 2013).

Accumulation of SQSTM1 comes from disturbances in the selective autophagy pathway (Deng et al., 2019). However, the pathogenic mechanism that contributes to SQSTM1-related impaired autophagy and degradation remains poorly understood. Similar to TDP-43 and FUS, SQSTM1 goes through liquid-liquid phase separation. Recent research shows that cytoplasmic DAXX drives SQSTM1/p62 phase condensation, an essential step in the activation of Nrf2-mediated stress response (Yang et al., 2019). Polyubiquitin chain-induced p62 phase separation leads

to the segregation of autophagic cargo (Herhaus and Dikic, 2018; Sun et al., 2018).

To date, no. p62 mouse model has been created to study the direct effect of p62 mutations in ALS/FTD. However, many mouse models exist that demonstrate a relationship between p62 and other ALS genes. Mitsui et al. (2018) previously reported that loss of SQSTM1 exacerbates disease phenotypes in SOD1H46R ALS mice. Following the initial report, the same authors demonstrated that SQSTM1 overexpression results in a significant increase in biochemically detectable insoluble SQSTM1 and poly-ubiquitinated proteins in the spinal cord of SQSTM1; SOD1H46R mice when compared to SOD1H46R mice. This observation suggests that overexpression of p62 in SOD1H46R mice accelerates disease onset by impairing the protein degradation pathways (Mitsui et al., 2018).

From the FTD perspective, apart from developing mature-onset obesity due to impaired glucose tolerance and insulin resistance, p62 knockout mice display significantly reduced life span and accelerated aging phenotypes. These mice develop cognitive impairment and anxiety, which are symptoms characteristic of human Alzheimer's disease (Kwon et al., 2012).

FUSED IN SARCOMA (FUS)

Fused in sarcoma (FUS) is an RNA-binding protein that was linked to ALS in 2009 (Kwiatkowski et al., 2009). Similar to TDP-43, FUS is involved in multiple aspects of RNA metabolism regulation, including alternative splicing, RNA translation, and transport (Kwiatkowski et al., 2009; Vance et al., 2009). Mutations in FUS are responsible for ∼1% of all ALS. They are also occasionally observed in behavioral FTD cases. In addition to these phenotypes, abnormal aggregates of FUS, independently of their mutations, are present in other neurodegenerative diseases such as hereditary essential tremor, the polyglutamine diseases, and Parkinson's disease.

Amyotrophic lateral sclerosis and FTD related mutations are clustered in highly conserved regions of the gene and affect the protein nuclear localization signal (NLS). Similar to TDP-43, mutations in the FUS gene are predominantly found in ALS patients. A limited number of FUS mutations (p.P106L, p.Gly174-Gly175 deletion GG, p.M254V) have been described in FTD patients without concomitant ALS (Van Langenhove et al., 2010; Huey et al., 2012).

Two mechanisms were proposed to explain FUS-related neurodegeneration. First of all, there is the toxic gain-of-function in which nuclear FUS aggregates in cytoplasm and spreads in a prion-like manner through neuronal tissues (Armstrong, 2017). Second, the depletion of FUS from the nucleus may impair transcription, alternative splicing, and DNA repair (Shang and Huang, 2016). A reasonable amount of evidence supports both mechanisms, and different mechanisms may stand behind different FUS mutations (Ishigaki and Sobue, 2018; An et al., 2019). Liquid–liquid phase separation (LLPS) of FUS has emerged recently as an alternative mechanism for FUS-related neurodegeneration. It is now established that LLPS is modulated by universal cellular actors such as ATP and nucleic acids through enhancement and dissolution (Kang et al., 2019). Other recent FUS studies expanded on LLPS functions, mechanism, and transformation (Berry et al., 2018; Kang et al., 2019; Murthy et al., 2019; Niaki et al., 2019).

Multiple mouse models have been created in an attempt to identify the pathogenic roles of FUS in neurodegeneration. FUS knockout mice display behavioral abnormalities such as hyperactivity and reduced anxiety-related behavior. However, they do not develop motor neuron impairment, suggesting that the ablation of the FUS gene alone is insufficient to cause ALS (Kino et al., 2015). Transgenic mice overexpressing exogenous FUS with nuclear localization signal deletion (ΔNLS-FUS) under Thy1 neuron-specific promoter develop progressive ALS phenotypes associated with the formation of ubiquitin/p62-positive FUS aggregates, neuronal loss, and gliosis. In FusΔNLS/ΔNLS mice, truncation of the NLS region leads to mislocalization of FUS protein from the nucleus to the cytoplasm in spinal motor neurons and cortical neurons where it leads to apoptosis (Scekic-Zahirovic et al., 2016). Furthermore, both FusΔNLS/+mice and knock-in mice carrying another C-terminal frameshift mutation (FusΔ14/+) develop progressive motor neuron loss in heterozygosity, recapitulating the early stages of disease (Scekic-Zahirovic et al., 2016; Devoy et al., 2017). More recent FUSR514G and FUSR521C transgenic mice models show that overriding the FUS autoregulation system triggers gain-of-function toxicity via an altered autophagy-lysosome pathway and impaired RNA metabolism (Ho and Ling, 2019; Ling et al., 2019).

VALOSIN CONTAINING PROTEIN (VCP)

Mutations in Valosin containing protein (VCP) was initially discovered as the cause of a clinical syndrome characterized by the triad of inclusion body myopathy, Paget's disease of bone, and frontotemporal dementia (IBMFTD) in 2004 (Watts et al., 2004). Mutations in this gene were subsequently identified as a cause of ALS, representing an early example of how genetic mutations in a single gene could underlie both ALS and FTD (Johnson et al., 2010). To date, 72 autosomal dominant mutations have been discovered in this gene, more than 30 of which are reported in ALS or FTD cases (including behavioral FTD, semantic dementia, and progressive non-fluent aphasia) (Al-Obeidi et al., 2018; Saracino et al., 2018; Bastola et al., 2019). Many of the reported VCP mutations are located on exon five within the N-terminal CDC48 domain, which is involved in ubiquitin-binding, meaning that mutations in this region may negatively affect the ubiquitin protein degradation pathway (Ganji et al., 2018; Twomey et al., 2019).

A recent study by Al-Obeidi et al. (2018) showed that VCP mutations are present in ∼9% of ALS, 4% of Parkinson's disease, and 2% of Alzheimer's disease patients. As of today, no definite correlation between the mutation type and the incidence of clinical features associated with VCP has been established (Al-Obeidi et al., 2018; Plewa et al., 2018).

Valosin Containing Protein encodes a member of the AAA-ATPase enzyme family with wide-ranging functions in

cell division (Ogura and Wilkinson, 2001), DNA repair, ubiquitin-dependent protein degradation, and suppression of apoptosis (Ogura and Wilkinson, 2001). Ludtmann et al. (2017) provides evidence that mutations in VCP lead to mitochondrial uncoupling due to a reduced ADP/ATP translocation by adenine nucleotide translocase. Such deficiency in mitochondrial bioenergetics makes neurons especially vulnerable as they require more energy than other cell types (Ludtmann et al., 2017).

Recent mouse models of VCP showed that activation of the NLRP3 inflammasome is associated with VCP protein myopathy. Nalbandian et al. (2017) reported a significant increase in the expression of NLRP3, Caspase 1, IL-1β, and IL-18 in the quadriceps of 12 and 24 months old VCPR155H/+heterozygous mice. Furthermore, a significant increase of IL-1β(+)F4/80(+)Ly6C(+) macrophages in the quadriceps and bones of the same mice were also observed and is positively correlated with high expression levels of TDP-43 and p62/SQSTM1 markers of VCP pathology and progressive muscle wasting (Nalbandian et al., 2017).

Another recent discovery showed that VCP plays a vital role in the maintenance of lysosomal homeostasis and TFEB activity in differentiated skeletal muscle (Arhzaouy et al., 2019). Arhzaouy et al. (2019) showed that selective inactivation of VCP in skeletal muscles of Myl1p-cre-vcp-/-mice, results in a necrotic myopathy with increased macroautophagic/autophagic proteins and damaged lysosomes. It was further demonstrated that the myofiber necrosis was preceded by the upregulation of LGALS3/Galectin-3, a marker of damaged lysosomes, and TFEB activation, suggesting early defects in the lysosomal system (Arhzaouy et al., 2019).

COILED-COIL-HELIX-COILED-COIL-HELIX DOMAIN CONTAINING 10 (CHCHD10)

Coiled-coil-helix-coiled-coil-helix domain-containing protein 10 (CHCHD10) is a mitochondrial protein associated with ALS and FTD, including the behavioral and primary progressive aphasia subtypes of this form of dementia (Ajroud-Driss et al., 2015; Cozzolino et al., 2015). The protein was discovered in 2014 by exome sequencing of a large French family affected by autosomal dominant FTD with or without ALS, cerebellar ataxia, and mitochondrial myopathy (Chaussenot et al., 2014). At least 30 variants have since been reported, and they are concentrated on exon two of the gene encoding the non-structured N-terminal (Taylor et al., 2016; Perrone et al., 2017; Zhou et al., 2017).

Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 10 is a multifunctional protein involved in the regulation of mitochondrial metabolism, synthesis of respiratory chain components, and modulation of cell apoptosis (Zhou et al., 2017). Perhaps not surprisingly, mutations in CHCHD10 lead to disassembly of the mitochondrial contact site complex, severe mitochondrial DNA repair deficiency after oxidative stress, disruption of oxygen consumption and ATP synthesis in cells, and disturbance of apoptotic mechanisms (Zhou et al., 2017).

Recent data shows enrichment of CHCHD10 expression at the postsynaptic membrane of neuromuscular junctions (Bannwarth et al., 2014; Zhou et al., 2017; Xiao et al., 2019). Deletion of CHCHD10 in skeletal muscle of HSA-CHCHD10-/- knockout mice results in motor defects and neurotransmission impairment, indicating that muscle CHCHD10 is required for normal neurotransmission between motoneurons and skeletal muscle fibers (Xiao et al., 2019). Furthermore, an examination of HSA-CHCHD10-/- mice mitochondria under an electron microscope revealed a large quantity of large lysosome-like vesicles, indicating active mitochondria degradation and suggesting that CHCHD10 is required for mitochondria structure and ATP production (Burstein et al., 2018; Xiao et al., 2019).

Two groups independently developed CHCHD10S55L knock-in mice, representative of human CHCHD10 S59L mutation, and found that these mice developed progressive motor deficits, myopathy, cardiomyopathy, and died prematurely (Anderson et al., 2019; Genin et al., 2019). Histological examination revealed that CHCHD10, together with its twin CHCHD2 forms aggregates resulting in abnormal organelle morphology and function. In contrast, knock out CHCHD10 mice containing a single adenine nucleotide insertion in exon two that results in a prematurely terminated protein, did not develop similar pathology, suggesting that tissue-specific toxic gain-of-function is the likely mechanism behind CHCHD10 S59L related neurodegeneration (Anderson et al., 2019).

TANK-BINDING KINASE 1 (TBK1)

TANK-binding kinase 1 (TBK1) gene was discovered in 2015 through the whole-exome sequencing analysis of a large case-control cohort (Cirulli et al., 2015; Freischmidt et al., 2015). In 2016, a large genome-wide association study (GWAS) also identified the TBK1 gene on chromosome 12q14.2 as a risk locus for ALS, thus confirming the gene's association with motor neuron degeneration (van Rheenen et al., 2016). TBK1 is a member of the IkB kinase family involved in autophagy, mitophagy, and innate immune signaling (Weidberg and Elazar, 2011). The protein is highly expressed in neuronal cells of the cerebral cortex, hippocampus, and lateral ventricle (Uhlen et al., 2015). It also interacts with other genes implicated in ALS, such as OPTN and SQSTM1, to form TBK1 autophagic adaptor complex (Ryzhakov and Randow, 2007; Morton et al., 2008; Li et al., 2016).

To date, more than 90 mutations have been discovered on TBK1. According to a recent meta-analysis study, TBK1 loss of function and missense mutations account for 1.0 and 1.8% in ALS/FTD patients, respectively (Lamb et al., 2019). The majority of TBK1 mutations are loss of function that result in the deletion of the C-terminal domain responsible for interaction with adaptor proteins that regulate the cellular distribution of TBK1 and activation of downstream signaling pathways (Ryzhakov and Randow, 2007). Indeed, mutations appear to lead to a significant decrease in TBK1 expression at the mRNA and protein levels (Freischmidt et al., 2015).

TANK-binding kinase 1 mutations are associated with bulbar onset ALS and fast progressing behavioral FTD (Freischmidt et al., 2015). In ALS patients, TBK1 mutations are pathologically characterized by TDP-43 positive and p62 positive inclusions in motor neurons, as well as TDP-43 inclusions in the cortex. Similar to that observed in ALS, FTD patients, harboring TBK1 mutations is also characterized by TDP-43 inclusions in numerous brain regions and cytoplasmic p62 and ubiquitin-positive inclusions in glial cells (Van Mossevelde et al., 2016).

Compelling evidence exists that loss-of-function is the pathological mechanism behind TBK1-related ALS and FTD (de Majo et al., 2018; Lamb et al., 2019; Weinreich et al., 2019). Germline deletion of TBK1 is lethal in embryonic mice suggesting that the protein plays a critical role in developmental homeostasis (Bonnard et al., 2000). More recent rodent models demonstrated that conditional neuron-specific knockout of Tbk1 in Tbk1fl/fl Nestin-Cre mice leads to the development of cognitive and motor dysfunction similar to ALS/FTD. Neuron-specific Tbk1 deletion induces morphological and biochemical alterations in neurons and glia such as abnormal dendrites, neurofibrillary tangles, reduced dendritic spine density, as well as cortical synapse loss. Furthermore, Tbk1 knockout impairs autophagy in motor neuron-like cells, while Tbk1 over-expression extends survival of ALS transgenic mice (Duan et al., 2019).

TANK-Binding Kinase 1 is a central regulator of selective autophagy and inflammatory responses via IFN type I signaling (Perry et al., 2004; Hu et al., 2018). Heterozygous deletion of the α-IFN receptor Ifnar1 significantly prolongs the life span of SOD1G93A ALS mice (Wang et al., 2011). In a 2019 study, Brenner et al. (2019) further elucidated on the connection between TBK1 and SOD1 in the mouse models. The group showed that at the early stage, heterozygous Tbk1 deletion impairs autophagy in motoneurons and prepones the clinical onset and muscular denervation in SOD1G93A/Tbk1 ± mice, while at the late disease stage, it significantly alleviates microglial neuroinflammation, decelerates disease progression, and extends mouse survival (Brenner et al., 2019).

Summary

After several decades of research, it is now clear that the same genes can cause ALS and FTD. Mutations in C9orf72, TARDBP, FUS, TBK1, VCP, CHCHD10, and SQSTM1 are the most closely associated with both diseases. Clinically, the ALS phenotype is most commonly associated with the behavioral variant of FTD, with other subtypes of FTD involving language occurring less commonly. The pathophysiology underlying this observation is poorly understood.

Nevertheless, this overlap is not complete: SOD1, FUS, and TDP-43 variants are most commonly associated with ALS and are only rarely found in FTD patients. Similarly, GRN is linked to FTD, but not to ALS. Clinically, the ALS phenotype is most commonly associated with the behavioral variant of FTD, with other subtypes of FTD involving language occurring less commonly. The pathophysiology underlying this observation is poorly understood.

It is striking how the same pathways are implicated repeatedly in ALS and FTD. Both disorders characterized by defects in RNA processing, protein clearance by autophagy, vesicle trafficking, mitochondrial dysfunction, and impaired protein homeostasis. The genes described in this review are the key players in these pathways. TDP-43 and FUS are responsible for RNA regulation; SQSTM1, C9orf72, VCP, and TBK1 are involved in autophagy and vesicle dynamics; TDP-43, FUS, and SQSTM1 are common components of nuclear and cytoplasmic inclusions (Weishaupt et al., 2016). Due to such significant genetic overlap between ALS and FTD, it is reasonable to look in FTD cases for mutations in ALS genes, and vice-versa.

The C9orf72 repeat expansion gives rise to a diverse range of inter-familial and intra-familial phenotypes, including age at disease onset, site of symptom onset, rate and pattern of progression, levels of cognitive impairment and motor neuron degeneration, as well as disease duration. This clinical heterogeneity likely indicates that both genetic and environmental factors play a significant role in the development and course of the disease. Environmental factors such as occupational exposure to heavy metals, toxic compounds, and extremely low-frequency electromagnetic frequencies have been previously reported to increase the risk of developing neurological disorders. Studies on personal habits revealed an increased risk of ALS among smokers, as well as an overall worse prognosis after disease onset. In contrast, alcohol consumption was associated with a reduced risk of ALS. Literature analysis of head trauma and the development of neurological disorders were inconclusive. More recently, advanced genetic analysis of a large genetic dataset implicated high cholesterol as driving the risk of ALS, as well as confirming an association with smoking and physical exercise (Bandres-Ciga et al., 2019).

Research shows that environmental factors can influence people's chances of developing ALS or FTD. Nevertheless, the studies were performed on case cohorts that were not genetically selected. Different sets of environmental factors may interact with different genes. Consequently, future genetic epidemiology efforts should focus on cohorts selected based on their underlying genetic risk. Studying such population-based cohorts that have been assiduously collected and phenotyped for clinical features, genetics, epigenetics, and environmental and lifestyle exposures will be essential to these efforts.

AUTHOR CONTRIBUTIONS

YA drafted the manuscript. PF, RC, and BT participated in critically revising the manuscript for important intellectual content. All authors read and approved the final manuscript.

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REFERENCES

Ajroud-Driss, S., Fecto, F., Ajroud, K., Lalani, I., Calvo, S. E., Mootha, V. K., et al. (2015). Mutation in the novel nuclear-encoded mitochondrial protein CHCHD10 in a family with autosomal dominant mitochondrial myopathy. *Neurogenetics* 16, 1–9. doi: 10.1007/s10048-014-0421-1

Al-Obeidi, E., Al-Tahan, S., Surampalli, A., Goyal, N., Wang, A. K., Hermann, A., et al. (2018). Genotype-phenotype study in patients with valosin-containing protein mutations associated with multisystem proteinopathy. *Clin. Genet.* 93, 119–125. doi: 10.1111/cge.13095

An, H., Skelt, L., Notaro, A., Highley, J. R., Fox, A. H., La Bella, V., et al. (2019). ALS-linked FUS mutations confer loss and gain of function in the nucleus by promoting excessive formation of dysfunctional paraspeckles. *Acta Neuropathol. Commun.* 7:7. doi: 10.1186/s40478-019-0658-x

Anderson, C. J., Bredvik, K., Burstein, S. R., Davis, C., Meadows, S. M., Dash, J., et al. (2019). ALS/FTD mutant CHCHD10 mice reveal a tissue-specific toxic gain-of-function and mitochondrial stress response. *Acta Neuropathol.* 138, 103–121. doi: 10.1007/s00401-019-01989-y

Arai, T., Nonaka, T., Hasegawa, M., Akiyama, H., Yoshida, M., Hashizume, Y., et al. (2003). Neuronal and glial inclusions in frontotemporal dementia with or without motor neuron disease are immunopositive for p62. *Neurosci. Lett.* 342, 41–44.

Arhzaouy, K., Papadopoulos, C., Schulze, N., Pittman, S. K., Meyer, H., and Weihl, C. C. (2019). VCP maintains lysosomal homeostasis and TFEB activity in differentiated skeletal muscle. *Autophagy* 15, 1082–1099. doi: 10.1080/15548627.2019.1569933

Armstrong, R. A. (2017). Neuronal cytoplasmic inclusions in tau, TDP-43, and FUS molecular subtypes of frontotemporal lobar degeneration share similar spatial patterns. *Folia Neuropathol.* 55, 185–192. doi: 10.5114/fn.2017.70482

Arthur, K. C., Calvo, A., Price, T. R., Geiger, J. T., Chio, A., and Traynor, B. J. (2016). Projected increase in amyotrophic lateral sclerosis from 2015 to 2040. *Nat. Commun.* 7:12408. doi: 10.1038/ncomms12408

Arzberger, T., Schludi, M. H., Lehmer, C., Schmid, B., and Edbauer, D. (2018). RNA versus protein toxicity in C9orf72 ALS/FTLD. *Acta Neuropathol.* 135, 475–479.

Bandres-Ciga, S., Noyce, A. J., Hemani, G., Nicolas, A., Calvo, A., Mora, G., et al. (2019). Shared polygenic risk and causal inferences in amyotrophic lateral sclerosis. *Ann. Neurol.* 85, 470–481. doi: 10.1002/ana.25431

Bannwarth, S., Ait-El-Mkadem, S., Chaussenot, A., Genin, E. C., Lacas-Gervais, S., Fragaki, K., et al. (2014). A mitochondrial origin for frontotemporal dementia and amyotrophic lateral sclerosis through CHCHD10 involvement. *Brain* 137(Pt 8), 2329–2345. doi: 10.1093/brain/awu138

Bastola, P., Bilkis, R., De Souza, C., Minn, K., and Chien, J. (2019). Heterozygous mutations in valosin-containing protein (VCP) and resistance to VCP inhibitors. *Sci. Rep.* 9:11002. doi: 10.1038/s41598-019-47085-9

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

Berry, J., Brangwynne, C. P., and Haataja, M. (2018). Physical principles of intracellular organization via active and passive phase transitions. *Rep. Prog. Phys.* 81:046601. doi: 10.1088/1361-6633/aaa61e

Bird, T. D., Nochlin, D., Poorkaj, P., Cherrier, M., Kaye, J., Payami, H., et al. (1999). A clinical pathological comparison of three families with frontotemporal dementia and identical mutations in the tau gene (P301L). *Brain* 122(Pt 4), 741–756.

Bodansky, A., Kim, J. M., Tempest, L., Velagapudi, A., Libby, R., and Ravits, J. (2010). TDP-43 and ubiquitinated cytoplasmic aggregates in sporadic ALS are low frequency and widely distributed in the lower motor neuron columns independent of disease spread. *Amyotroph. Lateral. Scler.* 11, 321–327. doi: 10.3109/17482961003602363

Bonnard, M., Mirtsos, C., Suzuki, S., Graham, K., Huang, J., Ng, M., et al. (2000). Deficiency of T2K leads to apoptotic liver degeneration and impaired NF-kappaB-dependent gene transcription. *EMBO J.* 19, 4976–4985.

Brenner, D., Sieverding, K., Bruno, C., Luningschror, P., Buck, E., Mungwa, S., et al. (2019). Heterozygous Tbk1 loss has opposing effects in early and late stages of ALS in mice. *J. Exp. Med.* 216, 267–278. doi: 10.1084/jem.20180729

Buratti, E., and Baralle, F. E. (2008). Multiple roles of TDP-43 in gene expression, splicing regulation, and human disease. *Front Biosci.* 13:867–878.

Buratti, E., and Baralle, F. E. (2012). TDP-43: gumming up neurons through protein-protein and protein-RNA interactions. *Trends Biochem. Sci.* 37, 237–247. doi: 10.1016/j.tibs.2012.03.003

Buratti, E., Dork, T., Zuccato, E., Pagani, F., Romano, M., and Baralle, F. E. (2001). Nuclear factor TDP-43 and SR proteins promote in vitro and in vivo CFTR exon 9 skipping. *EMBO J.* 20, 1774–1784.

Burrell, J. R., Kiernan, M. C., Vucic, S., and Hodges, J. R. (2011). Motor neuron dysfunction in frontotemporal dementia. *Brain* 134(Pt 9), 2582–2594. doi: 10.1093/brain/awr195

Burstein, S. R., Valsecchi, F., Kawamata, H., Bourens, M., Zeng, R., Zuberi, A., et al. (2018). In vitro and in vivo studies of the ALS-FTLD protein CHCHD10 reveal novel mitochondrial topology and protein interactions. *Hum. Mol. Genet.* 27, 160–177. doi: 10.1093/hmg/ddx397

Chaussenot, A., Le Ber, I., Ait-El-Mkadem, S., Camuzat, A., de Septenville, A., Bannwarth, S., et al. (2014). Screening of CHCHD10 in a French cohort confirms the involvement of this gene in frontotemporal dementia with amyotrophic lateral sclerosis patients. *Neurobiol. Aging* 35, e1–e4. doi: 10.1016/j.neurobiolaging.2014.07.022

Chio, A., Logroscino, G., Traynor, B. J., Collins, J., Simeone, J. C., Goldstein, L. A., et al. (2013). Global epidemiology of amyotrophic lateral sclerosis: a systematic review of the published literature. *Neuroepidemiology* 41, 118–130. doi: 10.1159/000351153

Choi, S. Y., Lopez-Gonzalez, R., Krishnan, G., Phillips, H. L., Li, A. N., Seeley, W. W., et al. (2019). C9ORF72-ALS/FTD-associated poly(GR) binds Atp5a1 and compromises mitochondrial function in vivo. *Nat. Neurosci.* 22, 851–862. doi: 10.1038/s41593-019-0397-0

Cirulli, E. T., Lasseigne, B. N., Petrovski, S., Sapp, P. C., Dion, P. A., Leblond, C. S., et al. (2015). Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. *Science* 347, 1436–1441. doi: 10.1126/science.aaa3650

Cleveland, D. W., and Rothstein, J. D. (2001). From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. *Nat. Rev. Neurosci.* 2, 806–819.

Conicella, A. E., Zerze, G. H., Mittal, J., and Fawzi, N. L. (2016). ALS mutations disrupt phase separation mediated by alpha-helical structure in the TDP-43 low-complexity C-terminal domain. *Structure* 24, 1537–1549. doi: 10.1016/j.str.2016.07.007

Cozzolino, M., Rossi, S., Mirra, A., and Carri, M. T. (2015). Mitochondrial dynamism and the pathogenesis of Amyotrophic Lateral Sclerosis. *Front. Cell Neurosci.* 9:31. doi: 10.3389/fncel.2015.00031

Davidson, Y., Kelley, T., Mackenzie, I. R., Pickering-Brown, S., Du Plessis, D., Neary, D., et al. (2007). Ubiquitinated pathological lesions in frontotemporal lobar degeneration contain the TAR DNA-binding protein. TDP-43. *Acta Neuropathol.* 113, 521–533.

de Majo, M., Topp, S. D., Smith, B. N., Nishimura, A. L., Chen, H. J., Gkazi, A. S., et al. (2018). ALS-associated missense and nonsense TBK1 mutations can both cause loss of kinase function. *Neurobiol. Aging* 71, e1–e10. doi: 10.1016/j.neurobiolaging.2018.06.015

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* 2011, 245–256. doi: 10.1016/j.neuron.2011.09.011

Deng, Z., Lim, J., Wang, Q., Purtell, K., Wu, S., Palomo, G. M., et al. (2019). ALS-FTLD-linked mutations of SQSTM1/p62 disrupt selective autophagy and NFE2L2/NRF2 anti-oxidative stress pathway. *Autophagy* doi: 10.1080/15548627.2019.1644076 [Epub ahead of print],

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

Devoy, A., Kalmar, B., Stewart, M., Park, H., Burke, B., Noy, S. J., et al. (2017). Humanized mutant FUS drives progressive motor neuron degeneration without aggregation in 'FUSDelta14' knockin mice. *Brain* 140, 2797–2805. doi: 10.1093/brain/awx248

Duan, W., Guo, M., Yi, L., Zhang, J., Bi, Y., Liu, Y., et al. (2019). Deletion of Tbk1 disrupts autophagy and reproduces behavioral and locomotor symptoms of FTD-ALS in mice. *Aging* 11, 2457–2476. doi: 10.18632/aging.101936

Ebstein, S. Y., Yagudayeva, I., and Shneider, N. A. (2019). Mutant TDP-43 causes early-stage dose-dependent motor neuron degeneration in a tardbp knockin mouse model of ALS. *Cell Rep* 26:364-373e4. doi: 10.1016/j.celrep.2018.12.045

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

Fiesel, F. C., Voigt, A., Weber, S. S., Van den Haute, C., Waldenmaier, A., Gorner, K., et al. (2010). Knockdown of transactive response DNA-binding protein (TDP-43) downregulates histone deacetylase 6. *EMBO J.* 29, 209–221. doi: 10.1038/emboj.2009.324

Foster, A., Scott, D., Layfield, R., and Rea, S. L. (2019). An FTLD-associated SQSTM1 variant impacts Nrf2 and NF-kappaB signalling and is associated with reduced phosphorylation of p62. *Mol. Cell Neurosci.* 98, 32–45. doi: 10.1016/j.mcn.2019.04.001

Fratta, P., Sivakumar, P., Humphrey, J., Lo, K., Ricketts, T., Oliveira, H., et al. (2018). Mice with endogenous TDP-43 mutations exhibit gain of splicing function and characteristics of amyotrophic lateral sclerosis. *EMBO J.* 37:e98684. doi: 10.15252/embj.201798684

Freibaum, B. D., Lu, Y., Lopez-Gonzalez, R., Kim, N. C., Almeida, S., Lee, K. H., et al. (2015). GGGGCC repeat expansion in C9orf72 compromises nucleocytoplasmic transport. *Nature* 525, 129–133. doi: 10.1038/nature14974

Freibaum, B. D., and Taylor, J. P. (2017). The role of dipeptide repeats in C9ORF72-related ALS-FTD. *Front. Mol. Neurosci.* 10:35. doi: 10.3389/fnmol.2017.00035

Freischmidt, A., Wieland, T., Richter, B., Ruf, W., Schaeffer, V., Muller, K., et al. (2015). Haploinsufficiency of TBK1 causes familial ALS and fronto-temporal dementia. *Nat. Neurosci.* 18, 631–636. doi: 10.1038/nn.4000

Ganji, R., Mukkavalli, S., Somanji, F., and Raman, M. (2018). The VCP-UBXN1 complex mediates triage of ubiquitylated cytosolic proteins bound to the BAG6 complex. *Mol. Cell Biol.* 38:MCB.00154-118. doi: 10.1128/MCB.00154-18

Gao, J., Perera, G., Bhadbhade, M., Halliday, G. M., and Dzamko, N. (2019). Autophagy activation promotes clearance of alpha-synuclein inclusions in fibril-seeded human neural cells. *J. Biol. Chem.* 294, 14241–14256. doi: 10.1074/jbc.RA119.008733

Genin, E. C., Madji Hounoum, B., Bannwarth, S., Fragaki, K., Lacas-Gervais, S., Mauri-Crouzet, A., et al. (2019). Mitochondrial defect in muscle precedes neuromuscular junction degeneration and motor neuron death in CHCHD10(S59L/+) mouse. *Acta Neuropathol.* 138, 123–145. doi: 10.1007/s00401-019-01988-z

Hao, Z., Liu, L., Tao, Z., Wang, R., Ren, H., Sun, H., et al. (2019). Motor dysfunction and neurodegeneration in a C9orf72 mouse line expressing poly-PR. *Nat. Commun.* 10:2906. doi: 10.1038/s41467-019-10956-w

Hensman Moss, D. J., Poulter, M., Beck, J., Hehir, J., Polke, J. M., Campbell, T., et al. (2014). C9orf72 expansions are the most common genetic cause of Huntington disease phenocopies. *Neurology* 82, 292–299. doi: 10.1212/WNL.0000000000000061

Hergesheimer, R. C., Chami, A. A., de Assis, D. R., Vourc'h, P., Andres, C. R., Corcia, P., et al. (2019). The debated toxic role of aggregated TDP-43 in amyotrophic lateral sclerosis: a resolution in sight? *Brain* 142, 1176–1194. doi: 10.1093/brain/awz078

Herhaus, L., and Dikic, I. (2018). Ubiquitin-induced phase separation of p62/SQSTM1. *Cell Res.* 28, 389–390.

Ho, W. Y., and Ling, S. C. (2019). Elevated FUS levels by overriding its autoregulation produce gain-of-toxicity properties that disrupt protein and RNA homeostasis. *Autophagy* 15, 1665–1667. doi: 10.1080/15548627.2019.1633162

Hu, Y. W., Zhang, J., Wu, X. M., Cao, L., Nie, P., and Chang, M. X. (2018). TANK-binding kinase 1 (TBK1) isoforms negatively regulate type I Interferon Induction by inhibiting TBK1-IRF3 interaction and IRF3 phosphorylation. *Front. Immunol.* 9:84. doi: 10.3389/fimmu.2018.00084

Huey, E. D., Ferrari, R., Moreno, J. H., Jensen, C., Morris, C. M., Potocnik, F., et al. (2012). FUS and TDP43 genetic variability in FTD and CBS. *Neurobiol. Aging* 33, e9–e17. doi: 10.1016/j.neurobiolaging.2011.08.004

Ishigaki, S., and Sobue, G. (2018). Importance of functional loss of FUS in FTLD/ALS. *Front. Mol. Biosci.* 5:44. doi: 10.3389/fmolb.2018.00044

Jiang, J., Zhu, Q., Gendron, T. F., Saberi, S., McAlonis-Downes, M., Seelman, A., et al. (2016). Gain of toxicity from ALS/FTD-linked repeat expansions in C9ORF72 Is alleviated by antisense oligonucleotides targeting GGGGCC-containing RNAs. *Neuron* 90, 535–550. doi: 10.1016/j.neuron.2016.04.006

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

Jovicic, A., Mertens, J., Boeynaems, S., Bogaert, E., Chai, N., Yamada, S. B., et al. (2015). Modifiers of C9orf72 dipeptide repeat toxicity connect nucleocytoplasmic transport defects to FTD/ALS. *Nat. Neurosci.* 18, 1226–1229. doi: 10.1038/nn.4085

Jung, K. T., and Oh, S. H. (2019). Polyubiquitination of p62/SQSTM1 is a prerequisite for Fas/CD95 aggregation to promote caspase-dependent apoptosis in cadmium-exposed mouse monocyte RAW264.7 cells. *Sci. Rep.* 9:12240. doi: 10.1038/s41598-019-48684-2

Kang, J., Lim, L., Lu, Y., and Song, J. (2019). A unified mechanism for LLPS of ALS/FTLD-causing FUS as well as its modulation by ATP and oligonucleic acids. *PLoS Biol.* 17:e3000327. doi: 10.1371/journal.pbio.3000327

Khalfallah, Y., Kuta, R., Grasmuck, C., Prat, A., Durham, H. D., and Vande Velde, C. (2018). TDP-43 regulation of stress granule dynamics in neurodegenerative disease-relevant cell types. *Sci. Rep.* 8:7551. doi: 10.1038/s41598-018-25767-0

Kino, Y., Washizu, C., Kurosawa, M., Yamada, M., Miyazaki, H., Akagi, T., et al. (2015). FUS/TLS deficiency causes behavioral and pathological abnormalities distinct from amyotrophic lateral sclerosis. *Acta Neuropathol. Commun.* 3:24. doi: 10.1186/s40478-015-0202-6

Koppers, M., Blokhuis, A. M., Westeneng, H. J., Terpstra, M. L., Zundel, C. A., Vieira de Sa, R., et al. (2015). C9orf72 ablation in mice does not cause motor neuron degeneration or motor deficits. *Ann. Neurol.* 78, 426–438. doi: 10.1002/ana.24453

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

Kwon, J., Han, E., Bui, C. B., Shin, W., Lee, J., Lee, S., et al. (2012). Assurance of mitochondrial integrity and mammalian longevity by the p62-Keap1-Nrf2-Nqo1 cascade. *EMBO Rep.* 13, 150–156. doi: 10.1038/embor.2011.246

Lagier-Tourenne, C., Polymenidou, M., and Cleveland, D. W. (2010). TDP-43 and FUS/TLS: emerging roles in RNA processing and neurodegeneration. *Hum. Mol. Genet.* 19, R46–R64. doi: 10.1093/hmg/ddq137

Lamb, R., Rohrer, J. D., Real, R., Lubbe, S. J., Waite, A. J., Blake, D. J., et al. (2019). A novel TBK1 mutation in a family with diverse frontotemporal dementia spectrum disorders. *Cold Spring Harb. Mol. Case Stud.* 5:a003913. doi: 10.1101/mcs.a003913

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.

Li, F., Xie, X., Wang, Y., Liu, J., Cheng, X., Guo, Y., et al. (2016). Structural insights into the interaction and disease mechanism of neurodegenerative disease-associated optineurin and TBK1 proteins. *Nat. Commun.* 7:12708. doi: 10.1038/ncomms12708

Ling, S. C., Dastidar, S. G., Tokunaga, S., Ho, W. Y., Lim, K., Ilieva, H., et al. (2019). Overriding FUS autoregulation in mice triggers gain-of-toxic dysfunctions in RNA metabolism and autophagy-lysosome axis. *eLife* 8:e40811. doi: 10.7554/eLife.40811

Ludtmann, M. H., Arber, C., Bartolome, F., de Vicente, M., Preza, E., Carro, E., et al. (2017). Mutations in valosin-containing protein (VCP) decrease ADP/ATP translocation across the mitochondrial membrane and impair energy metabolism in human neurons. *J. Biol. Chem.* 292, 8907–8917. doi: 10.1074/jbc.M116.762898

Mackenzie, I. R., Rademakers, R., and Neumann, M. (2010). TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia. *Lancet Neurol.* 9, 995–1007. doi: 10.1016/S1474-4422(10)70195-2

May, S., Hornburg, D., Schludi, M. H., Arzberger, T., Rentzsch, K., Schwenk, B. M., et al. (2014). C9orf72 FTLD/ALS-associated Gly-Ala dipeptide repeat proteins cause neuronal toxicity and Unc119 sequestration. *Acta Neuropathol.* 128, 485–503. doi: 10.1007/s00401-014-1329-4

Miller, R. G., Mitchell, J. D., and Moore, D. H. (2012). Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Cochrane Database Syst. Rev.* 3, CD001447.

Mitra, J., Guerrero, E. N., Hegde, P. M., Liachko, N. F., Wang, H., Vasquez, V., et al. (2019). Motor neuron disease-associated loss of nuclear TDP-43 is linked

to DNA double-strand break repair defects. *Proc. Natl. Acad. Sci. U.S.A.* 116, 4696–4705. doi: 10.1073/pnas.1818415116

Mitsui, S., Otomo, A., Nozaki, M., Ono, S., Sato, K., Shirakawa, R., et al. (2018). Systemic overexpression of SQSTM1/p62 accelerates disease onset in a SOD1(H46R)-expressing ALS mouse model. *Mol. Brain* 11:30. doi: 10.1186/s13041-018-0373-8

Morton, S., Hesson, L., Peggie, M., and Cohen, P. (2008). Enhanced binding of TBK1 by an optineurin mutant that causes a familial form of primary open angle glaucoma. *FEBS Lett.* 582, 997–1002. doi: 10.1016/j.febslet.2008.02.047

Murthy, A. C., Dignon, G. L., Kan, Y., Zerze, G. H., Parekh, S. H., Mittal, J., et al. (2019). Molecular interactions underlying liquid-liquid phase separation of the FUS low-complexity domain. *Nat. Struct. Mol. Biol.* 26, 637–648. doi: 10.1038/s41594-019-0250-x

Nalbandian, A., Khan, A. A., Srivastava, R., Llewellyn, K. J., Tan, B., Shukr, N., et al. (2017). Activation of the NLRP3 inflammasome is associated with valosin-containing protein myopathy. *Inflammation* 40, 21–41. doi: 10.1007/s10753-016-0449-5

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.

Niaki, A. G., Sarkar, J., Cai, X., Rhine, K., Vidaurre, V., Guy, B., et al. (2019). Loss of dynamic RNA interaction and aberrant phase separation induced by two distinct types of ALS/FTD-Linked FUS mutations. *Mol. Cell* 77:82-94.e4. doi: 10.1016/j.molcel.2019.09.022

Ogura, T., and Wilkinson, A. J. (2001). AAA+ superfamily ATPases: common structure–diverse function. *Genes Cells* 6, 575–597.

O'Rourke, J. G., Bogdanik, L., Muhammad, A. K., Gendron, T. F., Kim, K. J., Austin, A., et al. (2015). C9orf72 BAC transgenic mice display typical pathologic features of ALS/FTD. *Neuron* 88, 892–901. doi: 10.1016/j.neuron.2015.10.027

Park, S., Zuber, C., and Roth, J. (2019). Selective autophagy of cytosolic protein aggregates involves ribosome-free rough endoplasmic reticulum. *Histochem. Cell Biol.* doi: 10.1007/s00418-019-01829-w [Epub ahead of print],

Perrone, F., Nguyen, H. P., Van Mossevelde, S., Moisse, M., Sieben, A., Santens, P., et al. (2017). Investigating the role of ALS genes CHCHD10 and TUBA4A in Belgian FTD-ALS spectrum patients. *Neurobiol. Aging.* 51, e9–e16. doi: 10.1016/j.neurobiolaging.2016.12.008

Perry, A. K., Chow, E. K., Goodnough, J. B., Yeh, W. C., and Cheng, G. (2004). Differential requirement for TANK-binding kinase-1 in type I interferon responses to toll-like receptor activation and viral infection. *J. Exp. Med.* 199, 1651–1658.

Peters, O. M., Cabrera, G. T., Tran, H., Gendron, T. F., McKeon, J. E., Metterville, J., et al. (2015). Human C9ORF72 hexanucleotide expansion reproduces RNA Foci and dipeptide repeat proteins but not neurodegeneration in BAC transgenic mice. *Neuron* 88, 902–909. doi: 10.1016/j.neuron.2015.11.018

Plewa, J., Surampalli, A., Wencel, M., Milad, M., Donkervoort, S., Caiozzo, V. J., et al. (2018). A cross-sectional analysis of clinical evaluation in 35 individuals with mutations of the valosin-containing protein gene. *Neuromuscul. Disord.* 28, 778–786. doi: 10.1016/j.nmd.2018.06.007

Ratnavalli, E., Brayne, C., Dawson, K., and Hodges, J. R. (2002). The prevalence of frontotemporal dementia. *Neurology* 58, 1615–1621.

Rea, S. L., Walsh, J. P., Layfield, R., Ratajczak, T., and Xu, J. (2013). New insights into the role of sequestosome 1/p62 mutant proteins in the pathogenesis of Paget's disease of bone. *Endocr. Rev.* 34, 501–524. doi: 10.1210/er.2012-1034

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

Roczniak-Ferguson, A., and Ferguson, S. M. (2019). Pleiotropic requirements for human TDP-43 in the regulation of cell and organelle homeostasis. *bioRxiv [Preprint]* doi: 10.26508/lsa.20190035

Rothstein, J. D. (2017). Edaravone: a new drug approved for ALS. *Cell* 171:725. doi: 10.1016/j.cell.2017.10.011

Ryzhakov, G., and Randow, F. (2007). SINTBAD, a novel component of innate antiviral immunity, shares a TBK1-binding domain with NAP1 and TANK. *EMBO J.* 26, 3180–3190.

Saracino, D., Clot, F., Camuzat, A., Anquetil, V., Hannequin, D., Guyant-Marechal, L., et al. (2018). Novel VCP mutations expand the mutational spectrum of frontotemporal dementia. *Neurobiol. Aging* 72, e11–e14.

Scekic-Zahirovic, J., Sendscheid, O., El Oussini, H., Jambeau, M., Sun, Y., Mersmann, S., et al. (2016). Toxic gain of function from mutant FUS protein is crucial to trigger cell autonomous motor neuron loss. *EMBO J.* 35, 1077–1097. doi: 10.15252/embj.201592559

Shang, Y., and Huang, E. J. (2016). Mechanisms of FUS mutations in familial amyotrophic lateral sclerosis. *Brain Res.* 1647, 65–78. doi: 10.1016/j.brainres.2016.03.036

Shao, Q., Liang, C., Chang, Q., Zhang, W., Yang, M., and Chen, J. F. (2019). C9orf72 deficiency promotes motor deficits of a C9ALS/FTD mouse model in a dose-dependent manner. *Acta Neuropathol. Commun.* 7:32.

Shi, Y., Lin, S., Staats, K. A., Li, Y., Chang, W. H., Hung, S. T., et al. (2018). Haploinsufficiency leads to neurodegeneration in C9ORF72 ALS/FTD human induced motor neurons. *Nat. Med.* 24, 313–325. doi: 10.1038/nm.4490

Silva, P. R., Nieva, G. V., and Igaz, L. M. (2019). Suppression of conditional TDP-43 transgene expression differentially affects early cognitive and social phenotypes in TDP-43 Mice. *Front. Genet.* 10:369. doi: 10.3389/fgene.2019.00369

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

Strong, M. J., Volkening, K., Hammond, R., Yang, W., Strong, W., Leystra-Lantz, C., et al. (2007). TDP43 is a human low molecular weight neurofilament (hNFL) mRNA-binding protein. *Mol. Cell Neurosci.* 35, 320–327.

Sun, D., Wu, R., Zheng, J., Li, P., and Yu, L. (2018). Polyubiquitin chain-induced p62 phase separation drives autophagic cargo segregation. *Cell Res.* 28, 405–415. doi: 10.1038/s41422-018-0017-7

Tan, R. H., Yang, Y., Kim, W. S., Dobson-Stone, C., Kwok, J. B., Kiernan, M. C., et al. (2017). Distinct TDP-43 inclusion morphologies in frontotemporal lobar degeneration with and without amyotrophic lateral sclerosis. *Acta Neuropathol. Commun.* 5:76. doi: 10.1186/s40478-017-0480-2

Taylor, J. P., Brown, R. H. Jr., and Cleveland, D. W. (2016). Decoding ALS: from genes to mechanism. *Nature* 539, 197–206. doi: 10.1038/nature20413

Teyssou, E., Takeda, T., Lebon, V., Boillee, S., Doukoure, B., Bataillon, G., et al. (2013). Mutations in SQSTM1 encoding p62 in amyotrophic lateral sclerosis: genetics and neuropathology. *Acta Neuropathol.* 125, 511–522.

Tsai, R. M., and Boxer, A. L. (2014). Treatment of frontotemporal dementia. *Curr. Treat. Options Neurol.* 16:319. doi: 10.1007/s11940-014-0319-0

Twomey, E. C., Ji, Z., Wales, T. E., Bodnar, N. O., Ficarro, S. B., Marto, J. A., et al. (2019). Substrate processing by the Cdc48 ATPase complex is initiated by ubiquitin unfolding. *Science* 365:1033. doi: 10.1126/science.aax1033

Uhlen, M., Fagerberg, L., Hallstrom, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., et al. (2015). Proteomics. Tissue-based map of the human proteome. *Science* 347:1260419. doi: 10.1126/science.1260419

Van Langenhove, T., van der Zee, J., Sleegers, K., Engelborghs, S., Vandenberghe, R., Gijselinck, I., et al. (2010). Genetic contribution of FUS to frontotemporal lobar degeneration. *Neurology* 74, 366–371. doi: 10.1212/WNL.0b013e3181ccc732

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(Pt 2), 452–467. doi: 10.1093/brain/awv358

van Rheenen, W., Shatunov, A., Dekker, A. M., McLaughlin, R. L., Diekstra, F. P., Pulit, S. L., et al. (2016). Genome-wide association analyses identify new risk variants and the genetic architecture of amyotrophic lateral sclerosis. *Nat. Genet.* 48, 1043–1048. doi: 10.1038/ng.3622

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

Wang, R., Yang, B., and Zhang, D. (2011). Activation of interferon signaling pathways in spinal cord astrocytes from an ALS mouse model. *Glia* 59, 946–958. doi: 10.1002/glia.21167

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.

Weidberg, H., and Elazar, Z. (2011). TBK1 mediates crosstalk between the innate immune response and autophagy. *Sci. Signal.* 4:e39. doi: 10.1126/scisignal.2002355

Weinreich, M., Shepheard, S. R., Verber, N., Wyles, M., Heath, P. R., Highley, J. R., et al. (2019). Neuropathological characterization of a novel TANK binding kinase (TBK1) gene loss of function mutation associated with amyotrophic lateral sclerosis. *Neuropathol. Appl. Neurobiol.* doi: 10.1111/nan.12578 [Epub ahead of print].

Weishaupt, J. H., Hyman, T., and Dikic, I. (2016). Common molecular pathways in amyotrophic lateral sclerosis and frontotemporal dementia. *Trends Mol. Med.* 22, 769–783. doi: 10.1016/j.molmed.2016.07.005

White, M. A., Kim, E., Duffy, A., Adalbert, R., Phillips, B. U., Peters, O. M., et al. (2018a). Publisher Correction: TDP-43 gains function due to perturbed autoregulation in a Tardbp knock-in mouse model of ALS-FTD. *Nat. Neurosci.* 21:1138. doi: 10.1038/s41593-018-0160-y

White, M. A., Kim, E., Duffy, A., Adalbert, R., Phillips, B. U., Peters, O. M., et al. (2018b). TDP-43 gains function due to perturbed autoregulation in a Tardbp knock-in mouse model of ALS-FTD. *Nat. Neurosci.* 21, 552–563.

Xiao, Y., Zhang, J., Shu, X., Bai, L., Xu, W., Wang, A., et al. (2019). Loss of mitochondrial protein CHCHD10 in skeletal muscle causes neuromuscular junction impairment. *Hum. Mol. Genet.* doi: 10.1093/hmg/ddz154 [Epub ahead of print].

Yang, Y., Willis, T. L., Button, R. W., Strang, C. J., Fu, Y., Wen, X., et al. (2019). Cytoplasmic DAXX drives SQSTM1/p62 phase condensation to activate Nrf2-mediated stress response. *Nat. Commun.* 10:3759. doi: 10.1038/s41467-019-11671-2

Zaffagnini, G., Savova, A., Danieli, A., Romanov, J., Tremel, S., Ebner, M., et al. (2018). Phasing out the bad-How SQSTM1/p62 sequesters ubiquitinated proteins for degradation by autophagy. *Autophagy* 14, 1280–1282. doi: 10.1080/15548627.2018.1462079

Zhang, K., Donnelly, C. J., Haeusler, A. R., Grima, J. C., Machamer, J. B., Steinwald, P., et al. (2015). The C9orf72 repeat expansion disrupts nucleocytoplasmic transport. *Nature* 525, 56–61. doi: 10.1038/nature14973

Zhang, Y. J., Gendron, T. F., Ebbert, M. T. W., O'Raw, A. D., Yue, M., Jansen-West, K., et al. (2018). Poly(GR) impairs protein translation and stress granule dynamics in C9orf72-associated frontotemporal dementia and amyotrophic lateral sclerosis. *Nat. Med.* 24, 1136–1142. doi: 10.1038/s41591-018-0071-1

Zhou, Z. D., Saw, W. T., and Tan, E. K. (2017). Mitochondrial CHCHD-containing proteins: physiologic functions and link with neurodegenerative diseases. *Mol. Neurobiol.* 54, 5534–5546. doi: 10.1007/s12035-016-0099-5

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Clinical Phenotype and Inheritance in Patients With C9ORF72 Hexanucleotide Repeat Expansion: Results From a Large French Cohort

*Florence Esselin[1†], Kevin Mouzat[2†], Anne Polge[2], Raul Juntas-Morales[1], Nicolas Pageot[1], Elisa De la Cruz[1], Emilien Bernard[3], Emmeline Lagrange[4], Véronique Danel[5], Sébastien Alphandery[1], Laura Labar[1], Erika Nogué[6], Marie-Christine Picot[7], Serge Lumbroso[2] and William Camu[1]**

[1] Centre de référence SLA, CHU Gui de Chauliac, Montpellier, France, [2] Laboratoire de Biochimie et Biologie Moléculaire, CHU Nimes, Univ. Montpellier, Nimes, France, [3] Département de Neurologie, Centre SLA, CHU Wertheimer, Lyon, France, [4] EFSN, CHU Grenoble Alpes, Grenoble, France, [5] Centre de référence SLA, CHU Salengro, Lille, France, [6] CHU Montpellier, Unité de Recherche Clinique et Epidémiologie (Département Information Médicale), INSERM, Montpellier, France, [7] CHU Montpellier, Unité de Recherche Clinique et Epidémiologie (Département Information Médicale), INSERM, Centre d'Investigation Clinique 1411, Univ Montpellier, Montpellier, France

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***Correspondence:**
William Camu
w-camu@chu-montpellier.fr

[†] These authors have contributed equally to this work

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Background: In familial amyotrophic lateral sclerosis (ALS) cases, the presence of an abnormal C9ORF72 repeat expansion (C9RE) is the most frequent genetic cause identified. Various clinical phenotypes have been described in relation to the presence of C9RE, including psychiatric disorders or Huntington-like symptoms. In a subset of sporadic ALS, C9RE has also been described. In the present study, all index cases with ALS and C9RE identified in our center and their clinical profile, as well as neurological and psychiatric characteristics of identified family members, were described. Clinical characteristics of ALS patients were compared to 999 patients with sporadic ALS (SALS) from our database.

Results: From the 70 index cases with ALS identified, a total of 200 individuals were studied, 118 with ALS, 32 with fronto-temporal lobe degeneration (FTD), 37 with ALS/FTD, and 13 with psychiatric disorders. A familial history was present in 57 of the index cases (81%). In ALS and ALS/FTD cases with C9RE, the age of onset (AoO) was earlier than that in SALS cases, $p < 0.0001$ and $p = 0.008$, respectively. Sporadic cases with C9REALS ($n = 13$) had an earlier AoO compared to familial C9REALS ones, $p < 0.0001$. Within families, there was an earlier AoO in index cases and their siblings compared to their parental generation ($p < 0.01$). There was also a significant intrafamilial correlation for bulbar onset of ALS. The parental generation had significant female predominance compared to index cases and their siblings (sex ratio 0.47 vs. 1.4, $p = 0.004$), and this predominance was also present when considering parent–child pairs. In the group with psychiatric disorders, suicide was prominent ($n = 9$) and mean age was 54 years.

Conclusion: Although our sample size is rather limited, the earlier AoO in index cases and their siblings compared to the parental generation may suggest an anticipation. Reasons for predominance of female transmission are unclear, but the hypothesis that gender influences transmission of the genetic trait or C9RE size variation may be taken into account. Intrafamilial correlation suggests that genetic aspects underlie the occurrence of bulbar onset in ALS patients. Studies on larger samples are warranted to confirm those results.

Keywords: ALS, C9ORF72, clinical phenotype, familial, cohort

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by degeneration of upper and lower motoneurons, leading to death in a median time of 3 years after onset (Hardiman et al., 2011). The disease is inherited in 5–10% of the cases, and several causal mutations have been described (Volk et al., 2018). The most frequent one is the C9ORF72 repeat expansion (C9RE) that accounts for 30–50% of the familial cases in European and American countries, while the expansion is rare in Asia (DeJesus-Hernandez et al., 2011; Renton et al., 2011; Majounie et al., 2012). This expansion is now also recognized as the most frequent cause of familial fronto-temporal lobe degeneration (FTD). The clinical spectrum of C9RE cases may also extend to ALS/FTD and potentially to atypical parkinsonism, Huntington-like disease, or psychiatric disorders (Byrne et al., 2013; Cooper-Knock et al., 2014). The frequency as well as the clinical profile of ALS with the C9RE seem to be rather homogeneous, with a predominant bulbar onset, an age of onset of 58 years, and a mean disease duration shorter than for sporadic cases (Majounie et al., 2012; Snowden et al., 2013; Cooper-Knock et al., 2014; Umoh et al., 2016; Trojsi et al., 2019). Conversely, the other phenotypes associated with C9RE are not well defined and some remain in dispute (Ticozzi et al., 2014; Solje et al., 2016; Devenney et al., 2018; Martins et al., 2018; Sellami et al., 2019; Silverman et al., 2019). The proportion of ALS/FTD varies from 6 to 50%, reports of atypical phenotypes are scarce, and physicians are lacking a systematic study to help clarify the exact spectrum linked to C9RE. The difficulty is reinforced by the existence of C9RE in ∼3% of apparently sporadic cases and by an incomplete and age-related penetrance in both familial and sporadic cases (Majounie et al., 2012; Murphy et al., 2017; Volk et al., 2018). Incomplete familial enquiry might lead to potential underestimation of familial cases, as well as the existence of phenotypes not yet recognized as part of the disease spectrum, and difficulties asserting diagnosis. Improving knowledge in the phenotype of patients with C9RE is of clinical importance all the more as future gene therapies are being developed for treating patients. We thus undertook a systematic phenotypic study of all ALS families recruited in our ALS clinic, and thoroughly described phenotypes of all family members. Our secondary objective was to compare intra- and interfamilial phenotype variability.

METHODS

Patients

All patients and families were identified at the ALS clinic of Montpellier, France. As early as 1996, all FALS cases were collected and patients systematically followed quarterly. Between 1996 and 2012, blood collection was part of a DNA banking stored at Genethon, and all the patients gave written consent for participating to the genetic research. This research had been approved by the ethics committee of Pitié-Salpêtrière hospital. Since 2012, with the description of C9RE, genetic analysis of ALS cases was part of the diagnostic procedure, and all subjects also gave informed consent for genetic testing.

Phenotype Characterization

Clinical profiles of index cases and affected family members have been collected. For each patient, the following information was collected: age at onset, gender, site of onset, ALS functional rating scale–revised (ALSFRS-R) score, disease duration, and presence of dementia (Kimura et al., 2006). Familial inquiry considered relatives with ALS, ALS/FTD, FTD, psychiatric disorder, parkinsonism, and any other potential neurodegenerative disorder. Age at onset, disease duration, and/or age at death were collected for each family member identified, and neurologists as well as psychiatrists who followed a family member were contacted for diagnosis ascertainment.

Genetic Testing

Between 1996 and 2018, all index cases and other family members for whom blood collection was possible were studied for the presence of an abnormal C9RE by the Biochemistry and Molecular Biology department of CHU de Nîmes, as already described (Akimoto et al., 2014). All index cases were also checked for mutations in the main genes responsible for familial ALS: SOD1, TARDBP, and FUS genes. The study of these genes did not show any additional mutation.

Statistical Analysis

The patients' characteristics were reported and compared with data from sporadic ALS patients recorded in our prospective database (n = 999). Quantitative variables (age at onset, age at death, disease duration, ALSFRS-R score) were expressed by their mean ± standard deviation, and the qualitative variable (gender, site of onset) with frequencies and proportions (%) of the different categories.

Comparisons between groups for age of onset, age at death, disease duration, and ALSFRS-R score were done using Student's *t*-test. Gender and sites of onset were compared between groups using chi-squared test. The type I error rate was 0.05. Statistical analysis was performed using XLSTAT statistical and data analysis solution (Addinsoft, Paris, France)[1].

Phenotype similarities within families were expressed by the intracluster correlation coefficient (ICC), which compares the within-group variance to the between-group variance (Killip et al., 2004). Mathematically, it was the between-cluster variability divided by the sum of the within-cluster and between-cluster variabilities. In human studies, it is usually small. The 95% confidence intervals (CIs) of each ICC will be estimated by bootstrap with resampling including the delivery of 1,000 samples. A negative lower bound will be truncated to zero. The ICC associated with 95% CI containing the value 0 will be considered as not significantly different from 0. These tests were performed using SAS enterprise guide 7.13.

RESULTS

Between 1996 and 2018, we identified 70 individuals with ALS referred to our center who were shown to carry abnormal C9RE (index-cases). A familial history of ALS or FTD or both was identified in 57 of them; one family originated from North Africa (Tunisia), with all the others being of European descent. The pedigree study identified a total of 200 individuals with ALS ($n = 118$), FTD ($n = 32$), ALS/FTD ($n = 37$), as well as psychiatric disorders ($n = 13$).

Phenotype of Families

Families with only ALS cases and families with coexistence of ALS cases and FTD cases were the most represented, accounting for 58% of the 57 families (**Table 1**). The number of cases per family was higher in those with a coexistence of the three types of neurodegenerative disorders (4.5) and in families with ALS and ALS/FTD cases (3.4), compared to 2 and 2.6 for ALS/FTD- and ALS-only families, respectively. Psychiatric disorders were noted in almost all of the family types.

[1]https://www.xlstat.com

Patients' Characteristics

A total of 106 individuals out of the 200 patients of this cohort could be studied genetically, and the presence of C9RE was confirmed in all of them. The vast majority of the patients with neurological disorders had ALS ($n = 118$, **Table 2**). Clinical characteristics of these patients had some differences with the 999 sporadic ALS cases from our database, with a female predominance (gender ratio 0.9) compared to 1.25 in SALS, but this was not significant. In ALS patients with C9RE as well as in SALS, upper limb onset was underrepresented and lower limb onset was predominant (43%). Conversely, the age of onset was earlier in C9RE patients than in SALS cases, 57.5 years vs. 65.7 years, which was highly significant ($p < 0.0001$). There was no difference for mean disease duration. Interestingly, except for the gender ratio, ALS/FTD cases were rather similar to ALS patients, and the age of onset was also significantly earlier in ALS/FTD cases with C9RE, $p = 0.008$. When comparison between ALS and ALS/FTD patients was refined according to sites of onset, the only difference was disease duration, which was shorter in ALS/FTD cases (**Table 3**). On the contrary, FTD cases had a later onset and a much longer disease duration than ALS and ALS/FTD cases. There was no phenotype difference between women and men both in ALS and ALSFTD cases.

Apparently Sporadic Cases

A total of 13 patients, 9 with ALS and 4 with ALS/FTD, had no family history of ALS, ALS/FTD, or FTD. For two patients, the pedigree could not be found, and thus it was not possible to conclude precisely about the apparently sporadic nature of their disorder. In three cases, the parents of the patients died before the age of 70 years old, corresponding to a non-informative pedigree. In three patients, both parents were alive with age more than 80 years old. In two cases, one parent died with age between 70 and 80 years old, and the other was alive. In four index cases, psychiatric antecedents were recorded in six persons, including one suicide. Phenotype comparison with cases with familial history was limited due to the size of the samples (**Table 4**). However, the age of onset was significantly earlier in apparently sporadic ALS cases, 45.5 years vs. 58.8 years ($p < 0.0001$), but not in the ALS/FTD group, which was only composed of four patients. Conversely, in ALS/FTD cases, disease duration was shorter but the group was composed of only three patients.

TABLE 1 | Phenotype of families.

	ALS only	ALS/FTD only	ALS and FTD	ALS and ALS/FTD	ALS/FTD and FTD	ALS and ALS/FTD and FTD
Families (n)	19	3	14	10	5	6
ALS	50	–	28	19	–	13
ALS/FTD	–	6	–	15	5	7
FTD	–	–	18	–	6	7
Total	50	6	46	34	11	27
Cases per family	2.63	2	3.28	3.4	2.2	4.5
Psychiatric disorders (n)	4	0	2	3	2	1
Disease types	3 suicides 1 schizophrenia		1 bipolar 1 hypochondria	Suicides	1 suicide 1 unknown	Suicide

TABLE 2 | Characteristics of the population.

	ALS	ALS/FTD	FTD	Sporadic ALS
n	118	37	32	999
Gender: M/F (ratio)	56/62 (0.9)[a]	22/15 (1.46)[a]	14/18 (0.78)	555/444 (1.25)
Bulbar onset (%)	39	36.1	–	35.7
UL onset (%)	18	16.6	–	25.0
LL onset (%)	43	41.7	–	39.3
Dementia onset (%)	–	5.6	–	–
Age at onset	57.5 ± 10.4[b]	60.4 ± 7.0[c]	65.7 ± 9.4	65.7 ± 11.8
n	*94*	*34*	*10*	*999*
Age at death	60.7 ± 10.0	63.7 ± 6.7	72.3 ± 8.5	68.8 ± 11.5
n	*86*	*30*	*13*	*999*
Disease duration	40.3 ± 24.4[a]	30.0 ± 18.2[a]	87.6 ± 35.7	37.2 ± 31.7
Median survival	33.5	26.4	75	29.6
n	*86*	*29*	*8*	*999*

M/F, male/female; UL, upper limb; LL, lower limb.
[a]*Not statistically different vs. sporadic ALS cases.*
[b]*vs. sporadic ALS: $p < 0.0001$.*
[c]*vs. sporadic ALS: $p = 0.008$.*

TABLE 3 | Clinical characteristics of ALS and ALS/FTD patients.

	Age of onset	Age at death	Disease duration	ALSFRS-R score
ALS				
Bulbar onset	59.4 ± 10.0 (33)	61.1 ± 10.8 (30)	37.0 ± 28.7 (30)	39.0 ± 3.6 (25)
UL onset	56.4 ± 10.7 (16)	58.5 ± 9.1 (14)	42.1 ± 30.6 (14)	35.6 ± 9.5 (9)
LL onset	56.3 ± 10.5 (35)	59.9 ± 10.7 (32)	43.3 ± 19.8 (32)	40.0 ± 4.5 (24)
ALS/FTD				
Bulbar onset	60.0 ± 9.4 (12)	63.8 ± 8.8 (10)	22.6 ± 9.1 (10)	38.5 ± 8.1 (11)
UL onset	59.3 ± 5.8 (6)	61.4 ± 5.3 (5)	39.4 ± 26.4 (5)	31.4 ± 16.8 (6)
LL onset	61.0 ± 5.9 (14)	64.7 ± 6.0 (12)	30.7 ± 18.1 (12)	36.6 ± 11.0 (9)
Dementia onset	61.0 ± 4.8 (2)	64.3 ± 7.3 (2)	39.8 ± 30.5 (2)	
ALS				
Men	56.7 ± 10.6 (45)	60.0 ± 10.1 (38)	35.8 ± 19.0 (37)	39.6 ± 5.3 (32)
Women	58.0 ± 10.2 (48)	60.9 ± 10.1 (46)	43.7 ± 27.7 (49)	37.9 ± 5.9 (26)
ALS/FTD				
Men	59.4 ± 6.6 (21)	62.5 ± 6.5 (18)	30.5 ± 17.6 (18)	40.0 ± 6.7 (15)
Women	61.9 ± 7.6 (13)	65.5 ± 6.9 (12)	29.2 ± 19.9 (11)	31.8 ± 14.0 (11)

Values are means ± SD (n).
ALSFRS-R, amyotrophic lateral sclerosis rating scale-revised; UL, upper limb; LL, lower limb.

Influence of the Generation and Heritability

The study of the 57 families showed that either ALS, ALS/FTD, or FTD could be noted in 65 relatives belonging to the former generation (parents, uncles, aunts) of the index cases; 8 grandparents were also affected as well as 41 siblings (**Table 5**). In ALS cases, clinical comparison showed a significantly earlier age of onset in index cases compared to the former generation ($p = 0.005$) but not with their siblings. Correlatively, siblings also had an earlier age of onset compared to the former generation ($p = 0.027$). Disease duration was similar between the groups. A clear and significant female predominance could be noted in the former generation (gender ratio 0.44) compared to the index cases (1.45, $p = 0.011$); no female predominance could be noted in the siblings. The group of ALS/FTD patients was a smaller group, but the same differences could be noted, with a male predominance in index cases and siblings, and an earlier onset, compared to their parents, but those differences were not significant. On the contrary, in all the groups of ALS/FTD patients, the upper limb onset was underrepresented. In the group of FTD, once again, we can note a female predominance in parents and grandparents, but not in sibs.

To refine the study of clinical characteristics between generations, we constituted two generational groups, the first group comprising index cases and their sibs affected of ALS or ALS/FTD ($n = 108$), thus corresponding to the same generation, and the second group composed of subjects from the parental generation (parents, uncles, and aunts, $n = 44$, **Table 6**). The

TABLE 4 | Clinical characteristics of familial and apparently sporadic cases.

	ALS (*n* = 118)		ALS/FTD (*n* = 37)	
	AS cases	**Familial**	**AS cases**	**Familial**
Gender: M/F (ratio)	4/5 (0.8)	53/56 (0.9)	2/2 (1)	20/13 (1.5)
Bulbar onset (%)	22	41	50	36
UL onset (%)	0	20	25	16
LL onset (%)	78	39	25	42
Dementia onset (%)	–	–	–	6
Age of onset[a]	45.5 ± 6.5[b]	58.8 ± 9.9	64.6 ± 6.9	59.8 ± 7.0
n	*9*	*85*	*4*	*30*
Age at death[a]	49.4 ± 7.6	62.0 ± 9.5	65.8 ± 8.3	63.4 ± 6.7
n	*9*	*77*	*3*	*27*
Disease duration[a]	47.1 ± 21.1	39.5 ± 24.8	16.9 ± 12.5	31.5 ± 18.3
n	*9*	*77*	*3*	*26*
ALSFRS-R score[a]	36.0 ± 5.1	39.5 ± 5.4	46.3 ± 1.5	34.7 ± 11.2
n	*8*	*51*	*4*	*22*

AS, apparently sporadic; M/F, male/female; UL, upper limb; LL, lower limb; ALSFRS-R, amyotrophic lateral sclerosis rating scale-revised.
[a]*Value are means ± SD.*
[b]*vs. familial cases: p < 0.0001.*

comparison between these two groups showed a significant difference for gender ($p = 0.004$), age of onset ($p = 0.003$), and age at death ($p = 0.007$). There was no difference in the distribution of sites of onset nor for disease duration between the groups.

As the study of gender is potentially biased by an overrepresentation of women in some large families, we thus studied direct transmission of the trait by comparing pairs with mother-to-child transmission to those with father-to-child-transmission. There were 54 pairs with mother-to-child transmission and 33 with father-to-child, confirming the gender disequilibrium among parents. Comparison of the age of onset between parents and children from these pairs showed a significant difference with 62.7 ± 9.1 years in parents and 58.3 ± 8.5 years in children ($p = 0.015$). As an overrepresentation of women in pairs could also be due to their longer life span, and as penetrance of C9RE is age dependent, we also compared the age of onset of mothers and fathers from parent–child pairs. In women, the age of onset was 61.4 ± 11.3 years for ALS cases and 61.7 ± 8.1 years for FTD cases. In men, the mean age of onset was 59.6 ± 5.8 years for ALS cases and 72.2 ± 1.9 years for FTD cases. These differences were not significant.

Inter- and Intrafamilial Correlations

To compare intra- and interfamilial phenotype similarities, the ICCs with their 95% CI were estimated. There was a significant intrafamilial correlation (more similarities among the members of the same family than between families) for the bulbar onset of ALS, with an ICC = 0.35 (95% CI: 0.004–0.717). For the upper limb onset and the lower limb onset, the ICCs were 0.13 (0; 0.670) and 0.009 (0; 0.670), respectively, with a 95%CI including 0, non-significant. For the age at onset, duration of ALS, or gender, ICCs were close to 0, and thus non-significant.

Patients With Psychiatric Disorders

There were 13 patients with psychiatric disorders. Suicide was the most represented ($n = 9$), and other disorders included schizophrenia (in a nephew of the index case, $n = 1$), hypochondria (in a mother, $n = 1$), and bipolar disorder (in a son, $n = 1$). A last patient, father of an index case, was described as followed in a psychiatric hospital without any other detail. Suicide was noted in two fathers of index cases, one paternal uncle, three brothers, two sisters, and a nephew. Age at suicide could be ascertained for only six out of the nine relatives. The

TABLE 5 | Clinical characteristics according to parentality.

	n	Gender (M/F)	Site onset (B/UL/LL/D)	Age of onset[a]	Age at death[a]	Duration[a]
ALS						
Index cases	49	29/20[b]	17/10/21	55.2 ± 10.5 (49)[c]	58.9 ± 10.0 (44)	40.8 ± 24.8 (44)
Grand parents	2	2/0	2/0/0	69.0 ± *na* (1)	65.0 ± 9.9 (2)	36.0 ± *na* (1)
Parents	39	12/27	7/2/8	62.8 ± 10.4 (23)	64.2 ± 11.4 (20)	41.2 ± 25.0 (22)
Siblings	28	14/14[d]	8/4/8	56.4 ± 8.1 (21)[e]	60.5 ± 8.2 (20)	38.2 ± 24.6 (19)
ALS/FTD						
Index cases	21	13/8	8/6/7	60.6 ± 7.0 (21)	63.4 ± 7.1 (18)	29.7 ± 18.7 (18)
Parents	6	2/4	3/2/1	62.5 ± 6.2 (5)	65.0 ± 6.6 (5)	31.0 ± 18.1 (5)
Siblings	10	7/3	2/0/5/1	58.4 ± 7.8 (8)	63.4 ± 6.8 (7)	30.3 ± 20.0 (6)
FTD						
Grand parents	6	2/4	–	58.0 ± *na* (1)	62.5 ± 3.5 (2)	84.0 ± *na* (1)
Parents	20	9/11	–	64.8 ± 10.2 (7)	74.0 ± 8.3 (10)	88.1 ± 38.5 (7)
Siblings	3	3/3	–	73.0 ± 1.7 (2)	75.0 ± *na* (1)	

M/F, male/female; B/UL/LL/D, Bulbar/Upper limb/Lower limb/Dementia.
[a]*Value are means ± SD (n).*
[b]*Gender ratio vs. parents, p = 0.011.*
[c]*Age of onset vs. parents, p = 0.005.*
[d]*Gender ratio vs. parent, p = 0.027.*
[e]*Age of onset vs. parents, not significant.*

TABLE 6 | Clinical characteristics of index cases + sibs and parents with ALS and ALS/FTD.

	Parents	Index cases + sibs	*p*
Gender: M/F (ratio)	14/30 (0.47)	63/45 (1.4)	0.004
Age of onset[a]	63.0 ± 9.7 (27)	56.9 ± 9.3 (99)	0.003
Bulbar onset (%)	43.5	35.5	
Upper limb onset (%)	8.7	20	ns
Lower limb onset (%)	43.5	42.5	
Dementia (%)	4.3	2	
Age at death[a]	64.7 ± 10.7 (24)	60.5 ± 8.9 (89)	0.007
ALS duration[a]	39.6 ± 24.3 (26)	37.2 ± 23.5 (87)	0.710

M/F, male/female.
[a]*Value are means ± SD (n).*

mean age at suicide was 54 years, and ages were 30, 52, 54, 60, 60, and 68 years. One patient who committed suicide was the father of an apparently sporadic ALS case; all the other belonged to the FALS group. In families, suicide was noted in almost all the family types, but was more frequent in families with only ALS cases (**Table 1**). C9RE could be studied in one patient with psychiatric disorder, the individual with schizophrenia, and the presence of a pathological expansion was confirmed. His father had died of ALS.

DISCUSSION

This study describes one of the largest cohorts, to date, of patients with C9RE and their familial clinical profile. Out of the 70 index cases described, 18.6% of the patients had no apparent familial history of ALS or FTD. This is larger than estimated in previous works; however, familial inquiry was missing in two cases, and pedigree was not informative in three other cases. In only three cases (4%), parents were old and alive without any neurological or psychiatric disorder (Majounie et al., 2012). In the 57 families identified, no clear predominance of a particular phenotype was noted. Similarly, psychiatric antecedents in relatives were noted in almost all the familial phenotypes with no particular predominance of suicides in families with FTD or ALS/FTD cases.

Compared to our database of sporadic ALS cases, the only difference in clinical characteristics was the earlier age of onset of C9RE ALS and ALS/FTD cases, and this has already been underlined (Byrne et al., 2012; Majounie et al., 2012; Cooper-Knock et al., 2014). Disease duration was not shorter compared to other series. Although it had been already suggested that patients with C9RE had more frequent bulbar onset, we did not find such a predominance neither for ALS nor ALS/FTD C9RE patients in the present cohort. Sporadic ALS patients from our database have not been tested for C9RE. However, only 3–7% of sporadic ALS patients may carry an abnormal C9RE. This low percentage suggests that, even if some of those patients are present in this sporadic ALS group, it would have only a marginal effect on population characteristics.

ALS and ALS/FTD C9RE patients did not differ statistically in any of the clinical criteria even though the gender ratio clearly favored predominance of men in the ALS/FTD group conversely to the ALS group. Disease duration was shorter in ALS/FTD patients, as already described, but the size of the samples was limited and the great variability of duration within groups potentially explains the absence of statistical difference. The study of clinical characteristics between apparently sporadic patients and familial cases was also limited by the size of the samples. Nevertheless, a significantly earlier age at onset was noted in the group of apparently sporadic patients with ALS. The shortest duration was recorded in the group with ALS/FTD, but it only comprised three patients and no further interpretation seems possible at this stage.

One important question when facing new gene abnormalities and particularly those with expansion is the existence of an anticipation. This is all the more important as the exact determination of C9RE cannot be routinely done and it is the reason why anticipation with C9RE has been scarcely studied, but the rare works on that topic were in favor of an anticipation (Van Mossevelde et al., 2017). In our cohort, comparison of clinical characteristics between generations showed substantial differences. The age of onset between index cases and siblings was similar and significantly earlier than for their parents, uncles, and aunts by 7 years in ALS and 2 years in ALS/FTD cases, thus suggesting anticipation. This was reinforced by the study of parent–child pairs. However, it is not possible to determine the exact cause of a possible anticipation. It may be due to changes in the size of C9RE, to other genetic aspects, to epigenetic or environmental factors. One other interesting difference between these groups is the female predominance in the parental generation that reached significance, with a gender ratio of 0.47 in parents vs. 1.4 in index cases (p = 0.004). Although a female predominance in C9RE carriers has already been shown, the gender disequilibrium we describe between generations has not been previously described, to our knowledge (Curtis et al., 2017). This may suggest that C9RE is different between men and women, as it has been demonstrated in other neurological disorders such as myotonic dystrophy or Huntington's disease. The longer life span of women compared to men seems unlikely to explain such a gender disequilibrium as age of onset of parents with C9RE in the present cohort is not different between men and women.

It was interesting to compare the intrafamilial similarities of the phenotype to interfamilial ones. Bulbar onset was significantly less variable within families than between families suggesting that genetic aspects may underlie the occurrence of such onset in ALS. This was reinforced by the absence of intrafamilial correlation for gender. Indeed, as bulbar ALS patients are more frequently women (twice as more frequent than in men), this shows that gender was not a potential confounder for the intrafamilial similarity of bulbar onset between relatives.

Some but not all previous works have suggested that patients with C9RE may present with initial psychiatric symptoms. A higher rate of psychiatric disorders in ALS kindreds has also been described. The search for C9RE in individuals who died of suicide in Finland did not reveal any abnormal repeat expansion in the C9ORF72 region (Solje et al., 2016). In the present cohort, 9 patients had a suicide history in their family, and 4 other psychiatric disorders were found, for a total of 13, corresponding to 18.5% of index cases with a psychiatric disorder in their family and 12.8% with suicide. In France, the incidence of death by suicide is 11.8 per 100,000 people, and most of them occur between 45 and 64 years (Observatoire national du suicide, rapport 2014)[2]. While the mean age of suicide in our cohort is in the same range than in the general population, incidence of suicide in ALS families does not seem to be a chance association, all the more as the number of cases collected is likely to be underestimated. Indeed, familial information was obtained by a simple inquiry and a systematic medical ascertainment of causes of death may well have found more cases. Only one individual with psychiatric disorder could be genetically analyzed and carried C9RE. He was first diagnosed with schizophrenia at the age of 20. However, it is not possible to determine whether or not this disorder is a consequence of C9RE.

This study has some limitations. The cohort represents the recruitment of an ALS center, and it is possible that another source of recruitment, such as an FTD expert center for example, could have a somewhat significant clinical spectrum to describe. Moreover, we paid attention mainly to ALS cases, but FTD cases have not been studied neuropsychologically for subtype description. One other limit is that despite the size of the cohort, the limited number of subjects in subgroups precludes the analysis of all aspects of phenotypes with enough statistical power. Although we tried to ascertain diagnoses as much as possible, it was not possible for all the cases. Subsequently, it is not possible to exclude that some clinical phenotypes, not described in this cohort, may exist within ALS families. However, it seems unlikely that unusual phenotypes are frequent. Memory as well as knowledge biases may also underestimate some cases such as suicide for example, as this diagnosis may also be hidden within families. This is a descriptive and retrospective work without deep genetic analysis. It is also a center-based cohort, not a population-based study, and these points limit the scope of the conclusions. Subsequently, we cannot ascertain that genetic anticipation does exist even though the age of onset is significantly younger.

This cohort was composed of patients referred to our ALS center for suspicion of ALS. Among the 70 ALS index cases carrying C9RE, 57 familial cases were identified, with a total of 200 relatives with either ALS, ALS/FTD, FTD, or psychiatric disorder. No other neurological disorder was identified, making it unlikely that the pathological phenotype linked to C9RE is broad or frequent. Apparently, sporadic cases had an earlier age of onset. In familial cases, index cases and their siblings had an earlier age of onset compared to their parental generation, characterizing anticipation. In the parental generation, a significant female predominance was shown for which we have no formal explanation to date. A significant intrafamilial correlation of bulbar onset of ALS was also shown. In the families of index cases, a high incidence of suicide was described suggesting a direct link with C9RE, and physicians should be aware of that possibility. Studies on larger international cohorts with familial inquiry are warranted to confirm the existence of anticipation and to confirm and explain the reason for gender disequilibrium.

[2] https://drees.solidarites-sante.gouv.fr/etudes-et-statistiques/la-drees/observatoire-national-du-suicide-ons

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by CCPPRB, CHU Pitié-Salpêtrière, Paris, France. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FE, RJ-M, NP, EB, EL, VD, and WC recruited patients and obtained patient consent. SA and LL were responsible for the databases. KM, AP, and SL were responsible for the genetics studied. M-CP, EN, and WC were responsible for statistical analyses. WC and FE drafted the manuscript. All authors critically reviewed the manuscript.

REFERENCES

Akimoto, C., Volk, A. E., van Blitterswijk, M., Van den Broeck, M., Leblond, C. S., Lumbroso, S., et al. (2014). A blinded international study on the reliability of genetic testing for GGGGCC-repeat expansions in C9orf72 reveals marked differences in results among 14 laboratories. *J. Med. Genet.* 51, 419–424. doi: 10.1136/jmedgenet-2014-102360

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

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

Cooper-Knock, J., Shaw, P. J., and Kirby, J. (2014). The widening spectrum of C9ORF72-related disease; genotype/phenotype correlations and potential modifiers of clinical phenotype. *Acta Neuropathol*. 127, 333–345. doi: 10.1007/s00401-014-1251-9

Curtis, A. F., Masellis, M., Hsiung, G. R., Moineddin, R., Zhang, K., Au, B., et al. (2017). Sex differences in the prevalence of genetic mutations in FTD and ALS: a meta-analysis. *Neurology* 89, 1633–1642. doi: 10.1212/WNL.0000000000004494

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. 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

Hardiman, O., van den Berg, L. H., and Kiernan, M. C. (2011). Clinical diagnosis and management of amyotrophic lateral sclerosis. *Nat. Rev. Neurol.* 7, 639–649.

Killip, S., Mahfoud, Z., and Pearce, K. (2004). What is an intracluster correlation coefficient? crucial concepts for primary care researchers. *Ann. Fam. Med.* 2, 204–208. doi: 10.1370/afm.141

Kimura, F., Fujimura, C., and Ishida, S. (2006). Progression rate of ALSFRS-R-R at time of diagnosis predicts survival time in ALS. *Neurology* 66, 265–267. doi: 10.1212/01.wnl.0000194316.91908.8a

Majounie, E., Renton, A. E., Mok, K., Dopper, E. G., Waite, A., Rollinson, S., et al. (2012). Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study. *Lancet Neurol.* 11, 323–330.

Martins, J., Damásio, J., Mendes, A., Vila-Chã, N., Alves, J. E., Ramos, C., et al. (2018). Clinical spectrum of C9orf72 expansion in a cohort of Huntington's disease phenocopies. *Neurol. Sci.* 39, 741–744. doi: 10.1007/s10072-018-3268-7

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

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

Sellami, L., St-Onge, F., Poulin, S., and Laforce, R. Jr. (2019). Schizophrenia phenotype preceding behavioral variant frontotemporal dementia related to C9orf72 repeat expansion. *Cogn. Behav. Neurol.* 32, 120–123. doi: 10.1097/WNN.0000000000000189

Silverman, H. E., Goldman, J. S., and Huey, E. D. (2019). Links between the C9orf72 repeat expansion and psychiatric symptoms. *Curr. Neurol. Neurosci. Rep.* 19:93. doi: 10.1007/s11910-019-1017-9

Snowden, J. S., Harris, J., Richardson, A., Rollinson, S., Thompson, J. C., Neary, D., et al. (2013). Frontotemporal dementia with amyotrophic lateral sclerosis: a clinical comparison of patients with and without repeat expansions in C9orf72. 12. *Amyotroph. Lateral Scler. Frontotemporal. Degener.* 14, 172–176. doi: 10.3109/21678421.2013.765485

Solje, E., Riipinen, P., Helisalmi, S., Särkioja, T., Laitinen, M., Hiltunen, M., et al. (2016). The role of the FTD-ALS associated C9orf72 expansion in suicide victims. *Amyotroph. Lateral Scler. Frontotemporal. Degener.* 17, 589–592. doi: 10.1080/21678421.2016.1203337

Ticozzi, N., Tiloca, C., Calini, D., Gagliardi, S., Altieri, A., Colombrita, C., et al. (2014). C9orf72 repeat expansions are restricted to the ALS-FTD spectrum. *Neurobiol. Aging* 35, e13–e17. doi: 10.1016/j.neurobiolaging.2013.09.037

Trojsi, F., Siciliano, M., Femiano, C., Santangelo, G., Lunetta, C., Calvo, A., et al. (2019). Comparative analysis of C9orf72 and sporadic disease in a large multicenter ALS population: the effect of male sex on survival of C9orf72 positive patients. *Front. Neurosci.* 13:485. doi: 10.3389/fnins.2019.00485

Umoh, M. E., Fournier, C., Li, Y., Polak, M., Shaw, L., Landers, J. E., et al. (2016). Comparative analysis of C9orf72 and sporadic disease in an ALS clinic population. *Neurology* 87, 1024–1030. doi: 10.1212/WNL.0000000000003067

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

Volk, A. E., Weishaupt, J. H., Andersen, P. M., Ludolph, A. C., and Kubisch, C. (2018). Current knowledge and recent insights into the genetic basis of amyotrophic lateral sclerosis. *Med. Genet.* 30, 252–258. doi: 10.1007/s11825-018-0185-3

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REVIEW
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Multifaceted Genes in Amyotrophic Lateral Sclerosis-Frontotemporal Dementia

*Ramya Ranganathan[1†], Shaila Haque[1,2†], Kayesha Coley[1], Stephanie Shepheard[1], Johnathan Cooper-Knock[1] and Janine Kirby[1]**

[1] *Sheffield Institute for Translational Neuroscience (SITraN), The University of Sheffield, Sheffield, United Kingdom,*
[2] *Department of Biochemistry and Biotechnology, University of Barishal, Barishal, Bangladesh*

Amyotrophic lateral sclerosis and frontotemporal dementia are two progressive, adult onset neurodegenerative diseases, caused by the cell death of motor neurons in the motor cortex and spinal cord and cortical neurons in the frontal and temporal lobes, respectively. Whilst these have previously appeared to be quite distinct disorders, in terms of areas affected and clinical symptoms, identification of cognitive dysfunction as a component of amyotrophic lateral sclerosis (ALS), with some patients presenting with both ALS and FTD, overlapping features of neuropathology and the ongoing discoveries that a significant proportion of the genes underlying the familial forms of the disease are the same, has led to ALS and FTD being described as a disease spectrum. Many of these genes encode proteins in common biological pathways including RNA processing, autophagy, ubiquitin proteasome system, unfolded protein response and intracellular trafficking. This article provides an overview of the ALS-FTD genes before summarizing other known ALS and FTD causing genes where mutations have been found primarily in patients of one disease and rarely in the other. In discussing these genes, the review highlights the similarity of biological pathways in which the encoded proteins function and the interactions that occur between these proteins, whilst recognizing the distinctions of *MAPT*-related FTD and *SOD1*-related ALS. However, mutations in all of these genes result in similar pathology including protein aggregation and neuroinflammation, highlighting that multiple different mechanisms lead to common downstream effects and neuronal loss. Next generation sequencing has had a significant impact on the identification of genes associated with both diseases, and has also highlighted the widening clinical phenotypes associated with variants in these ALS and FTD genes. It is hoped that the large sequencing initiatives currently underway in ALS and FTD will begin to uncover why different diseases are associated with mutations within a single gene, especially as a personalized medicine approach to therapy, based on a patient's genetics, approaches the clinic.

Keywords: ALS, FTD, C9orf72, RNA processing, autophagy, protein aggregation

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****Correspondence:***
Janine Kirby
j.kirby@sheffield.ac.uk

[†]These authors share first authorship

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is an adult onset neurodegenerative disorder caused by progressive loss of upper motor neurons in the motor cortex and brainstem and lower motor neurons in the spinal cord (Hardiman et al., 2017). It has an incidence of 2–3 per 100,000 and a lifetime risk of 1 per 400 individuals (Brown and Al-Chalabi, 2017). Disease onset occurs most frequently in the limbs, characterized by a loss of dexterity in the fingers or a mild limp, whilst bulbar onset, which occurs in 20–25% of cases, is characterized by a slurring of speech (dysarthria) or difficulty swallowing (dysphagia). Less than 3% of cases are due to respiratory onset, with shortness of breath (dyspnea) being the most common symptom (Gautier et al., 2010). As the disease rapidly progresses, muscle wasting, fasciculations and weight loss occur, with death usually due to respiratory failure 32 months following symptom onset (Cooper-Knock et al., 2013).

Amyotrophic lateral sclerosis clinical features may also be accompanied by cognitive impairment in up to 50% of patients, whilst up to 15% may develop symptoms which are clinically diagnosed as frontotemporal dementia (FTD), resulting in a clinical diagnosis of ALS-FTD (Nguyen et al., 2018). FTD is the second most common form of presenile dementia after Alzheimer's disease, accounting for 3–26% of cases of dementia in individuals under 65 years of age, depending on population (Bang et al., 2015). Whilst FTD is a clinical diagnosis, evidence of degeneration of the neurons in the frontal and temporal lobes upon post-mortem allows the pathological diagnosis of frontotemporal lobar degeneration (FTLD) (Mackenzie and Neumann, 2016). FTD can be divided into three different clinical subtypes. Behavioral variant FTD (bvFTD) is characterized by personality changes such as disinhibited behavior, apathy and loss of empathy. In contrast, semantic variant primary progressive aphasia (svPPA or svFTD) is characterized by individuals having difficulties understanding the meaning of words or naming objects or people, whereas non-fluent variant PPA (nfvPPA or nfvFTD) is when individuals have difficulties in pronunciation, grammar and fluency of speech (Bang et al., 2015).

Whilst the majority of ALS and FTD cases are sporadic (sALS and sFTD), with no family history of disease, in around 10% of cases ALS is inherited, usually in an autosomal dominant manner with an adult onset (fALS) (Brenner and Weishaupt, 2019). In FTD, it is estimated that 10–30% shows autosomal dominant inheritance (fFTD), though this figure may be increased to approximately 40% when a history of neurodegenerative disease is included (Sirkis et al., 2019). To date, 5 ALS-FTD genes have been recognized along with 24 ALS-associated genes and 3 FTD-associated genes (**Table 1**). This review will firstly summarize the genes that have been recognized as ALS-FTD genes (defined as FTDALS loci on Online Mendelian Inheritance in Man[1]) before summarizing other known ALS and FTD causing genes where mutations have been found primarily in patients of one disease and rarely in the other. Finally, a brief comment on the other ALS and FTD genes allocated an ALS or FTD loci number is provided for completeness and to highlight the similarity of biological pathways that are implicated in both disorders, supporting the proposal that these two disorders represent either end of a disease spectrum. Whilst some of these genes result in Mendelian inheritance of ALS, others act as risk factors. However, understanding why a particular mutation in a gene leads to ALS, FTD or both, currently remains unknown.

[1]https://www.ncbi.nlm.nih.gov/omim

ALS-FTD GENES

Mutations in five genes have been recognized as being implicated in ALS-FTD families. These are the GGGGCC (G4C2) hexanucleotide repeat expansion (HRE) in *C9orf72* (FTDALS1), and missense and/or loss of function mutations in *CHCHD10* (FTDALS2), *SQSTM1* (FTDALS3), and *TBK1* (FTDALS4). In addition, *CCNF* has also been reported as an ALS-FTD gene and is referred in this review as FTDALS5. All of these genes encode proteins with a function in autophagy, except CHCHD10, which localizes to the mitochondria.

FTDALS1: Chromosome 9 Open Reading Frame 72 (*C9orf72*)

Linkage and genome wide association studies in several families presenting with ALS/FTD, ALS or FTD revealed the diseases to segregate with a locus on chromosome 9p21 (Morita et al., 2006; Vance et al., 2006; Laaksovirta et al., 2010; Shatunov et al., 2010). In 2011, the pathogenic G4C2 hexanucleotide repeat expansion (HRE) in intron 1 of chromosome 9 open reading frame 72 (*C9orf72*) (**Figure 1**) was found to be the most common cause of familial ALS and FTD (DeJesus-Hernandez et al., 2011; Renton et al., 2011) with ∼40% fALS and 25% fFTD carrying the *C9orf72* repeat expansion (Majounie et al., 2012). However, the frequency of *C9orf72*-related ALS-FTD (and *C9orf72*-ALS and *C9orf72*-FTD) patients positive for the presence of repeat expansion in *C9orf72* varies between different populations and ethnicities; whilst the *C9orf72* G4C2 HRE is the most frequent cause of ALS-FTD, ALS and FTD in European and North American populations, it was found to be extremely rare in Asia and the Middle East (Majounie et al., 2012). Whilst it is currently unclear why some patients with the *C9orf72* expansion develop ALS or FTD while others manifest a combination of both, the discovery of *C9orf72* in causing both ALS and FTD has strengthened the genetic link between these neurodegenerative disorders. *C9orf72*-related ALS-FTD has an autosomal dominant mode of inheritance with evidence of incomplete penetrance (Majounie et al., 2012). Anticipation has been reported in some families (Gijselinck et al., 2016; Van Mossevelde et al., 2017b), however, this phenomenon has been found to be inconsistent, with other studies having reported no association between age of onset and repeat length (Dols-Icardo et al., 2014) or even an inverse correlation between expansion size and age of onset in successive generations, with contractions in the repeat size also reported in subsequent generations (Fournier et al., 2019; Jackson et al., 2020). Some of these contradictory findings may be attributed to the age at collection of sample, owing to the dynamic nature of repeat size relative to age, the methodology

TABLE 1 | Overview of known ALS-FTD, ALS, and FTD loci.

ALS Loci number	Chromosomal location	Gene	Onset	Inheritance	Implicated pathogenic mechanisms	Original References
ALS-FTD genes						
FTDALS1	9p21.2	*C9orf72*	Adult	AD	RNA processing; nucleocytoplasmic transport defects; proteasome impairment; autophagy; inflammation; protein aggregation (DPRs)	DeJesus-Hernandez et al., 2011; Renton et al., 2011
FTDALS2	22q11.23	*CHCHD10*	Adult	AD	Mitochondrial function, synaptic dysfunction	Bannwarth et al., 2014
FTDALS3	5q35.3	*SQSTM1*	Adult	AD	Proteasome impairment; autophagy; protein aggregation; axonal defects; oxidative stress	Fecto et al., 2011
FTDALS4	12q14.2	*TBK1*	Adult	AD	Autophagy; inflammation; mitochondrial dysfunction	Cirulli et al., 2015; Freischmidt et al., 2015
FTDALS5	16p13.3	*CCNF*	Adult	AD	Autophagy, axonal defects, protein aggregation	Williams et al., 2016
Predominantly ALS genes also found with FTD						
ALS6	16p11.2	*FUS*	Adult	AD (AR)	RNA processing; nucleocytoplasmic transport defects; stress granule function; protein aggregation	Kwiatkowski et al., 2009; Vance et al., 2009
ALS10	1p36.22	*TARDBP*	Adult	AD	RNA processing; nucleocytoplasmic transport defects; stress granule function; protein aggregation	Sreedharan et al., 2008
ALS12	10p13	*OPTN*	Adult	AD (AR)	Autophagy; protein aggregation; inflammation	Maruyama et al., 2010
ALS15	Xp11.21	*UBQLN2*	Adult	X-LD	Proteasome impairment; autophagy; protein aggregation; oxidative stress; axonal defects	Deng et al., 2011
ALS22	2q35	*TUBA4A*	Adult	AD	Cytoskeleton	Smith et al., 2014
ALS13	12q24.12	*ATXN2*	Adult	AD	RNA processing	Elden et al., 2010
Predominantly FTD genes also found with ALS						
ALS14	9p13.3	*VCP*	Adult	AD	Autophagy; proteasome impairment; defects in stress granules; protein aggregation; mitochondrial dysfunction	Johnson et al., 2010
ALS17	3p11.2	*CHMP2B*	Adult	AD	Autophagy; protein aggregation	Parkinson et al., 2006
Known ALS genes						
ALS1	21q22.11	*SOD1*	Adult	AD (AR)	Oxidative stress; protein aggregation; mitochondrial dysfunction, axonal defects, proteasome impairment apoptosis	Rosen et al., 1993
ALS2	2q33.1	*ALS2*	Juvenile	AR	Intracellular trafficking	Hadano et al., 2001; Yang et al., 2001
ALS4	9q34.13	*SETX*	Juvenile	AD	RNA processing	Chen et al., 2004
ALS5	15q21.1	*SPG11*	Juvenile	AR	Axonal defects	Orlacchio et al., 2010
ALS8	20q13. 32	*VAPB*	Adult	AD	Proteasome impairment; intracellular trafficking	Nishimura et al., 2004
ALS9	14q11.2	*ANG*	Adult	AD	RNA processing	Greenway et al., 2006
ALS11	6q21	*FIG4*	Adult	AD	Intracellular trafficking	Chow et al., 2009
ALS16	9p13.3	*SIGMAR1*	Juvenile	AD and AR	Proteasome impairment; intracellular trafficking	Luty et al., 2010; AL-Saif et al., 2011
ALS18	17p13.2	*PFN1*	Adult	AD	Axonal defects	Wu et al., 2012
ALS19	2q34	*ERBB4*	Adult	AD	Neuronal development	Takahashi et al., 2013
ALS20	12q13.13	*hnRNPA1*	Adult	AD	RNA processing	Kim et al., 2013
ALS21	5q31.2	*MATR3*	Adult	AD	RNA processing	Johnson et al., 2014
ALS23	10q22.2	*ANXA11*	Adult	AD	Intracellular trafficking	Smith et al., 2014
ALS24	4q33	*NEK1*	Adult	AD	Intracellular trafficking	Brenner et al., 2016
ALS25	12q13.3	*KIF5A*	Adult	AD	Axonal defects; intracellular trafficking	Nicolas et al., 2018
ALS	3p21.1	*GLT8D1*	Adult	AD	Ganglioside synthesis	Cooper-Knock et al., 2019
Known FTD genes						
FTD	17q21.2	*MAPT*	Adult	AD	Axonal defects, protein aggregation	Hutton et al., 1998
FTD	17q21.31	*GRN*	Adult	AD	Inflammation; protein aggregation	Baker et al., 2006; Cruts et al., 2006
FTD	6q27	*TBP*	Adult	AD	RNA processing	Olszewska et al., 2019

AD, autosomal dominant; AR, autosomal recessive; X-LD, X-linked inheritance.

used and the source of samples being compared (peripheral blood versus brain autopsy tissue) (van Blitterswijk et al., 2013a; Fournier et al., 2019).

Although, the exact cut-off for repeat size that would result in pathogenicity has not been clearly defined, neurologically healthy non-carriers typically present with less than 20 repeats, while repeat expansion mutation carriers show more than 30 repeats on repeat primed PCR (Renton et al., 2011). Southern blotting of the region has estimated that repeat lengths of several hundred to several thousand are associated with *C9orf72*-related ALS-FTD, though smaller repeat sizes have been found to co-segregate with disease (Van Mossevelde et al., 2017a). Conventional techniques such as short-read next generation sequencing (NGS) limit accurate repeat sizing of the larger repeats, and somatic instability has been reported, thereby contributing to the variability in repeat size in different tissues from the same individual, such as in comparisons between blood and central nervous system (CNS) tissue (Vatsavayai et al., 2019). Studies have described an inverse relationship between repeat expansion size and disease duration with *C9orf72*-related ALS-FTD presenting an earlier age of symptom onset, a higher incidence of bulbar onset and shorter disease duration (Majounie et al., 2012; van Blitterswijk et al., 2013b; Suh et al., 2015; Trojsi et al., 2019). However, other studies have also reported disease durations between 1 and 22 years (Majounie et al., 2012; Woollacott and Mead, 2014). Sex has been reported to be a risk factor in driving phenotype with one study showing females have a higher prevalence for *C9orf72* HRE in ALS in a meta-analysis study (Trojsi et al., 2019) whilst males presented with a shorter survival time based on Cox proportional hazard regression multivariate analysis (Curtis et al., 2017). It is noteworthy that *C9orf72* repeat expansions have been shown to be associated with a number of neurodegenerative conditions including Parkinson disease, Alzheimer's disease, Huntington-disease like syndrome among others (Woollacott and Mead, 2014; Cooper-Knock et al., 2015c).

The function of endogenous C9orf72 protein is not fully characterized although it has been identified as a guanine exchange factor (GEF), with both Rho and Rab-GTPase GEF activity (Iyer et al., 2018). It has also been shown that the C9orf72 protein interacts with the Rab1a and Unc-51-like kinase 1 (ULK1) autophagy initiation complex, with the C9orf72 protein regulating the trafficking of the ULK1 complex to the phagophore (Webster et al., 2016). As such, a reduction in C9orf72 protein would lead to reduced autophagy and accumulation of p62-positive aggregates, similar to those seen upon neuropathological examination of patients.

Whilst the exact mechanism of action of how the HRE in *C9orf72* causes neurodegeneration remains to be fully elucidated, three mutually compatible mechanisms have been proposed including haploinsufficiency of endogenous C9orf72 protein, loss of function and/or toxic gain of function following the formation of RNA foci and toxic gain of function of the dipeptide repeat (DPR) protein inclusions (**Figure 1**).

Haploinsufficiency of C9orf72

The *C9orf72* gene contains 12 exons (1a, 1b, 2–11) and has three well characterized transcripts which produce two protein isoforms (**Figure 1**) (Balendra and Isaacs, 2018) though additional alternatively spliced and protein coding transcripts have been identified (see Ensembl ENSG00000147894). The HRE is located in the pre-mRNA transcript of variant 1 and 3 but in the promoter region of variant 2. It is noteworthy that variants 1 and 3 are predominantly expressed in the brain. Additionally, HRE dependent epigenetic changes such as hypermethylation of the *C9orf72* gene locus has been reported and associated with disease duration and advanced age of onset (Nordin et al., 2015; Gijselinck et al., 2016; Zhang M. et al., 2017). Studies have shown that transcript sequences upstream of the repeat are increased relative to those downstream which might imply that the transcription was aborted due to the presence of repeat. Consequently, reduced levels of the transcript variants have been reported in blood lymphocytes (Ciura et al., 2013), induced pluripotent stem cells (iPSCs)-derived neurons (Shi et al., 2018) and post mortem tissue of *C9orf72*-related ALS and FTD patients (van Blitterswijk et al., 2013b). *C9orf72* knockdown in zebrafish was shown to produce defective axon generation and motor deficits (Ciura et al., 2013) indicating that C9orf72 protein might play a role in neuronal health. In contrast, specific knockdown of *C9orf72* in mouse brain by antisense oligonucleotides (Lagier-Tourenne et al., 2013) or full ablation of *C9orf72* (*C9orf72*$^{-/-}$) in neuron-specific (Atanasio et al., 2016) or all tissues (Sudria-Lopez et al., 2016) in mice models showed no neurodegenerative phenotype, although ablated mice developed an autoimmune phenotype and showed reduced survival. This suggests that haploinsufficiency alone may not be sufficient to cause disease and a combination of aberrant pathways such as gain of toxic function with loss of endogenous C9orf72 protein function might therefore be required for disease pathogenesis.

RNA Foci Formation

The hexanucleotide repeat DNA sequences are bidirectionally transcribed resulting in the production of G4C2 sense and C2G4 antisense transcripts retaining the repeat expansions. Hexanucleotide repeat-retaining RNA forms secondary structures (such as a G-quadruplex) in which the abnormal RNA accumulate to form RNA foci. These RNA foci have been shown to be present in post-mortem brain and spinal cord tissues of *C9orf72*-related ALS-FTD patients whilst being absent in age-matched non-ALS/FTD neurologically healthy controls (Zu et al., 2013). Sense and antisense RNA transcripts get transported to the cytoplasm and have been detected in the cytoplasm of patient post mortem tissue (Mizielinska et al., 2013; Cooper-Knock et al., 2015b) and RNA foci have been found in many cell lines and patient biosamples, such as leukocytes, fibroblasts, and iPSC-derived motor neurons (Gendron and Petrucelli, 2018). The aggregation of RNA foci are dynamic and result from association and dissociation of RNA binding proteins (RBPs) which results in loss of their function. Research has shown that antisense foci were observed to be higher in the Purkinje neurons in cerebellum and motor neurons whereas sense foci were significantly increased in the granule neurons in the cerebellum obtained post mortem from *C9orf72*-related ALS or *C9orf72*-related ALS-FTD patients, as detected by fluorescence

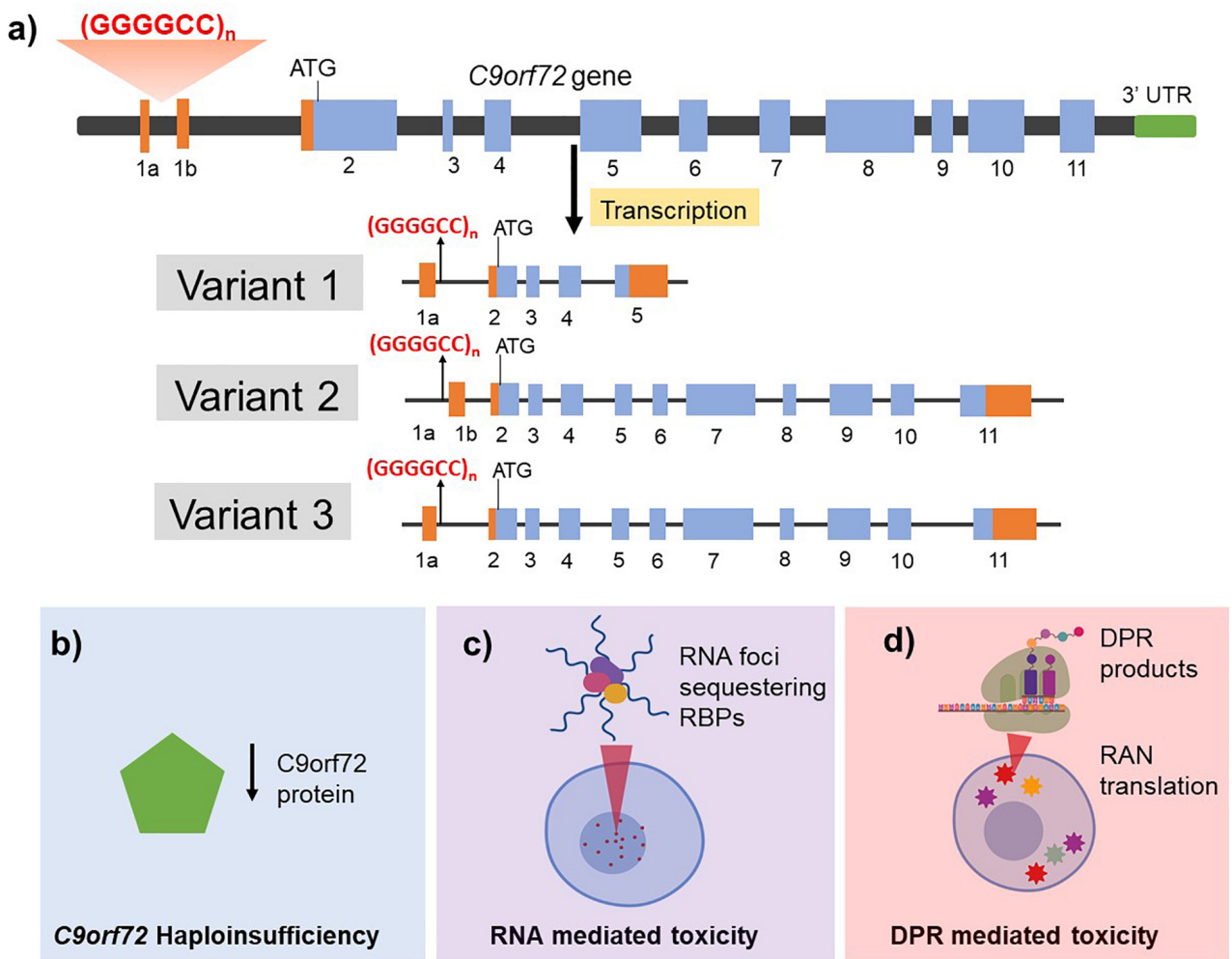

FIGURE 1 | Structure of the *C9orf72* gene **(a)** and the proposed mechanisms of *C9orf72* related toxicity in driving ALS/FTD pathogenesis **(b–d)**. **(a)** *C9orf72* has 11 exons and is transcribed into three different mRNA transcripts: variant 1 results in translation of the short protein isoform while variants 2 and 3 both generate the long protein isoform. The non-coding introns are represented in orange and the coding exons are shown in blue. The hexanucleotide repeat expansion is present within intron 1 region and is retained in the pre-mRNA transcript in variants 1 and 3 whereas it is present in the promoter region in variant 2. The G4C2 HRE is proposed to cause neurotoxicity by three mutually compatible mechanisms. **(b)** Haploinsufficiency of endogenous C9orf2 protein by incorporation of the repeat in the transcript leading to reduced production and function of normal C9orf72 protein. **(c)** RNA mediated toxicity by the formation of RNA foci that sequester RNA binding proteins (RBPs) and **(d)** DPR mediated toxicity resulting from repeat associated non-AUG (RAN) translation of mRNA transcripts retaining hexanucleotide repeats thus generating five different dipeptide repeat (DPR) products.

in situ hybridization (Cooper-Knock et al., 2014, 2015b) and RT-PCR (Zu et al., 2013).

Several studies have aimed to identify the RBPs that are sequestered within the C9orf72 RNA foci and have found hnRNPA1/3, PUR-a, ADARB2, Nucleolin, SRSF2, and ALYREF in post mortem CNS tissue, iPSCs-derived cortical neurons reprogrammed from C9orf72-related ALS-FTD patient fibroblasts, neuronal cell lines or a *Drosophila* model of *C9orf72* ALS (Donnelly et al., 2013; Sareen et al., 2013; Cooper-Knock et al., 2014; Hautbergue et al., 2017). Gene ontology and transcriptomic analyses have revealed that the formation of foci result in transcriptional profiles unique to *C9orf72*-related ALS and FTD when compared to other genetic causes of ALS and FTD. Changes in gene expression are associated with various cellular pathways including unfolded protein response (UPR), RNA splicing, inflammatory response, cell signaling and synaptic transmission (Cooper-Knock et al., 2015a; Prudencio et al., 2015). Taken together, these studies provide evidence for both a loss of function of the RNA binding proteins and a potential gain of toxic function of the downstream effects of the RNA foci formation in driving *C9orf72*-related ALS-FTD pathogenesis.

Toxicity by DPR

The *C9orf72* HRE sense and antisense RNA transcripts can get translated by a non-canonical mechanism of repeat associated non-ATG (RAN) translation resulting in the production of dipeptide repeats. RAN translation occurs in the absence of an AUG start codon resulting in multiple reading frames of

a single repeat transcript. These DPRs can be generated from the sense and/or antisense transcripts resulting in the formation of five different products: poly-GA and poly-GR are translated from the sense GGGGCC strand whereas poly-PA and poly-PR are translated from the antisense CCGGGG strand; poly-GP is translated from both strands. DPRs have been shown to aggregate in the cytoplasm and appear as star-shaped inclusions in both neurons and glia (Mann et al., 2013; Schludi et al., 2015). Sense-derived poly-GA aggregated seem to be more frequent than others, however, both sense and antisense DPRs have been observed in neocortex, hippocampus, and thalamus (Cooper-Knock et al., 2012; Schludi et al., 2015). poly-GA and poly-PR inclusions were found to be more frequent in the granular layer of cerebellum and CA3/4 regions, respectively, in C9orf72-FTD compared to *C9orf72*-ALS and *C9orf72*-related ALS-FTD patients (Schludi et al., 2015). In contrast, DPRs were rarely observed in the brainstem and spinal cord. Arginine rich DPRs (poly-PR and poly-GR) have been documented to be toxic and contributory to neurodegeneration although these studies were performed on primary neuronal cultures and animal models (May et al., 2014; Mizielinska et al., 2014; Moens et al., 2017). DPR inclusions stain positive for p62 (SQSTM1), which is a component of ubiquitin-proteasome system (and which is also mutated in ALS-FTD) whilst TDP-43 pathology is found to be variable, with TDP-43 inclusions not always present in the same neurons as DPR inclusions (Cooper-Knock et al., 2012, 2015a; Mann et al., 2013; Schludi et al., 2015; Hautbergue et al., 2017).

In a *Drosophila* model of *C9orf72*-related ALS and FTD, it was demonstrated that neurodegeneration was mediated through DPRs rather than RNA foci (Mizielinska et al., 2014; Tran et al., 2015). Firstly, only pure repeats rather than stop-codon interrupted "RNA only" repeats led to a neurodegenerative-like phenotype in the flies (Mizielinska et al., 2014). A second study generated several uninterrupted repeat constructs including 5, 12, 40, and 160 G4C2 repeats with flanking intron and exon sequences (Tran et al., 2015). Although the fly expressing this 160 G4C2 repeat formed abundant RNA foci in neurons and glia, no DPR were produced and it did not develop neurotoxicity (Tran et al., 2015). In contrast, BAC transgenic mice models of *C9orf72*-related ALS-FTD showed RNA foci and DPR inclusions without development of a neurodegenerative-like phenotype or behavioral abnormalities (O'Rourke et al., 2015; Peters et al., 2015). Recently, a DPR-only mouse expressing poly$(GA)_{149}$ conjugated to cyan fluorescent protein developed p62-positive poly-GA inclusions in motor neurons and interneurons of spinal cord, brain stem and in cerebellar nuclei, with motor deficits occurring at 4 months. However, there was no impairment to learning and memory (Schludi et al., 2017).

Expansions of G4C2 which are translated to form DPRs have been shown in *Drosophila* models and *C9orf72*-ALS iPSC derived neurons to disrupt nucleocytoplasmic transport (NCT), both export of nuclear RNA and import of nuclear proteins, through binding to many of the nuclear pore complex proteins (Freibaum et al., 2015). RanGAP, a key regulator of nucleocytoplasmic transport, was found to bind to both the G4C2 RNA and the DPR protein poly(GA) thereby causing defective nucleocytoplasmic transport in *Drosophila* and mouse models of *C9orf72*-ALS and iPSC derived neurons (Zhang et al., 2015, 2016). Poly(PR) and poly(GR) were also found to interact with RNA binding proteins and other low complexity domain proteins, including those in the nuclear pore complex (Lee et al., 2016) and using *Xenopus laevis* oocytes, poly(PR) was shown to bind and block the central channel of the pore (Shi et al., 2017). However, in the SHSY-5Y neuronal cell line and iPSC-derived neurons, it was shown that poly(GR) and poly(PR) had no effect on active nucleocytoplasmic transport, though poly(GA) deficits were observed (Vanneste et al., 2019). Thus, there are likely to be specific pathogenic mechanisms associated with the different DPRs.

In summary, many studies have been conducted to elucidate the pathogenesis of the *C9orf72* HRE in causing neurodegeneration and cognitive dysfunction without obtaining clear conclusions. Several factors including background of the animal studies, overexpression of mutation and experimental design can contribute to the variable results. It remains to be determined whether loss of C9orf72 protein function and toxic gain of function by RNA foci formation and DPR inclusion act in a concerted manner to manifest neurodegeneration in ALS and patient-derived cell models may be a more appropriate model for addressing these questions as they carry the HRE in a natural genetic context and the protein is expressed at physiological levels. However, a recent paper described that knocking down one or both endogenous *C9orf72* alleles in transgenic mice expressing either 66 repeats or 450 repeats led to reduced autophagy and enhanced DPR accumulations, cognitive deficits, hippocampal neuron loss and glial activation (Zhu et al., 2020). Thus, this work demonstrated that there is a synergy between the loss of C9orf72 protein and the toxic gain of function mechanisms. In addition, these mechanisms offer targets for novel therapeutics and the antisense oligomer strategy currently in clinical trials may offer a disease-modifying therapy.

FTDALS2: Coiled-Coil Helix Coiled-Coil Helix Domain Containing Protein 10 (CHCHD10)

CHCHD10 was initially associated with ALS when it was shown to segregate with disease in a family presenting with a complex phenotype including ALS, FTD, cerebellar ataxia, and myopathy (Bannwarth et al., 2014). Subsequently, several cohorts of ALS and ALS-FTD patients were screened for *CHCHD10* mutations and a number of candidate rare, predicted deleterious mutations were identified (Johnson et al., 2014; Dols-Icardo et al., 2015). However, it has been shown more recently that many of the proposed mutations are present at similar frequency in controls (Marroquin et al., 2016), perhaps because exome sequencing studies typically give poor coverage of the *CHCHD10* gene which leads to a propensity for false positives. Whole genome sequencing, where coverage of the *CHCHD10* is complete, revealed that there was no significant burden of disease-associated mutations in sporadic ALS patients (Project MinE ALSSC, 2018). In fact, with increased coverage of control cohorts most of the ALS-associated mutations have been found at comparable frequency in controls despite *in vitro* and *in vivo* evidence for toxicity. Of the remaining mutations there is a

notable association with complex phenotypes including motor neuron degeneration but not typical ALS. The exception is c.44G > T (p.Arg15Leu) which has been identified in both sporadic and familial ALS cases with variable penetrance but is virtually absent in control databases (Project MinE ALSSC, 2018). Some of these cases have additional phenotypes such as hearing loss which may represent a distinct pathogenic process. However, assessment of TDP-43 pathology in these cases, which is arguably the molecular hallmark of ALS, is not yet available. CHCHD10 is localized to the mitochondria and patients with mutations in *CHCHD10* demonstrated abnormal mitochondrial morphology and respiratory chain deficiencies (Bannwarth et al., 2014).

FTDALS3: Sequestosome 1 (*SQSTM1*):

SQSTM1, also known as p62, is a ubiquitin binding protein that is present in a variety of ubiquitinated inclusions associated neurodegenerative diseases including ALS and FTD. Mutations in this gene were originally associated with Paget's disease of bone (PDB), a progressive skeletal disorder associated with an increased bone turnover producing localized lesions and bone pain (Rea et al., 2014). Missense mutations in the ubiquitin associated domain (UBA) or truncation mutations that cause partial or complete remove of the UBA account for 25–50% of familial PDB and 5–10% of sporadic PDB. However, the mutations in *SQSTM1* associated with ALS, FTD, and ALS-FTD cases are found throughout the gene, including the UBA, thereby impacting on many of the other pathways that the SQSTM1 protein participates in Rea et al. (2014). Functional domains include a light chain 3 (LC3) interacting region (LIR) that interacts with LC3 to promote autophagy, a KEAP1 interacting region (KIR) which binds KEAP1 competitively with NRF2 to regulate oxidative stress response and the PB1 domain, that interacts with several proteins which impact on neuronal survival and inflammation (Ma et al., 2019). Whilst *SQSTM1* mutations have been found in individuals who also carry a *C9orf72* expansion (Almeida et al., 2016; Kovacs et al., 2016), the pathogenicity of *SQSTM1* mutations has been demonstrated in zebrafish, which showed behavioral abnormalities as well as disrupted autophagy and shorter axons following knockdown of the *SQSTM1* ortholog. Importantly, these features were rescued by human SQSTM1 protein but not by the p.P392L common SQSTM1 mutation (Lattante et al., 2015). In addition, the KEAP1-NRF2 signaling pathway and oxidative response has been shown to be disrupted by *SQSTM1* mutations (Deng et al., 2019; Foster et al., 2019), a pathway originally found to be dysregulated in SOD1-ALS (Kirby et al., 2005), which was subsequently implicated in sALS (Sarlette et al., 2008). Finally, it has recently been shown that SQSTM1 co-localizes with misfolded MAPT and degrades the insoluble forms of the protein (Xu et al., 2019). In transgenic rTg4510 mutant MAPT mice, AAV-*SQSTM1* was administered to increase SQSTM1 protein expression and this resulted in reduced mutant insoluble MAPT and improved pathology, including reduced astrogliosis and microgliosis.

FTDALS4: TANK-Binding Kinase (*TBK1*)

TBK1 mutations were first linked to ALS (Cirulli et al., 2015; Freischmidt et al., 2015), FTD (Gijselinck et al., 2015; Le Ber et al., 2015) and ALS-FTD in 2015 (Pottier et al., 2015). Loss of function (LoF) mutations, including frameshifts, splice-site alterations, read-throughs and nonsense mutations have been reported to show definite or probable pathogenicity. The pathogenicity of missense mutations is less certain as some have also been found in controls (van der Zee et al., 2017) but such mutations in functional domains which impair target protein binding, or target or TBK1 phosphorylation, can also cause loss of function at the protein level (Freischmidt et al., 2015; Pozzi et al., 2017; van der Zee et al., 2017; de Majo et al., 2018). In addition, both LoF and missense mutations increase the risk of ALS/FTD (odds ratio 11.78 and 1.62, respectively) (Cui et al., 2018).

The mutation frequency of *TBK1* in ALS, FTD and ALS-FTD is reported to be from 0.4 to 1.7% (Gijselinck et al., 2015; van der Zee et al., 2017). More recently, a meta-analysis found LoF mutations in 1.0% and missense mutations in 1.8% of ALS and/or FTD, and suggests a higher prevalence in European populations compared to Asian populations (Cui et al., 2018). A separate paper also showed *TBK1* mutations to be the most important cause of ALS-FTD after *C9orf72* (Dols-Icardo et al., 2018).

TBK1 codes the TBK1 (TANK-binding kinase 1) protein, a kinase which binds and phosphorylates proteins involved in innate immunity (Pilli et al., 2012), autophagy (Korac et al., 2013), and mitophagy (Heo et al., 2015). Protein targets include optineurin (*OPTN*) (ALS12) and p62 (*SQSTM1*) (FTDALS3), two ALS-FTD associated genes (Maruyama et al., 2010; Rea et al., 2014) and mutations in both of these genes have been found along with *TBK1* mutations in patients (Pottier et al., 2015; Borghero et al., 2016; Dols-Icardo et al., 2018; Lattante et al., 2019).

TBK1 mutations have also been identified alongside the *C9orf72* expansion (Gijselinck et al., 2015; van der Zee et al., 2017), and mutations in *FUS* (Lattante et al., 2019), *TARDBP* (Freischmidt et al., 2015; de Majo et al., 2018), or *DCTN1* and *FUS* together (Muller et al., 2018). Interestingly, those harboring *TBK1* and *TARDBP* (de Majo et al., 2018) or *TBK1* and *FUS* (Freischmidt et al., 2015) mutations, showed earlier disease onset than those with *TBK1* alone (Freischmidt et al., 2015; Pozzi et al., 2017). One case showing *TBK1* and *C9orf72* mutations had a later disease onset; however, this was attributed to a shorter *C9orf72* expansion size of 59 repeats and variable penetrance of the *TBK1* mutation (Gijselinck et al., 2015). No further genotype-phenotype associations have been identified between *TBK1* mutation type or position, and clinical phenotype. Mutations occur throughout the *TBK1* gene, though missense variants cluster within the kinase and ubiquitin like domains (de Majo et al., 2018). *TBK1*-linked clinical phenotypes show variable age of onset, different rates of progression, and survival length (Gijselinck et al., 2015; Borghero et al., 2016; Pozzi et al., 2017; van der Zee et al., 2017; de Majo et al., 2018; Weinreich et al., 2019).

Cyclin F (*CCNF*)

Mutation of *CCNF* was first identified using genome-wide linkage followed by exome sequencing within a large ALS-FTD pedigree (Williams et al., 2016). Subsequently additional variants in both ALS and FTD cases were identified accounting for 0.6–3.3% of fALS-FTD patients among different populations (Williams et al., 2016; Pan et al., 2017). Cyclin F, encoded by *CCNF*, is one

of the components of an E3 ubiquitin-protein ligase complex also known as $SCF^{Cyclin\,F}$ (Skp1-Cul1-F-box E3 ubiquitin ligase complex) (Galper et al., 2017). Mutation of *CCNF* in neuronal cells causes errors in ubiquitination leading to ubiquitinated protein accumulation of $SCF^{CyclinF}$ and TDP-43 as well as impairment of autophagosome-lysosome fusion (Williams et al., 2016; Lee et al., 2018). Recently, a *CCNF* mutation in a zebrafish model has been shown to have disrupted axonal outgrowth (Galper et al., 2017; Hogan et al., 2017). Further support for *CCNF* as an ALS-FTD gene comes from the finding that CCNF interacts with valosin containing protein (VCP) (ALS14), increasing VCP's ATPase activity, which in turn promotes TDP-43 aggregation (Yu et al., 2019). Thus, for the purposes of this review, *CCNF* is described as FTDALS5.

ALS GENES SUBSEQUENTLY ASSOCIATED WITH ALS-FTD AND FTD

There are many genes which have been characterized as causative for ALS where potentially pathogenic variants have also been described in FTD cases. Whilst this is rare, the co-occurrence of ALS and FTD being associated with mutations in these genes further strengthens the genetic linkage between these two disorders. These genes encode proteins associated with autophagy/ubiquitin proteasome system (UPS) or RNA processing. The exception is *TUBA4A*, which encodes a microtubule associated protein. In addition, intermediate CAG repeat expansions in *ATXN2* have been reported as a risk factor in ALS and ALS-FTD and a disease modifier in both ALS and FTD.

ALS6: Fused in Sarcoma (*FUS*)

Fused in srcoma was initially identified as part of a fusion oncogene, *FUS-CHOP*, resulting from a *t*(12;16) (q13;p11) translocation event in malignant liposarcoma (Crozat et al., 1993; Rabbitts et al., 1993). Located at chromosome 16p11.2, *FUS* encodes a predominantly nuclear DNA/RNA binding protein which belongs to the FET protein family. As a functional component of the hnRNP complex, FUS is involved in many RNA processing activities, including transcription regulation, RNA transport and trafficking, pre-mRNA splicing, and miRNA processing. *FUS* consists of 15 exons which encode 526 amino acids. FUS has a multidomain structure consisting of an N-terminal glutamine-glycine-serine-tyrosine (QGSY) domain, three arginine-glycine-glycine rich domains (RGG1-3), an RRM, zinc finger motif (ZnF), and a highly conserved C-terminal NLS (Deng et al., 2014).

Mutations in *FUS* were first associated with autosomal recessive fALS, with additional screening revealing mutations in *FUS* to be causal in autosomal dominant ALS (Kwiatkowski et al., 2009; Vance et al., 2009). Further studies have shown that *FUS* mutations account for approximately 4% of fALS cases, and 1% of sALS cases. The vast majority of mutations are missense, with in-frame insertions and deletions occurring rarely. Although ALS-associated mutations occur throughout the whole length of the gene, most mutations are located in exons 3–6, encoding the N-terminal transcriptional activation domain, QGSY, and the nucleic acid binding domain RGG1, or in exons 12–15 which encode C-terminal nuclear binding domains RGG2 and RGG3, a ZnF domain and an NLS domain (Deng et al., 2014). Mutations within exons 12–15 have been found to be functional, whilst those in exons 3–6, which are also more commonly found in sALS, do not always segregate with disease. This indicates the presence of non-pathogenic variations, and incomplete penetrance, highlighting the complexity of the role of *FUS* in ALS pathogenesis. Screening of FTD patients subsequently identified several FUS mutations in patients with bv-FTD either with or without concurrent ALS though the frequency of FUS mutations is much rarer in FTD than ALS cases (Ticozzi et al., 2009; Blair et al., 2010; Van Langenhove et al., 2010; Huey, Ferrari et al., 2012).

Fused in srcoma plays an important role in RNA processing. Therefore, mutations in *FUS* have a negative impact on RNA transcription, alternative splicing, and mRNA transport and stabilization. It is evident that this results in widespread neuronal dysfunction, contributing to the ALS phenotype, although, how this occurs is not well understood. Several theories have been proposed, including gain- and loss-of-function mechanisms (Deng et al., 2014). Wild-type FUS is predominantly located in the nucleus, however, disease-causing mutations in the C-terminal NLS of *FUS*, including the most common *FUS* mutation, p.R521C, leads to FUS-positive neuronal cytoplasmic inclusions. The accumulation of FUS aggregates has been found in the neuronal cytoplasm and dendrites of ALS and FTLD patients. This disruption of nuclear import may result in toxic gain of cytoplasmic function and loss of nuclear function (Deng et al., 2014; Lopez-Erauskin et al., 2018). FUS inclusions have also been found in atypical FTLD cases (aFTLD-U) as one of the proteins in the ubiquitinated neuronal inclusions, as well as being found in glial cells (Neumann et al., 2009). None of these cases had mutations in the *FUS* gene.

Fused in srcoma was first found to have a role in RNA transcription when nuclear depletion of RNA polymerase II (RNAPII) resulted in an increase in cytoplasmic FUS (Zinszner et al., 1997). Further studies demonstrated the role of FUS in pre-mRNA splicing. FUS mediates the interaction between RNAPII and U1 snRNP, a splicing factor responsible for recognizing the 5′ splice junction (Yu and Reed, 2015). Beyond this, FUS has been identified as component of the spliceosome, and also interacts with other important splicing factors such as hnRNPA1 (Rappsilber et al., 2002; Zhou et al., 2013; Kamelgarn et al., 2016). Loss of FUS functionality affects the splicing of its target genes, contributing to widespread splicing dysfunction of genes involved in neuronal functions, such as *PPP2R2C* which is required for neurogenesis and *ACTL6B* which has a role in dendritic development (Reber et al., 2016). Beyond this, *FUS* mutations also result in the mislocalisation of U1 snRNP to the cytoplasm, and the aggregation of FUS, hnRNPA1, hnRNPA2 and SMN1 into stress granules (Takanashi and Yamaguchi, 2014; Yu et al., 2015).

Transcriptome analysis of human MNs generated from mutant FUS iPSCs, identified changes in expression levels of genes involved in cellular processes which have previously been associated with neurodegenerative disease, including cell

adhesion. Also, TAF15 which is also a member of the FET family was found to be differentially expressed in FUS mutant MNs (De Santis et al., 2017). More recently, mutant FUS has been shown to affect important processes vital for neuronal functionality in mice. Studies using transgenic mice demonstrated that ALS/FTD-linked mutant FUS accumulates within axons, reducing intra-axonal translation which, in turn, causes early activation of the integrated stress response (ISR) and increased phosphorylation of eIF2α. Ultimately, this inhibits the protein synthesis of important RNAs, including those encoding ion channels and transporters essential for synaptic function (Lopez-Erauskin et al., 2018). Furthermore, suppressed protein synthesis and disrupted regulation of nonsense medicated decay was detected in fibroblast cells derived from FUS-related ALS cases (Kamelgarn et al., 2018).

ALS10: TAR DNA Binding Protein (*TARDBP*)

TARDBP is located on chromosome 1p36.22 and encodes the transactive response DNA-binding protein 43 (TDP-43). Like FUS, TDP-43 is a predominantly nuclear DNA/RNA binding protein which is a member of the heterogeneous nuclear ribonucleoprotein (hnRNP) family (Sreedharan et al., 2008). The *TARDBP* gene consists of 6 exons, and has a similar structure to *FUS*; an N-terminal domain (NTD), 2 RNA recognition motifs (RRM1-2) which are involved in RNA and DNA binding, a nuclear localization signal and nuclear export signal, and a C-terminal glycine-rich domain (GRD) which is responsible for protein–protein interactions (Lagier-Tourenne et al., 2010; Baralle et al., 2013). TDP-43 was initially recognized as a transcription repressor protein which binds to the TAR regulatory element of human immunodeficiency virus-1 (HIV-1) (Ou et al., 1995). Further investigations have shown that TDP-43 has other important roles in RNA processing, including RNA transcription, pre-mRNA and pre-miRNA splicing, RNA transport and mRNA stability (Scotter et al., 2015).

Mutations in *TARDBP* are responsible for 4–5% of fALS cases and 1% of sALS cases, and are inherited in an autosomal dominant manner (Millecamps et al., 2010). *TARDBP* mutations cause an ALS phenotype consisting of classic ALS symptoms. Mutations in *TARDBP* have also been reported in patients with FTD, with and without ALS (Benajiba et al., 2009; Borroni et al., 2009; Kovacs et al., 2009; Pesiridis et al., 2009). The frequency of *TARDBP* mutations in patients with FTD is estimated at 1%, the majority presenting with bvFTD, though some patients do present with svFTD or nfvFTD at onset (Caroppo et al., 2016).

The majority of mutations are located in exon 6, which encodes the aggregation-prone C-terminal GRD. These mutations increase the aggregation potential of this protein. Ubiquitinated aggregates of TDP-43 are found in the cytoplasm of MNs of ALS and FTD patients, not just patients with *TARDBP* mutations (Neumann et al., 2006; Mackenzie and Rademakers, 2008; Johnson et al., 2009; Kirby et al., 2010). Given that 97% of fALS and sALS patients are positive for TDP-43 cytoplasmic inclusions, it is evident TDP-43 plays an important role in MN degeneration and disease pathogenesis (Sreedharan et al., 2008; Qin et al., 2014). In addition, TDP-43 positive inclusions are also found in 50% of FTLD cases (Neumann et al., 2006; Mackenzie et al., 2010). Although it is unknown how this occurs, it has been hypothesized that this may be due to toxic gain of cytoplasmic function and loss of nuclear function (Kabashi et al., 2010).

TDP-43 is functionally homologous to FUS and also has important functions in RNA metabolism. Mutations in *TARDBP* result in aberrant RNA processing on multiple levels; transcription regulation, alternative splicing and mRNA stability (Buratti and Baralle, 2008). Beyond regulating its own expression level by binding to the 3′ untranslated region (3′ UTR) of its mRNA, TDP-43 is also essential for maintaining normal expression levels and splicing patterns of over 1,000 mRNAs (Ayala et al., 2008; Polymenidou et al., 2011). Specifically, TDP-43 dysfunction results in the dysregulated expression of other ALS-associated proteins which also have roles in RNA metabolism, including FUS, ATXN2, and progranulin (PGRN) (Polymenidou et al., 2011; Sephton et al., 2011; Highley et al., 2014). Furthermore, dysfunction of TDP-43 results in defective alternative splicing of its target genes, including *hnRNAPA1,* which negatively impacts cellular stability (Butti and Patten, 2018). Additionally, TDP-43 is involved in the splicing of cryptic exons of particular mRNAs, such as *ATG4B* (autophagy related 4B cysteine peptidase). Splicing of cryptic exons produces aberrant mRNA products. These have been observed in the CNS of ALS and FTD patients and have been recently been linked to impaired autophagy (Ling et al., 2015; Torres et al., 2018). It is known that TDP-43 is a component of stress granules, but how this contributes to the ALS phenotype is unknown (Aulas and Vande Velde, 2015).

ALS12: Optineurin (OPTN)

Amyotrophic lateral sclerosis-associated mutations in *OPTN,* which was previously implicated in glaucoma, were first identified in 2010 in six affected members of a Japanese pedigree with consanguineous marriages presenting with three different types of mutations: a homozygous deletion of exon 5, a homozygous nonsense p.Q398X mutation and a heterozygous missense p.E478G mutation (Maruyama et al., 2010). Subsequently, more than 20 mutations have been described although not all have been investigated in *in vitro* and *in vivo* disease models. The incidence of *OPTN* mutations in FTD is still under debate, as one study reported copy number variants in *OPTN* in 4.8% of FTD cases (Pottier et al., 2015) while another study, recruiting a larger cohort of 371 FTD cases, did not detect any mutations using whole exome sequencing (Rollinson et al., 2012). More recently, a patient with ALS-FTD was reported with compound heterozygous mutations, resulting in a 75–80% reduction in OPTN (Pottier et al., 2018).

OPTN is a highly conserved hexameric protein that is ubiquitously expressed with significantly high expression in skeletal muscles (Toth and Atkin, 2018). OPTN is known to interact with TBK1 (FTDALS4); in fact, a series of evolutionarily conserved serine residues precedes the hydrophobic core sequence in OPTN which bears homology to TBK1-binding site of TANK, another substrate of TBK1 (Wild et al., 2011).

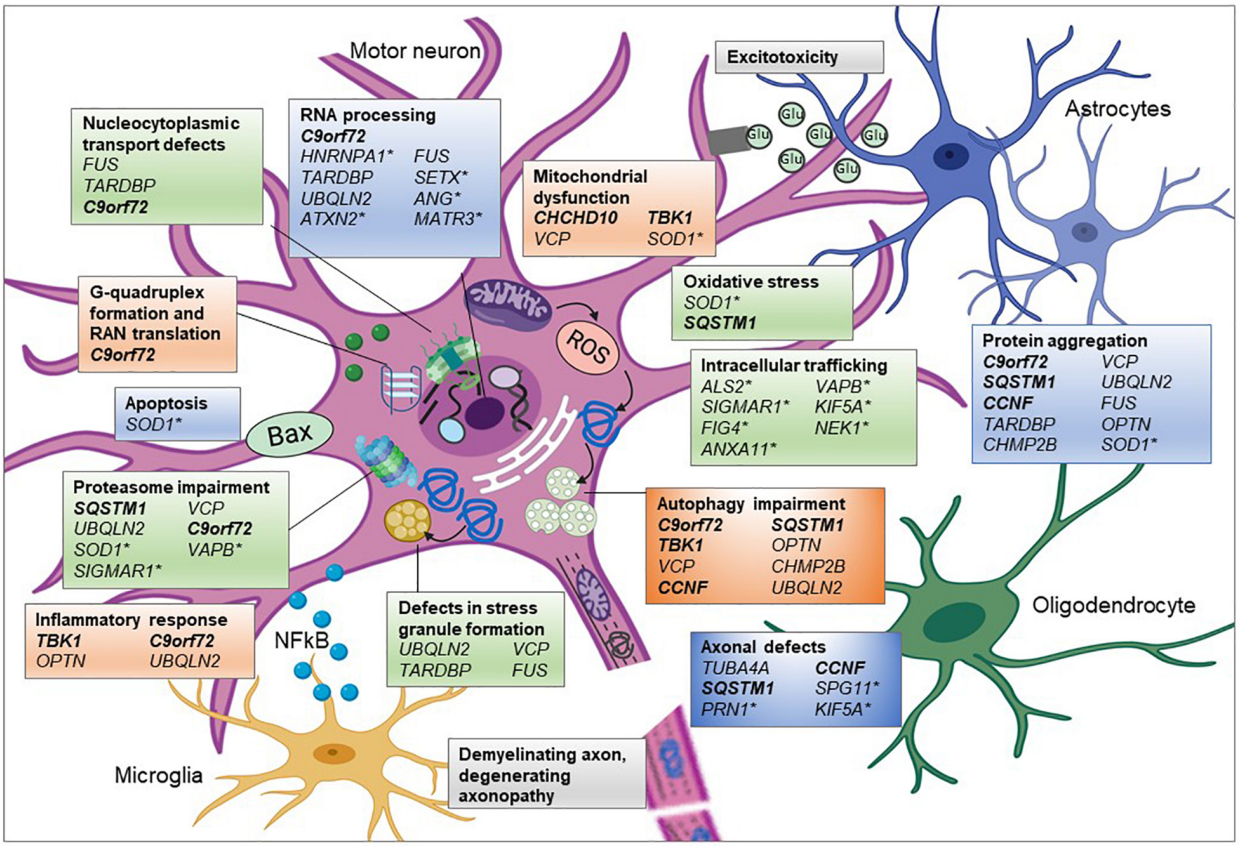

FIGURE 2 | Pathogenic pathways associated with genetic variants of ALS. ALS is a complex disease affecting multiple interconnecting cellular pathways and dysfunction of these pathways has been associated with many of the genetic mutations. Proposed aberrant mechanisms include abnormal nucleocytoplasmic transport of RNA and RNA binding proteins (RBP) and altered RNA metabolism resulting from mislocalisation of RBPs. Mutations in RBP can undergo liquid-liquid phase separation thereby altering stress granule formation and propagating cytoplasmic protein aggregation. Overload of these misfolded proteins could burden the proteasome-ubiquitin system affecting timely clearance of abnormal proteins and downstream processes such as autophagy. Protein aggregation could influence microtubule dynamics resulting in abnormal anterograde and retrograde axonal transport of vesicular cargoes and mitochondria. See text for full information on how each of these genes associates with the mechanism shown. Glu, glutamate; genes in bold and italic, ALS-FTD genes;* = genes only associated with ALS and the remainder are associated with ALS and FTD in differing proportions. Created using Biorender.com.

OPTN is involved in several cellular functions including autophagy, vesicular trafficking, Golgi maintenance [as evident from Golgi apparatus fragmentation in spinal motor neurons and glia in post mortem tissue obtained from an ALS-FTD patient (Kamada et al., 2014) and neuroinflammation (Toth and Atkin, 2018; McCauley and Baloh, 2019)]. OPTN has also been shown to regulate NFκB signaling wherein ALS associated mutations in *OPTN* showed increased immunoreactivity of microglia (McCauley and Baloh, 2019). OPTN-positive cytoplasmic inclusions in the CNS are not only seen in cases with *OPTN* mutations, but also in *C9orf72*, *FUS,* and *SOD1*-related cases (Bury et al., 2016). Interestingly, conditional loss of OPTN by Cre-loxP system in different cell types using a murine model (*Cnp-cre, Lyz2-cre, Gfap-cre,* and *Mnx1-cre* mice) showed RIPK1-mediated necroptosis resulting in axonal myelination pathology when OPTN was depleted in oligodendrocytes and myeloid cells, whereas no pathology was observed when OPTN expression was selectively removed in astrocytes and motor neurons (Ito et al., 2016), further confirming non-cell autonomous toxicity in driving neurodegeneration.

ALS15: Ubiquilin 2 (*UBQLN2*)

Mutations in *UBQLN2,* which is localized on the X chromosome, were first identified in large ALS-FTD family in 2011 (Deng et al., 2011). Four mutations located within the proline-X-X (PXX) repeat region of the protein were subsequently found through additional screening of fALS cases with no male to male transmission. Further variants within or adjacent to the PXX repeat region have been identified in ALS, FTD, or ALS-FTD patients, though at rare frequencies (Williams et al., 2012; Gellera et al., 2013; Ugwu et al., 2015). As a member of the ubiquilin family, the protein is actively associated in the degradation of misfolded and redundant proteins through macroautophagy and the ubiquitin-proteasome system (Renaud et al., 2019). Mutations cause defective binding to the proteasome leading to interruption of the protein degradation, triggering mislocalisation of OPTN from Rab-11 positive endosomal

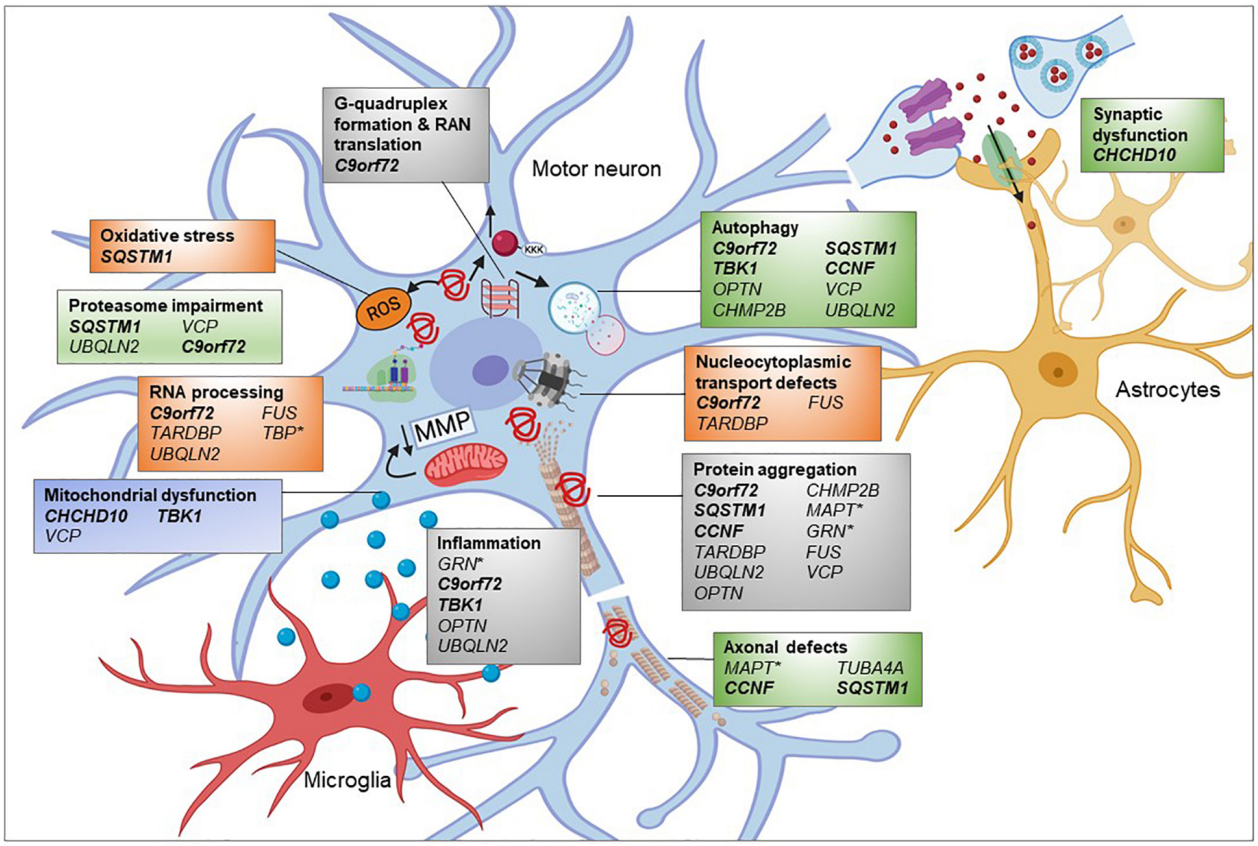

FIGURE 3 | Pathogenic pathways associated with genetic variants of FTD. FTD is a complex disease attributed to multiple genetic mutations affecting several cellular pathways. Many of the proteins encoded by these genes are found to be aggregated into inclusions. Overload of these misfolded proteins might impair the proteasome and autophagy, affecting timely clearance of abnormal proteins. Microtubule dysfunction results in abnormal anterograde and retrograde axonal transport of vesicular cargoes and mitochondria and axonal degeneration. Decreased mitochondrial membrane potential (MMP) could damage mitochondrial function and subsequently promote exiting of TDP-43 from the nucleus contributing to cytoplasmic TDP-43 inclusions. RNA processing and nucleocytoplasmic transport defects impact upon many cellular pathways, depending on the regulatory RNAs and proteins being affected. Mutations in FTD-related genes such as progranulin (GRN) could promote inflammatory response by microglia which is toxic to neuronal health. Genes in bold and italic = ALS-FTD genes,* = genes only associated with FTD and the remainder are associated with ALS and FTD in differing proportions. Created using Biorender.com.

vesicles as well as loss of binding of UBQLN2 to hnRNP proteins, including hnRNPA1 (ALS20) resulting in impaired RNA metabolism (Chang and Monteiro, 2015; Gilpin et al., 2015; Osaka et al., 2015). ALS-linked mutations in UBQLN2 gene were also found to be associated with dysfunction of autophagy, neuroinflammation, as well as the formation of stress granules, where mutations disrupted interaction with FUS (ALS6) and FUS-RNA complexes (Picher-Martel et al., 2015; Hjerpe et al., 2016; Alexander et al., 2018; Dao et al., 2018; Renaud et al., 2019).

ALS22: Tubulin Alpha 4A (*TUBA4A*)

Mutations in Tubulin alpha 4A (*TUBA4A*) were identified as a very rare cause of ALS following the discovery of non-synonymous variants during whole exome sequencing of fALS index cases (Smith et al., 2014). Whilst patients had spinal-onset ALS, two cases developed FTD, whilst a third case had FTD in a first degree relative. Additional studies identified further cases of FTD and ALS with mutations in *TUBA4A*, though they were exceptionally rare, whilst other papers failed to find any *TUBA4A* mutations in ALS or FTD cohorts (Dols-Icardo et al., 2016; Perrone et al., 2017; Li et al., 2018). *TUBA4A* encodes an alpha tubulin subunit which combines with other alpha and beta tubulins to form microtubules. Mutant TUBA4A proteins showed altered incorporation into microtubules, thereby reducing the stability of the microtubule network in a dominant negative manner (Smith et al., 2014). Interestingly, a study identifying miR-1825 as decreased in both serum and plasma of sALS and fALS, was shown to directly target tubulin-folding co-factor b (TBCB) expression and this was associated with depolymerisation and degradation of TUBA4A protein in HEK293 cells (Helferich et al., 2018). Additional studies in zebrafish embryos expressing human TBCB displayed reduced levels of the TUBA4A zebrafish homolog and reduced axonal length and branching, whilst TBCB and TUBA4A proteins levels were inversely correlated in post-mortem brain cortex of fALS and sALS. Thus, *TUBA4A* is implicated not only through genetic mutations, but also by dysregulation of an upstream miRNA in both fALS and sALS cases.

TABLE 2 | Clinical phenotypes also associated with ALS and FTD related genes.

ALS loci	Gene name	Alternative clinical phenotypes	Inheritance
FTDALS2	*CHCHD1*	SMA, Jokela type (SMAJ); Myopathy isolated mitochondrial, autosomal dominant (IMMD)	AD AD
FTDALS3	*SQSTM1*	Paget disease of bone 3 (PDB3); Myopathy, distal, with rimmed vacuoles (DMRV); Neurodegeneration with ataxia, dystonia and gaze palsy, childhood onset (NADGP)	AD AD AR
FTDALS4	*TBK1*	Encephalopathy, acute, infection-induced 8 (IIAE8) (susceptibility to)	AD
ALS1	*SOD1*	Spastic tetraplegia and axial hypotonia, progressive (STAHP)	AR
ALS2	*ALS2*	Primary lateral sclerosis, juvenile (PLSJ); Spastic paralysis, infantile onset ascending (IAHSP)	AR AR
ALS4	*SETX*	Spinocerebellar ataxia, with axonal neuropathy 2 (SCAN2)	AR
ALS5	*SPG11*	Spastic paraplegia 11 (SPG11); Charcot-Marie-Tooth disease, axonal, type 2X (CMT2X)	AR AR
ALS6	*FUS*	Tremor, hereditary essential 4 (ETM4)	AD
ALS8	*VAPB*	Spinal muscular atrophy (SMA), late onset, Finkel type (SMAFK)	AD
ALS11	*FIG4*	Charcot-Marie-Tooth disease, type 4J (CMT4J); Yunis-Varon syndrome Polymicrogyria, bilateral temporo-occitpital (BTOP)	AR AR AR
ALS12	*OPTN*	Glaucoma, primary open angle (POAG); Glaucoma, normal tension (susceptibility to)	AD
ALS13	*ATXN2*	Spinocerebellar ataxia 2 (SCA2); Parkinson's disease, late onset (susceptibility to)	AD AD
ALS14	*VCP*	Charcot-Marie-Tooth disease, type 2Y (CMT2Y); Inclusion body myopathy with early onset Paget disease and frontotemporal dementia 1 (IBMPFD1)	AD AD
ALS16	*SIGMAR1*	SMA, distal, autosomal recessive 2 (DSMA2)	AR
ALS20	*hnRNPA1*	Inclusion body myopathy with early onset Paget disease and frontotemporal dementia 3 (IBMPFD3)	AD
ALS24	*NEK1*	Short-rib thoracic dysplasia 6, with or without polydactyly (SRTD6)	AR
ALS25	*KIF5A*	Spastic paraplegia 10 (SPG10); Myoclonus, intractable, neonatal (NEIMY)	AD AD

Data obtained from Gene-Phenotype relationship data available on OMIM (http://www.ncbi.nlm.nih.gov/omim).

ALS13: Ataxin 2 (*ATXN2*)

A CAG repeat expansion encoding a polyglutamine repeat is found in ataxin 2 (*ATXN2*), a ubiquitously expressed protein involved in RNA processing, stress granule formation, endocytosis, calcium signaling and controlling metabolism and energy balance. In the normal population, the size varies between 13 and 31 CAG repeats, though 22–23 repeat are the most common (Velazquez-Perez et al., 2017). Repeats of over 35 are associated with fully penetrant spinocerebellar ataxia 2, with those 32–34 showing variable penetrance. Following identification that ATXN2 interacts with TDP-43, intermediate repeats of 27–33 were found to be a risk factor for ALS (Elden et al., 2010), with the intermediate CAG repeat interrupted with a CAA codon (Corrado et al., 2011). Subsequently, a meta-analysis of 9 studies highlighted that whilst there was an increased risk of ALS from 29 CAG/CAA repeats, significance was only reached for 31–33 repeats (Neuenschwander et al., 2014). Interestingly, repeat sizes of 27–28 were found to lower risk of ALS. More recently, a meta-analysis of 16 published studies, along with two large unpublished cohorts of ALS demonstrated an increased risk of ALS with 29–32 CAG/CAA repeats, and this risk increased with the number of repeats (Sproviero et al., 2017). This study also found 27 repeats to have a protective effect.

Following the link with ALS, the role of *ATXN2* intermediate repeats in FTD was investigated. Screening of ALS and FTD alongside other neurodegenerative diseases identified 30–33 repeats to be associated with ALS but not FTD (Ross et al., 2011). Subsequently, *ATXN2* CAG repeats of ≥29 were also found to be associated with ALS and familial ALS-FTD but not sporadic ALS-FTD or FTD (Lattante et al., 2014). A further study of 368 cases also found no significant correlation between FTD and *ATXN2* CAG repeat size though they did find that intermediate repeats (≥27) were associated with an earlier age at onset of FTD (Rubino et al., 2019).

Screening of ATXN2 has also identified expansions >34 in rare cases of both ALS (Corrado et al., 2011; Ross et al., 2011; Van Damme et al., 2011) and FTD (Baumer et al., 2014; Fournier et al., 2018) although no signs of ataxia were reported and neuropathological examination confirmed a diagnosis of ALS. As well as interacting with TDP-43, ATXN2 has also shown to bind to mutant FUS, with intermediate repeats binding both WT and mutant FUS proteins (Farg et al., 2013).

FTD GENES SUBSEQUENTLY ASSOCIATED WITH ALS-FTD AND ALS

Several ALS genes identified through next generation sequencing have previously been identified as being associated with FTD or a syndrome incorporating FTD, such as inclusion body myopathy with Paget's disease of bone and frontotemporal dementia (IBMPFD). These include *VCP* and *CHMP2B*.

ALS14: Valosin Containing Protein (*VCP*)

Mutations in *VCP* have been described in ALS, FTD and inclusion body myopathy with Paget's disease of bone and FTD (IBMPFD) which is an adult onset disorder characterized by muscle weakness, early onset PDB (see section "Cyclin F (*CCNF*)") and FTD, though episodic memory is preserved (Kimonis, 1993). Mutations in VCP account for 1–2% of fALS cases, are found to be rare in sALS (Koppers et al., 2012) and whilst FTD is recognized in a third of IBMPFD patients,

mutations have been found in FTD cases (Saracino et al., 2018; Wong et al., 2018).

Valosin containing protein (also called as CDC48 or p97) is a hexameric ATPase that is ubiquitously expressed and involved in diverse cellular functions including autophagy, endoplasmic reticulum (ER)- associated degradation (ERAD), chromatin remodeling, DNA repair and other protein quality control pathways (Wang et al., 2016; Shahheydari et al., 2017). ATPase has two domains, D1 and D2 and a regulatory N-domain. A majority of the mutations in *VCP* have been documented in the N-domain in patients with ALS and/or FTD (Abrahao et al., 2016; Wang et al., 2016; Shahheydari et al., 2017) although additional ALS and FTD mutations have been reported in the D1 domain (Wong et al., 2018). A study reported that mutations in the N-domain, an evolutionarily conserved region in VCP, results in poor hexamer assembly and reduced small ubiquitin-like modifier (SUMO)-ylation of VCP that diminishes its recruitment to stress granules and consequently affects ERAD in a *Drosophila* model of ALS/FTD. In contrast, a recent study that screened 48 patients with FTD reported identified 3 mutations that lie within the D1 domain of VCP and are hypothesized to affect ATPase binding activity (Wong et al., 2018). Interestingly, it has been reported that VCP interacts with FUS (ALS6) (Wang et al., 2015) and Cyclin F (CCNF) (proposed FTDALS5) (Yu et al., 2019) both of which are implicated in ALS. Mutations in *FUS*/*CCNF* were shown to increase ATPase activity of VCP in the cytoplasm, causing VCP to mislocalize to the cytoplasm (Yu et al., 2019) and trigger accumulation of polyubiquitinated proteins (Wang et al., 2015). VCP is also vital in mitochondrial quality control and IBMFTD patient fibroblasts carrying a mutation in VCP showed uncoupling of mitochondria, reduced mitochondrial membrane potential and ATP production (Bartolome et al., 2013), a feature that is also evident in *SIGMAR1* (ALS16) mutations.

ALS17: Chromatin Modifying Protein 2B (*CHMP2B*)

Mutation of *CHMP2B* was initially identified in a large Danish family with FTD linked to chromosome 3 (termed FTD-3) (Skibinski et al., 2005). The splice site mutation c.532-1G > C results in the formation of two transcripts encoding two different proteins with a defective carboxyl terminus: $CHMP2B^{intron5}$, where the intronic sequence between exons 5 and 6 is retained and a single valine is incorporated instead of the final 36 amino acids encoded by exon 6 and $CHMP2B^{del10}$, where a cryptic splice site is used 10 bp from exon 6, resulting in the insertion of 29 novel amino acids. Subsequently, a Belgian family with FTD-3 was identified, where the c.493C > T mutation lead to truncation of the protein, with the loss of 49 amino acids (van der Zee et al., 2008). In contrast, mutations identified in *CHMP2B* that were associated with ALS were missense mutations (Parkinson et al., 2006).

CHMP2B is a component of the endosomal sorting complex required for transport III (ESCRT-III) complex, which is involved in the maturation of endosomes and autophagosomes. Using cellular and animal models, mutations in CHMP2B (both truncated and missense mutations) have been shown to disrupt endosomal-lysosomal trafficking, through accumulation and enlargement of endosomes (Cox et al., 2010; Zhang Y. et al., 2017; Vandal et al., 2018). The pathology of FTD-3 cases is distinguished by the presence of ubiquitin and p62 (*SQSTM1*; FTDALS3) positive inclusions, which are negative for TDP-43 and Tau (Holm et al., 2007).

OTHER ALS AND FTD GENES

In addition to those genes described above, there are other genes associated solely with fALS and fFTD, including "Pure" ALS genes such as SOD1 and "Pure" FTLD genes MAPT and Progranulin (Bennion Callister and Pickering-Brown, 2014). However, it is notable that many of these pure ALS genes also encode proteins that cluster into functional pathways associated with ALS-FTD genes, with mutations in the pure ALS gene affecting similar biological pathways. For example, numerous genes are associated with autophagy/proteasome impairment (*C9orf72, SQSTM1, TBK1, OPTN, VCP, UBQLN2*, and *CHMP2B*) and/or their proteins are found to be aggregated in cytoplasmic inclusions (C9orf72, SQSTM1, OPTN, VCP, UBQLN2, CCNF, FUS, TDP-43, and CHMP2B) (**Figure 2**). Many of these genes also encode proteins that have a role in RNA processing (*C9orf72*, *FUS, TARDBP*, and *UBQLN2*), whilst others are associated with and dysregulate the mitochondria (*CHCHD10; TBK1*; and *VCP*) or the cytoskeleton (*TUBA4A, CCNF*, and *SQSTM1*). SOD1-ALS, accounting for around 10% of fALS cases, is distinctive in that it is not associated with TDP-43 inclusions, unlike the majority of ALS cases. However, mutations in *SOD1* are associated with similar pathogenic mechanisms, such as disruption to protein quality control, mitochondrial dysfunction, dysregulated axonal transport and RNA processing, in addition to oxidative stress and excitotoxicity. Due to the wide range of biological pathways, gene silencing of SOD1 is currently in clinical trials as a therapeutic strategy for SOD1-ALS patients (van Zundert and Brown, 2017).

Many of the additional ALS genes can also be categorized into these pathways (**Table 1** and **Figure 2**), such as *SETX, ANG, ATXN2, hnRNPA1,* and *MATR3,* which are all involved in RNA processing and *SPG11, KIF5A,* and *PFN1* that are associated with the cytoskeleton and mutations in which cause axonal defects (Alsultan et al., 2016). Many of the genes also encode proteins involved in trafficking components within the cell, such as endosomes (*ALS2, FIG4*, and *NEK1*) or in the unfolded protein response (*VAPB* and *SIGMAR1*). However, as research has investigated the effect of mutations within these genes, additional secondary pathways have been implicated, such as mutant SOD1 protein's effect on protein homeostasis, gene expression and axonal transport, resulting in a complex interactome of direct and indirect effects, which ultimately lead to neurodegeneration.

Whilst there are far fewer genes associated with only FTD, *GRN* (responsible for 5–20% of fFTD) and *MAPT* (responsible for 10–20% of fFTD) are also involved in similar pathways (**Figure 3**). The progranulin gene (*GRN*) encodes a secreted glycoprotein that is taken up by the cell and cleaved into multiple smaller granulins. The precise function of granulin is

still to be determined, though it has been shown to be involved in multiple pathways including neuronal survival, neurite outgrowth, neuroinflammation, and autophagy (Olszewska et al., 2016). Mutations in *GRN,* leading to haploinsufficiency, are thought to cause FTD though lysosomal defects and reduced clearance of proteins (Ferrari et al., 2019). As with ALS, TDP-43 inclusions are also present, of the FTLD-TDP Type A form (Mackenzie et al., 2011). *MAPT*, encoding the microtubule associated protein tau, stabilizes microtubules through binding to tubulin. Mutations in *MAPT* disrupt this binding and lead to hyperphosphorylated tau aggregates. Recently, mutations in the TATA-box-binding gene (*TBP*), normally associated with spinocerebellar ataxia 17 (SCA17), were identified in a patient with FTD whose MRI showed cerebellar atrophy (Olszewska et al., 2019). The variant was found to co-segregate with disease. Thus, this new FTD gene, which encodes a transcription initiation factor can be categorized as an RNA processing gene.

The identification of a novel gene in a pathway that has not previously been associated with the genetics of ALS or FTD is particularly valuable for highlighting new disease biology and subsequently novel therapeutic targets. In this respect, the identification of mutations in *GLT8D1* and *DNAJC7* in fALS cases are notable discoveries. GLT8D1 is a glycosyltransferase with an enrichment of familial ALS-associated mutations proximal to the substrate binding site (Cooper-Knock et al., 2019). It was demonstrated that the mutations negatively impact enzyme activity suggesting a loss of function mechanism. Whilst the exact role of GLT8D1 remains to be discovered, however, glycosyltransferases are known to be involved in the synthesis of gangliosides which are signaling molecules important for motor neuron function (Harschnitz et al., 2014). As such, it is perhaps not surprising that glycosyltransferase dysfunction has already been associated with other neurodegenerative diseases such as Parkinson's, Huntington's and Alzheimer's disease.

DNAJC7 encodes a heat shock protein (HSP40) which alongside HSP70 facilitates protein homeostasis, through folding new peptides and removing misfolded proteins. Rare protein truncating mutations were identified in *DNAJC7* in ALS cases and were absent from controls and subsequent screening identified further loss of function mutations as well as several rare missense mutations, predicted to be damaging (Farhan et al., 2020). In fibroblasts from a patient with a p.Arg156Ter mutation, protein levels were reduced, suggesting that these mutations may lead to protein aggregation, a characteristic feature of ALS. Further screening of *DNAJC7* and *GLT8D1* in additional cohorts of ALS, ALS-FTD, and FTD cases will establish the contribution of these genes and the roles of their proteins in disease pathogenesis.

CONCLUSION

Amyotrophic lateral sclerosis and FTD have been described as forming a spectrum of disease, with converging mechanisms of neurodegeneration involving RNA processing, stress granules, protein aggregation and autophagy supporting this proposal (Ling et al., 2013; Deng et al., 2017; Nguyen et al., 2019; Baradaran-Heravi et al., 2020). However, it also clear that some genes, such as *MAPT*, are quite distinct and therefore it is argued that these should be kept separate (Hardy and Rogaeva, 2014). This distinction is also supported by the neuropathology, as whilst the majority of genetic (and sporadic) ALS cases have TDP-43-positive inclusions, along with *C9orf72* and *GRN*-FTD, *MAPT*- FTD does not, similar to *SOD1*-ALS. Thus, these distinctions are important to consider when pursuing diagnostic and prognostic biomarkers or therapeutic strategies.

The application of next generation sequencing, either in the form of targeted, whole exome or whole genome sequencing (WGS) has had a significant impact on the identification of genes associated with these diseases. However, it is also: (i) broadening the range of diseases that we see associated with variants in these genes (**Table 2**), (ii) broadening the range of genes that you would conventionally associate with ALS and FTD (Blauwendraat et al., 2018; Tripolszki et al., 2019), (iii) increasing the frequency of variants in known ALS and FTD genes within apparently sporadic cases, highlighting the variable penetrance of many of these proposed mutations (Tripolszki et al., 2019), (iv) identifying multiple variants in disease-associated genes within an individual (Cady et al., 2015), which will become increasingly important as personalized medicine based on your genetic mutation enters the clinic and finally (v) illustrating both the variability in frequencies of known genes across populations worldwide (Majounie et al., 2012; Wei et al., 2019), but also the inequality as the majority of these studies are undertaken in the northern hemisphere. It is hoped that WGS of large international cohorts of ALS and FTD such as Project MinE[2] and GENFI[3] will begin to fully understand the genetic contribution to disease and potentially answer why individuals with a particular variant go on to develop ALS, FTD or ALS-FTD.

AUTHOR CONTRIBUTIONS

RR, SH, KC, SS, JC-K, and JK wrote the sections of the manuscript. JK and RR designed and drew the figures. SH drafted **Table 1**. JK drafted **Table 2**. JK completed the review of all sections, final edits, and formatting. All authors contributed to the article and approved the submitted version.

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[2]www.projectmine.com

[3]http://genfi.org.uk/

REFERENCES

Abrahao, A., Abath Neto, O., Kok, F., Zanoteli, E., Santos, B., Acary, S. B. O., et al. (2016). One family, one gene and three phenotypes: a novel vcp (valosin-containing protein) mutation associated with myopathy with rimmed vacuoles, amyotrophic lateral sclerosis and frontotemporal dementia. *J. Neurol. Sci.* 368, 352–358. doi: 10.1016/j.jns.2016.07.048

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

Alexander, E. J., Ghanbari Niaki, A., Zhang, T., Sarkar, J., Liu, Y., Wang, J., et al. (2018). Ubiquilin 2 modulates als/ftd-linked fus-rna complex dynamics and stress granule formation. *Proc. Natl. Acad. Sci. U.S.A.* 115, E11485–E11494.

Almeida, M. R., Letra, L., Pires, P., Santos, A., Rebelo, O., Isabel, S., et al. (2016). Characterization of an ftld-pdb family with the coexistence of sqstm1 mutation and hexanucleotide (g(4)c(2)) repeat expansion in c9orf72 gene. *Neurobiol. Aging* 40:191.e1-191.e8.

Alsultan, A. A., Waller, R., Heath, P. R., and Kirby, J. (2016). The genetics of amyotrophic lateral sclerosis: current insights. *Degener. Neurol. Neuromuscul. Dis.* 6, 49–64.

Atanasio, A., Decman, V., White, D., Ramos, M., Ikiz, B., Bai, Y., et al. (2016). C9orf72 ablation causes immune dysregulation characterized by leukocyte expansion, autoantibody production, and glomerulonephropathy in mice. *Sci. Rep.* 6:23204.

Aulas, A., and Vande Velde, C. (2015). Alterations in stress granule dynamics driven by tdp-43 and fus: a link to pathological inclusions in als? *Front. Cell Neurosci.* 9:423. doi: 10.3389/fncel.2015.00423

Ayala, Y. M., Misteli, T., and Baralle, F. E. (2008). Tdp-43 regulates retinoblastoma protein phosphorylation through the repression of cyclin-dependent kinase 6 expression. *Proc. Natl. Acad. Sci. U.S.A.* 105, 3785–3789. doi: 10.1073/pnas.0800546105

Balendra, R., and Isaacs, A. M. (2018). C9orf72-mediated als and ftd: multiple pathways to disease. *Nat. Rev. Neurol.* 14, 544–558. doi: 10.1038/s41582-018-0047-2

Bang, J., Spina, S., and Miller, B. L. (2015). Frontotemporal dementia. *Lancet* 386, 1672–1682.

Bannwarth, S., Ait-El-Mkadem, S., Chaussenot, A., Genin, E. C., Lacas-Gervais, S., Brice, A., et al. (2014). A mitochondrial origin for frontotemporal dementia and amyotrophic lateral sclerosis through chchd10 involvement. *Brain* 137, 2329–2345. doi: 10.1093/brain/awu138

Baradaran-Heravi, Y., Van Broeckhoven, C., and van der Zee, J. (2020). Stress granule mediated protein aggregation and underlying gene defects in the ftd-als spectrum. *Neurobiol. Dis.* 134:104639. doi: 10.1016/j.nbd.2019.104639

Baralle, M., Buratti, E., and Baralle, F. E. (2013). The role of tdp-43 in the pathogenesis of als and ftld. *Biochem. Soc. Trans.* 41, 1536–1540. doi: 10.1042/bst20130186

Bartolome, F., Wu, H. C., Burchell, V. S., Preza, E., Wray, S., Colin, J. M., et al. (2013). Pathogenic vcp mutations induce mitochondrial uncoupling and reduced atp levels. *Neuron* 78, 57–64. doi: 10.1016/j.neuron.2013.02.028

Baumer, D., East, S. Z., Tseu, B., Zeman, A., Hilton, D., Kevin, T., et al. (2014). Ftld-als of tdp-43 type and sca2 in a family with a full ataxin-2 polyglutamine expansion. *Acta Neuropathol.* 128, 597–604. doi: 10.1007/s00401-014-1277-z

Benajiba, L., Le Ber, I., Camuzat, A., Lacoste, M., Thomas-Anterion, C., Camu, W., et al. (2009). Tardbp mutations in motoneuron disease with frontotemporal lobar degeneration. *Ann. Neurol.* 65, 470–473. doi: 10.1002/ana.21612

Baker, M., Mackenzie, I. R., Pickering-Brown, S. M., Gass, J., Rademakers, R., Lindholm, C., et al. (2006). Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. *Nature* 442, 916–919. doi: 10.1038/nature05016

Bennion Callister, J., and Pickering-Brown, S. M. (2014). Pathogenesis/genetics of frontotemporal dementia and how it relates to als. *Exp. Neurol.* 262(Pt B), 84–90. doi: 10.1016/j.expneurol.2014.06.001

Blair, I. P., Williams, K. L., Warraich, S. T., Durnall, J. C., Thoeng, A. D., Jim, M., et al. (2010). Fus mutations in amyotrophic lateral sclerosis: clinical, pathological, neurophysiological and genetic analysis. *J. Neurol. Neurosurg. Psychiatry* 81, 639–645. doi: 10.1136/jnnp.2009.194399

Blauwendraat, C., Wilke, C., Simon-Sanchez, J., Jansen, I. E., Reifschneider, A., Anja, C., et al. (2018). The wide genetic landscape of clinical frontotemporal dementia: systematic combined sequencing of 121 consecutive subjects. *Genet. Med.* 20, 240–249. doi: 10.1038/gim.2017.102

Borghero, G., Pugliatti, M., Marrosu, F., Marrosu, M. G., Murru, M. R., et al. (2016). Tbk1 is associated with als and als-ftd in sardinian patients. *Neurobiol. Aging* 43, e1–e5.

Borroni, B., Bonvicini, C., Alberici, A., Buratti, E., Agosti, C., Tea, B. C., et al. (2009). Mutation within tardbp leads to frontotemporal dementia without motor neuron disease. *Hum. Mutat.* 30, E974–E983.

Brenner, D., Müller, K., Wieland, T., Weydt, P., Böhm, S., Lulé, D., et al. (2016). Nek1 mutations in familial amyotrophic lateral sclerosis. *Brain* 139:e28. doi: 10.1093/brain/aww033

Brenner, D., and Weishaupt, J. H. (2019). Update on amyotrophic lateral sclerosis genetics. *Curr. Opin. Neurol.* 32, 735–739.

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

Buratti, E., and Baralle, F. E. (2008). Multiple roles of tdp-43 in gene expression, splicing regulation, and human disease. *Front. Biosci.* 13, 867–878. doi: 10.2741/2727

Bury, J. J., Highley, J. R., Cooper-Knock, J., Goodall, E. F., Higginbottom, A. (2016). Oligogenic inheritance of optineurin (optn) and c9orf72 mutations in als highlights localisation of optn in the tdp-43-negative inclusions of c9orf72-als. *Neuropathology* 36, 125–134. doi: 10.1111/neup.12240

Butti, Z., and Patten, S. A. (2018). RNA dysregulation in amyotrophic lateral sclerosis. *Front. Genet.* 9:712. doi: 10.3389/fgene.2018.00712

Cady, J., Allred, P., Bali, T., Pestronk, A., Goate, A., Robi, D. M., et al. (2015). Amyotrophic lateral sclerosis onset is influenced by the burden of rare variants in known amyotrophic lateral sclerosis genes. *Ann. Neurol.* 77, 100–113. doi: 10.1002/ana.24306

Caroppo, P., Camuzat, A., Guillot-Noel, L., Thomas-Anterion, C., Couratier, P., Serena, L., et al. (2016). Defining the spectrum of frontotemporal dementias associated with tardbp mutations. *Neurol Genet.* 2:e80. doi: 10.1212/nxg.0000000000000080

Chang, L., and Monteiro, M. J. (2015). Defective proteasome delivery of polyubiquitinated proteins by ubiquilin-2 proteins containing als mutations. *PLoS One* 10:e0130162. doi: 10.1371/journal.pone.0130162

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

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

Cirulli, E. T., Lasseigne, B. N., Petrovski, S., Sapp, P. C., Dion, P. A., Julien, C., et al. (2015). Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. *Science* 347, 1436–1441.

Ciura, S., Lattante, S., Le Ber, I., Latouche, M., Tostivint, H., Brice, A., et al. (2013). Loss of function of c9orf72 causes motor deficits in a zebrafish model of amyotrophic lateral sclerosis. *Ann. Neurol.* 74, 180–187.

Cooper-Knock, J., Bury, J. J., Heath, P. R., Wyles, M., Higginbottom, A., Robin, J. H., et al. (2015a). C9orf72 ggggcc expanded repeats produce splicing dysregulation which correlates with disease severity in amyotrophic lateral sclerosis. *PLoS One* 10:e0127376. doi: 10.1371/journal.pone.0127376

Cooper-Knock, J., Higginbottom, A., Stopford, M. J., Highley, J. R., Ince, P. G., Shaw, P. J., et al. (2015b). Antisense rna foci in the motor neurons of c9orf72-als patients are associated with tdp-43 proteinopathy. *Acta Neuropathol.* 130, 63–75. doi: 10.1007/s00401-015-1429-9

Cooper-Knock, J., Kirby, J., Highley, R., and Shaw, P. J. (2015c). The spectrum of c9orf72-mediated neurodegeneration and amyotrophic lateral sclerosis. *Neurotherapeutics* 12, 326–339. doi: 10.1007/s13311-015-0342-1

Cooper-Knock, J., Hewitt, C., Highley, J. R., Brockington, A., Milano, A., Man, S., et al. (2012). Clinico-pathological features in amyotrophic lateral sclerosis with expansions in c9orf72. *Brain* 135, 751–764. doi: 10.1093/brain/awr365

Cooper-Knock, J., Jenkins, T., and Shaw, P. J. (2013). Clinical and molecular aspects of motor neuron disease. *Colloquium Ser. Genomic Mol. Med.* 2, 1–60. doi: 10.4199/c00093ed1v01y201309gmm004

Cooper-Knock, J., Moll, T., Ramesh, T., Castelli, L., Beer, A., Fox, I., et al. (2019). Mutations in the glycosyltransferase domain of glt8d1 are associated with familial amyotrophic lateral sclerosis. *Cell Rep.* 26, 2298e–2306e.

Cooper-Knock, J., Walsh, M. J., Higginbottom, A., Robin Highley, J., Dickman, M. J., Paul, G. I., et al. (2014). Sequestration of multiple rna recognition motif-containing proteins by c9orf72 repeat expansions. *Brain* 137, 2040–2051. doi: 10.1093/brain/awu120

Corrado, L., Mazzini, L., Oggioni, G. D., Luciano, B., Godi, M., Brusco, A., et al. (2011). Atxn-2 cag repeat expansions are interrupted in als patients. *Hum. Genet.* 130, 575–580. doi: 10.1007/s00439-011-1000-2

Cox, L. E., Ferraiuolo, L., Goodall, E. F., Heath, P. R., Higginbottom, A., Heather, M., et al. (2010). Mutations in chmp2b in lower motor neuron predominant amyotrophic lateral sclerosis (als). *PLoS One* 5:e9872. doi: 10.1371/journal.pone.0009872

Crozat, A., Aman, P., Mandahl, N., and Ron, D. (1993). Fusion of chop to a novel rna-binding protein in human myxoid liposarcoma. *Nature* 363, 640–644. doi: 10.1038/363640a0

Cruts, M., Gijselinck, I., van der Zee, J., Engelborghs, S., Wils, H., Pirici, D., et al. (2006). Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature* 442, 920–924. doi: 10.1038/nature05017

Cui, R., Tuo, M., Li, P., and Zhou, C. (2018). Association between tbk1 mutations and risk of amyotrophic lateral sclerosis/frontotemporal dementia spectrum: a meta-analysis. *Neurol. Sci.* 39, 811–820. doi: 10.1007/s10072-018-3246-0

Curtis, A. F., Masellis, M., Hsiung, G. R., Moineddin, R., Zhang, K., Bonnie, A., et al. (2017). Sex differences in the prevalence of genetic mutations in ftd and als: a meta-analysis. *Neurology* 89, 1633–1642. doi: 10.1212/wnl.0000000000004494

Dao, T. P., Kolaitis, R. M., Kim, H. J., O'Donovan, K., Martyniak, B., Erica, C., et al. (2018). Ubiquitin modulates liquid-liquid phase separation of ubqln2 via disruption of multivalent interactions. *Mol. Cell.* 69:965-978.e6.

de Majo, M., Topp, S. D., Smith, B. N., Nishimura, A. L., Chen, H. J., Chun, H. W., et al. (2018). Als-associated missense and nonsense tbk1 mutations can both cause loss of kinase function. *Neurobiol. Aging* 71:266.e1-266.e10.

De Santis, R., Santini, L., Colantoni, A., Peruzzi, G., de Turris, V., Irene, B., et al. (2017). Fus mutant human motoneurons display altered transcriptome and microrna pathways with implications for als pathogenesis. *Stem Cell Rep.* 9, 1450–1462. doi: 10.1016/j.stemcr.2017.09.004

DeJesus-Hernandez, M., Mackenzie, I. R., Boeve, B. F., Boxer, A. L., Baker, M., Nicola, J. R., 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

Deng, H., Gao, K., and Jankovic, J. (2014). The role of fus gene variants in neurodegenerative diseases. *Nat. Rev. Neurol.* 10, 337–348. doi: 10.1038/nrneurol.2014.78

Deng, H. X., Chen, W., Hong, S. T., Boycott, K. M., Gorrie, G. H., Yang, Y., 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

Deng, Z., Lim, J., Wang, Q., Purtell, K., Wu, S., Tan, H., et al. (2019). Als-ftld-linked mutations of sqstm1/p62 disrupt selective autophagy and nfe2l2/nrf2 anti-oxidative stress pathway. *Autophagy* 16, 917–931. doi: 10.1080/15548627.2019.1644076

Deng, Z., Sheehan, P., Chen, S., and Yue, Z. (2017). Is amyotrophic lateral sclerosis/frontotemporal dementia an autophagy disease? *Mol. Neurodegener.* 12:90.

Dols-Icardo, O., Garcia-Redondo, A., Rojas-Garcia, R., Borrego-Hernandez, D., Illan-Gala, I., Luna, N. D., et al. (2018). Analysis of known amyotrophic lateral sclerosis and frontotemporal dementia genes reveals a substantial genetic burden in patients manifesting both diseases not carrying the c9orf72 expansion mutation. *J. Neurol. Neurosurg. Psychiatry* 89, 162–168. doi: 10.1136/jnnp-2017-316820

Dols-Icardo, O., Garcia-Redondo, A., Rojas-Garcia, R., Sanchez-Valle, R., Noguera, A., Pau, P., et al. (2014). Characterization of the repeat expansion size in c9orf72 in amyotrophic lateral sclerosis and frontotemporal dementia. *Hum. Mol. Genet.* 23, 749–754.

Dols-Icardo, O., Iborra, O., Valdivia, J., Pastor, P., Ruiz, A., Juan, F., et al. (2016). Assessing the role of tuba4a gene in frontotemporal degeneration. *Neurobiol. Aging* 38:215.e13-215.e14.

Dols-Icardo, O., Nebot, I., Gorostidi, A., Ortega-Cubero, S., Hernandez, I., Albert, L., et al. (2015). Analysis of the chchd10 gene in patients with frontotemporal dementia and amyotrophic lateral sclerosis from spain. *Brain* 138: e400.

Donnelly, C. J., Zhang, P. W., Pham, J. T., Haeusler, A. R., Mistry, N. A., Hoover, B., et al. (2013). Rna toxicity from the als/ftd c9orf72 expansion is mitigated by antisense intervention. *Neuron* 80, 415–428. doi: 10.1016/j.neuron.2013.10.015

Elden, A. C., Kim, H. J., Hart, M. P., Chen-Plotkin, A. S., Johnson, B. S., Fang, X., et al. (2010). Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for als. *Nature* 466, 1069–1075. doi: 10.1038/nature09320

Farg, M. A., Soo, K. Y., Warraich, S. T., Sundaramoorthy, V., Blair, I. P., and Atkin, J. D. (2013). Ataxin-2 interacts with fus and intermediate-length polyglutamine expansions enhance fus-related pathology in amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 22, 717–728. doi: 10.1093/hmg/dds479

Farhan, S. M. K., Howrigan, D. P., Abbott, L. E., Klim, J. R., Topp, S. D., Wuu, J., et al. (2020). Publisher correction: exome sequencing in amyotrophic lateral sclerosis implicates a novel gene, dnajc7, encoding a heat-shock protein. *Nat. Neurosci.* 23:295. doi: 10.1038/s41593-019-0570-5

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

Ferrari, R., Manzoni, C., and Hardy, J. (2019). Genetics and molecular mechanisms of frontotemporal lobar degeneration: an update and future avenues. *Neurobiol. Aging* 78, 98–110. doi: 10.1016/j.neurobiolaging.2019.02.006

Foster, A., Scott, D., Layfield, R., and Rea, S. L. (2019). An ftld-associated sqstm1 variant impacts nrf2 and nf-kappab signalling and is associated with reduced phosphorylation of p62. *Mol. Cell. Neurosci.* 98, 32–45. doi: 10.1016/j.mcn.2019.04.001

Fournier, C., Anquetil, V., Camuzat, A., Stirati-Buron, S., Sazdovitch, V., Laura, M. P., et al. (2018). Interrupted cag expansions in atxn2 gene expand the genetic spectrum of frontotemporal dementias. *Acta Neuropathol. Commun.* 6:41.

Fournier, C., Barbier, M., Camuzat, A., Anquetil, V., Lattante, S., Clot, F., et al. (2019). Relations between c9orf72 expansion size in blood, age at onset, age at collection and transmission across generations in patients and presymptomatic carriers. *Neurobiol. Aging* 74:234.e1-234.e8.

Freibaum, B. D., Lu, Y., Lopez-Gonzalez, R., Kim, N. C., Almeida, S., Nisha, B., et al. (2015). Ggggcc repeat expansion in c9orf72 compromises nucleocytoplasmic transport. *Nature* 525, 129–133. doi: 10.1038/nature14974

Freischmidt, A., Wieland, T., Richter, B., Ruf, W., Schaeffer, V., Muller, K., et al. (2015). Haploinsufficiency of tbk1 causes familial als and fronto-temporal dementia. *Nat. Neurosci.* 18, 631–636.

Galper, J., Rayner, S. L., Hogan, A. L., Fifita, J. A., Lee, A., Roger, S. C., et al. (2017). Cyclin f: a component of an e3 ubiquitin ligase complex with roles in neurodegeneration and cancer. *Int. J. Biochem. Cell Biol.* 89, 216–220. doi: 10.1016/j.biocel.2017.06.011

Gautier, G., Verschueren, A., Monnier, A., Attarian, S., Salort-Campana, E., and Pouget, J. (2010). Als with respiratory onset: clinical features and effects of non-invasive ventilation on the prognosis. *Amyotroph. Lateral Scler.* 11, 379–382. doi: 10.3109/17482960903426543

Gellera, C., Tiloca, C., Del, Bo, R., Corrado, L., Pensato, V., Cereda, C., et al. (2013). Ubiquilin 2 mutations in italian patients with amyotrophic lateral sclerosis and frontotemporal dementia. *J. Neurol. Neurosurg. Psychiatry* 84, 183–187.

Gendron, T. F., and Petrucelli, L. (2018). Disease mechanisms of c9orf72 repeat expansions. *Cold Spring Harb. Perspect. Med.* 8:a024224. doi: 10.1101/cshperspect.a024224

Gijselinck, I., Van Mossevelde, S., van der Zee, J., Sieben, A., Engelborghs, S., Deryck, O., et al. (2016). The c9orf72 repeat size correlates with onset age of disease, DNA methylation and transcriptional downregulation of the promoter. *Mol. Psychiatry* 21, 1112–1124. doi: 10.1038/mp.2015.159

Gijselinck, I., Van Mossevelde, S., van der Zee, J., Sieben, A., Philtjens, S., Bavo, H., et al. (2015). Loss of tbk1 is a frequent cause of frontotemporal dementia in a belgian cohort. *Neurology* 85, 2116–2125. doi: 10.1212/wnl.0000000000002220

Gilpin, K. M., Chang, L., and Monteiro, M. J. (2015). Als-linked mutations in ubiquilin-2 or hnrnpa1 reduce interaction between ubiquilin-2

and hnrnpa1. *Hum. Mol. Genet.* 24, 2565–2577. doi: 10.1093/hmg/ddv020

Greenway, M. J., Andersen, P. M., Russ, C., Ennis, S., and Cashman, S. (2006). ANG mutations segregate with familial and 'sporadic' amyotrophic lateral sclerosis. *Nat. Genet.* 38, 411–413. doi: 10.1038/ng1742

Hadano, S., Hand, C. K., Osuga, H., Yanagisawa, Y., Otomo, A., Devon, R. S., et al. (2001). A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. *Nat. Genet.* 29, 166–173. doi: 10.1038/ng1001-166

Hardiman, O., Al-Chalabi, A., Chio, A., Corr, E. M., Logroscino, G., Wim, R., et al. (2017). Amyotrophic lateral sclerosis. *Nat. Rev. Dis. Primers* 3:17071.

Hardy, J., and Rogaeva, E. (2014). Motor neuron disease and frontotemporal dementia: sometimes related, sometimes not. *Exp. Neurol.* 262(Pt B), 75–83. doi: 10.1016/j.expneurol.2013.11.006

Harschnitz, O., Jongbloed, B. A., Franssen, H., Straver, D. C., van der Pol, W. L., and van den Berg, L. H. (2014). Mmn: from immunological cross-talk to conduction block. *J. Clin. Immunol.* 34(Suppl. 1), S112–S119.

Hautbergue, G. M., Castelli, L. M., Ferraiuolo, L., Sanchez-Martinez, A., Cooper-Knock, J., Dodd, J. E., et al. (2017). Srsf1-dependent nuclear export inhibition of c9orf72 repeat transcripts prevents neurodegeneration and associated motor deficits. *Nat. Commun.* 8:16063.

Helferich, A. M., Brockmann, S. J., Reinders, J., Deshpande, D., Holzmann, K., David, B., 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

Heo, J. M., Ordureau, A., Paulo, J. A., Rinehart, J., and Harper, J. W. (2015). The pink1-parkin mitochondrial ubiquitylation pathway drives a program of optn/ndp52 recruitment and tbk1 activation to promote mitophagy. *Mol. Cell.* 60, 7–20. doi: 10.1016/j.molcel.2015.08.016

Highley, J. R., Kirby, J., Jansweijer, J. A., Webb, P. S., Hewamadduma, C. A., Paul, R. H., et al. (2014). Loss of nuclear tdp-43 in amyotrophic lateral sclerosis (als) causes altered expression of splicing machinery and widespread dysregulation of rna splicing in motor neurones. *Neuropathol. Appl. Neurobiol.* 40, 670–685. doi: 10.1111/nan.12148

Hjerpe, R., Bett, J. S., Keuss, M. J., Solovyova, A., McWilliams, T. G., Clare, J., et al. (2016). Ubqln2 mediates autophagy-independent protein aggregate clearance by the proteasome. *Cell* 166, 935–949. doi: 10.1016/j.cell.2016.07.001

Hogan, A. L., Don, E. K., Rayner, S. L., Lee, A., Laird, A. S., Watchon, M., et al. (2017). Expression of als/ftd-linked mutant ccnf in zebrafish leads to increased cell death in the spinal cord and an aberrant motor phenotype. *Hum. Mol. Genet.* 26, 2616–2626. doi: 10.1093/hmg/ddx136

Holm, I. E., Englund, E., Mackenzie, I. R., Johannsen, P., and Isaacs, A. M. (2007). A reassessment of the neuropathology of frontotemporal dementia linked to chromosome 3. *J. Neuropathol. Exp. Neurol.* 66, 884–891. doi: 10.1097/nen.0b013e3181567f02

Huey, Ferrari, R., Moreno, J. H., Jensen, C., Morris, C. M., Felix, P., et al. (2012). Fus and tdp43 genetic variability in ftd and cbs. *Neurobiol. Aging* 33, e9–e17.

Hutton, M., Lendon, C. L., Rizzu, P., Baker, M., Froelich, S., Houlden, H., et al. (1998). Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature* 393, 702–705. doi: 10.1038/31508

Ito, Y., Ofengeim, D., Najafov, A., Das, S., Saberi, S., Li, Y., et al. (2016). Ripk1 mediates axonal degeneration by promoting inflammation and necroptosis in als. *Science* 353, 603–608. doi: 10.1126/science.aaf6803

Iyer, S., Subramanian, V., and Acharya, K. R. (2018). C9orf72, a protein associated with amyotrophic lateral sclerosis (als) is a guanine nucleotide exchange factor. *PeerJ* 6:e5815. doi: 10.7717/peerj.5815

Jackson, J. L., Finch, N. A., Baker, M. C., Kachergus, J. M., DeJesus-Hernandez, M., Rosa, R., et al. (2020). Elevated methylation levels, reduced expression levels, and frequent contractions in a clinical cohort of c9orf72 expansion carriers. *Mol. Neurodegener.* 15:7.

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

Johnson, B. S., Snead, D., Lee, J. J., McCaffery, J. M., Shorter, J., and Gitler, A. D. (2009). Tdp-43 is intrinsically aggregation-prone, and amyotrophic lateral sclerosis-linked mutations accelerate aggregation and increase toxicity. *J. Biol. Chem.* 284, 20329–20339. doi: 10.1074/jbc.m109.010264

Johnson, J. O., Glynn, S. M., Gibbs, J. R., Nalls, M. A., Sabatelli, M., Bryan, J. T., et al. (2014). Mutations in the chchd10 gene are a common cause of familial amyotrophic lateral sclerosis. *Brain* 137:e311. doi: 10.1093/brain/awu26

Kabashi, E., Lin, L., Tradewell, M. L., Dion, P. A., Bercier, V., Samar, B. J., et al. (2010). Gain and loss of function of als-related mutations of tardbp (tdp-43) cause motor deficits in vivo. *Hum. Mol. Genet.* 19, 671–683. doi: 10.1093/hmg/ddp534

Kamada, M., Izumi, Y., Ayaki, T., Nakamura, M., Kagawa, S., Kaji, R., et al. (2014). Clinicopathologic features of autosomal recessive amyotrophic lateral sclerosis associated with optineurin mutation. *Neuropathology* 34, 64–70. doi: 10.1111/neup.12051

Kamelgarn, M., Chen, J., Kuang, L., Arenas, A., Zhai, J., Gal, J., et al. (2016). Proteomic analysis of fus interacting proteins provides insights into fus function and its role in als. *Biochim. Biophys. Acta* 1862, 2004–2014. doi: 10.1016/j.bbadis.2016.07.015

Kamelgarn, M., Chen, J., Kuang, L., Jin, H., Kasarskis, E. J., and Zhu, H. (2018). Als mutations of fus suppress protein translation and disrupt the regulation of nonsense-mediated decay. *Proc. Natl. Acad. Sci. U.S.A.* 115, E11904–E11913.

Kim, H. J., Kim, N. C., Wang, Y. D., Scarborough, E. A., Moore, J., Diaz, Z., et al. (2013). Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS. *Nature* 495, 467–473. doi: 10.1038/nature11922

Kimonis, V. (1993). "Inclusion body myopathy with paget disease of bone and/or frontotemporal dementia," in *Genereviews((r))*, eds M. P. Adam, R. A. Pagon, S. E. Wallace, L. J. H. Bean, et al. (Seattle, WA: University of Washington).

Kirby, J., Goodall, E. F., Smith, W., Highley, J. R., Masanzu, R., Shaw, P. J., et al. (2010). Broad clinical phenotypes associated with tar-DNA binding protein (tardbp) mutations in amyotrophic lateral sclerosis. *Neurogenetics* 11, 217–225. doi: 10.1007/s10048-009-0218-9

Kirby, J., Halligan, E., Baptista, M. J., Allen, S., Heath, P. R., Shaw, P. J., et al. (2005). Mutant sod1 alters the motor neuronal transcriptome: implications for familial als. *Brain* 128, 1686–1706. doi: 10.1093/brain/awh503

Koppers, M., van Blitterswijk, M. M., Vlam, L., Rowicka, P. A., van Vught, P. W., Jan, H. V., et al. (2012). Vcp mutations in familial and sporadic amyotrophic lateral sclerosis. *Neurobiol. Aging* 33, e7–e13.

Korac, J., Schaeffer, V., Kovacevic, I., Clement, A. M., Jungblut, B., Ivan, D., et al. (2013). Ubiquitin-independent function of optineurin in autophagic clearance of protein aggregates. *J. Cell Sci.* 126, 580–592. doi: 10.1242/jcs.114926

Kovacs, G. G., Murrell, J. R., Horvath, S., Haraszti, L., Majtenyi, K., Spina, S., et al. (2009). Tardbp variation associated with frontotemporal dementia, supranuclear gaze palsy, and chorea. *Mov. Disord.* 24, 1843–1847.

Kovacs, G. G., van der Zee, J., Hort, J., Kristoferitsch, W., Leitha, T., Matej, R., et al. (2016). Clinicopathological description of two cases with sqstm1 gene mutation associated with frontotemporal dementia. *Neuropathology* 36, 27–38. doi: 10.1111/neup.12233

Kwiatkowski, T. J. Jr., Bosco, D. A., Leclerc, A. L., Tamrazian, E., Vanderburg, C. R., Haines, J. L., et al. (2009). Mutations in the fus/tls gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* 323, 1205–1208.

Laaksovirta, H., Peuralinna, T., Schymick, J. C., Scholz, S. W., Lai, S. L., Bryan, J. T., et al. (2010). Chromosome 9p21 in amyotrophic lateral sclerosis in finland: a genome-wide association study. *Lancet Neurol.* 9, 978–985. doi: 10.1016/s1474-4422(10)70184-8

Lagier-Tourenne, C., Baughn, M., Rigo, F., Sun, S., Liu, P., Hutt, K. R., et al. (2013). Targeted degradation of sense and antisense c9orf72 rna foci as therapy for als and frontotemporal degeneration. *Proc. Natl. Acad. Sci. U.S.A.* 110, E4530–E4539.

Lagier-Tourenne, C., Polymenidou, M., and Cleveland, D. W. (2010). Tdp-43 and fus/tls: emerging roles in rna processing and neurodegeneration. *Hum. Mol. Genet.* 19, R46–R64.

Lattante, S., de Calbiac, H., Le Ber, I., Brice, A., Ciura, S., and Kabashi, E. (2015). Sqstm1 knock-down causes a locomotor phenotype ameliorated by rapamycin in a zebrafish model of als/ftld. *Hum. Mol. Genet.* 24, 1682–1690. doi: 10.1093/hmg/ddu580

Lattante, S., Doronzio, P. N., Marangi, G., Conte, A., Bisogni, G., Mario, S., et al. (2019). Coexistence of variants in tbk1 and in other als-related genes elucidates an oligogenic model of pathogenesis in sporadic als. *Neurobiol. Aging* 84:239.e9-239.e14.

Lattante, S., Millecamps, S., Stevanin, G., Rivaud-Pechoux, S., Moigneu, C., Edor, K., et al. (2014). Contribution of atxn2 intermediary polyq expansions in a spectrum of neurodegenerative disorders. *Neurology* 83, 990–995. doi: 10.1212/wnl.0000000000000778

Le Ber, I., De Septenville, A., Millecamps, S., Camuzat, A., Caroppo, P., Brice, A., et al. (2015). Tbk1 mutation frequencies in french frontotemporal dementia and amyotrophic lateral sclerosis cohorts. *Neurobiol. Aging* 36:3116.e5-3116.e8.

Lee, A., Rayner, S. L., Gwee, S. S. L., De Luca, A., Shahheydari, H., Ian, B. P., et al. (2018). Pathogenic mutation in the als/ftd gene, ccnf, causes elevated lys48-linked ubiquitylation and defective autophagy. *Cell Mol. Life. Sci.* 75, 335–354. doi: 10.1007/s00018-017-2632-8

Lee, K. H., Zhang, P., Kim, H. J., Mitrea, D. M., Sarkar, M., Bai, B., et al. (2016). C9orf72 dipeptide repeats impair the assembly, dynamics, and function of membrane-less organelles. *Cell* 167, 774-788.e17.

Li, J., He, J., Tang, L., Chen, L., Ma, Y., and Fan, D. (2018). Screening for tuba4a mutations in a large chinese cohort of patients with als: re-evaluating the pathogenesis of tuba4a in als. *J. Neurol. Neurosurg. Psychiatry* 89, 1350–1352. doi: 10.1136/jnnp-2017-317560

Ling, J. P., Pletnikova, O., Troncoso, J. C., and Wong, P. C. (2015). Tdp-43 repression of nonconserved cryptic exons is compromised in als-ftd. *Science* 349, 650–655. doi: 10.1126/science.aab0983

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

Lopez-Erauskin, J., Tadokoro, T., Baughn, M. W., Myers, B., McAlonis-Downes, M., Cruz, S. D., et al. (2018). Als/ftd-linked mutation in fus suppresses intra-axonal protein synthesis and drives disease without nuclear loss-of-function of fus. *Neuron* 100, 816e–830e.

Luty, A. A., Kwok, J. B., Dobson-Stone, C., Loy, C. T., Coupland, K. G., Helena, K., et al. (2010). Sigma nonopioid intracellular receptor 1 mutations cause frontotemporal lobar degeneration-motor neuron disease. *Ann. Neurol.* 68, 639–649. doi: 10.1002/ana.22274

Ma, S., Attarwala, I. Y., and Xie, X. Q. (2019). Sqstm1/p62: a potential target for neurodegenerative disease. *ACS Chem. Neurosci.* 10, 2094–2114. doi: 10.1021/acschemneuro.8b00516

Mackenzie, I. R., and Neumann, M. (2016). Molecular neuropathology of frontotemporal dementia: insights into disease mechanisms from postmortem studies. *J. Neurochem.* 138(Suppl. 1), 54–70. doi: 10.1111/jnc.13588

Mackenzie, I. R., Neumann, M., Baborie, A., Sampathu, D. M., Du Plessis, D., Lee, V. M. Y., et al. (2011). A harmonized classification system for ftld-tdp pathology. *Acta Neuropathol.* 122, 111–113. doi: 10.1007/s00401-011-0845-8

Mackenzie, I. R., and Rademakers, R. (2008). The role of transactive response DNA-binding protein-43 in amyotrophic lateral sclerosis and frontotemporal dementia. *Curr. Opin. Neurol.* 21, 693–700. doi: 10.1097/wco.0b013e3283168d1d

Mackenzie, I. R., Rademakers, R., and Neumann, M. (2010). Tdp-43 and fus in amyotrophic lateral sclerosis and frontotemporal dementia. *Lancet Neurol.* 9, 995–1007. doi: 10.1016/s1474-4422(10)70195-2

Majounie, E., Renton, A. E., Mok, K., Dopper, E. G., Waite, A., Pall, H., et al. (2012). Frequency of the c9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study. *Lancet Neurol.* 11, 323–330.

Mann, D. M., Rollinson, S., Robinson, A., Bennion Callister, J., Thompson, J. C., Davidson, Y., et al. (2013). Dipeptide repeat proteins are present in the p62 positive inclusions in patients with frontotemporal lobar degeneration and motor neurone disease associated with expansions in c9orf72. *Acta Neuropathol. Commun.* 1:68.

Marroquin, N., Stranz, S., Muller, K., Wieland, T., Ruf, W. P., Jochen, H. W., et al. (2016). Screening for chchd10 mutations in a large cohort of sporadic als patients: no evidence for pathogenicity of the p.P34s variant. *Brain* 139:e8. doi: 10.1093/brain/awv218

Maruyama, H., Morino, H., Ito, H., Izumi, Y., Kato, H., Kaji, R., et al. (2010). Mutations of optineurin in amyotrophic lateral sclerosis. *Nature* 465, 223–226.

May, S., Hornburg, D., Schludi, M. H., Arzberger, T., Rentzsch, K., Felix, M., et al. (2014). C9orf72 ftld/als-associated gly-ala dipeptide repeat proteins cause neuronal toxicity and unc119 sequestration. *Acta Neuropathol.* 128, 485–503. doi: 10.1007/s00401-014-1329-4

McCauley, M. E., and Baloh, R. H. (2019). Inflammation in als/ftd pathogenesis. *Acta Neuropathol.* 137, 715–730. doi: 10.1007/s00401-018-1933-9

Millecamps, S., Salachas, F., Cazeneuve, C., Gordon, P., Bricka, B., Eric, L. G., et al. (2010). Sod1, ang, vapb, tardbp, and fus mutations in familial amyotrophic lateral sclerosis: genotype-phenotype correlations. *J. Med. Genet.* 47, 554–560. doi: 10.1136/jmg.2010.077180

Mizielinska, S., Gronke, S., Niccoli, T., Ridler, C. E., Clayton, E. L., Adrian, M. I., et al. (2014). C9orf72 repeat expansions cause neurodegeneration in drosophila through arginine-rich proteins. *Science* 345, 1192–1194. doi: 10.1126/science.1256800

Mizielinska, S., Lashley, T., Norona, F. E., Clayton, E. L., Ridler, C. E., Adrian, M. I., et al. (2013). C9orf72 frontotemporal lobar degeneration is characterised by frequent neuronal sense and antisense rna foci. *Acta Neuropathol.* 126, 845–857. doi: 10.1007/s00401-013-1200-z

Moens, T. G., Partridge, L., and Isaacs, A. M. (2017). Genetic models of c9orf72: what is toxic? *Curr. Opin. Genet. Dev.* 44, 92–101. doi: 10.1016/j.gde.2017.01.006

Morita, M., Al-Chalabi, A., Andersen, P. M., Hosler, B., Sapp, P., Brown, R. H., et al. (2006). A locus on chromosome 9p confers susceptibility to als and frontotemporal dementia. *Neurology* 66, 839–844. doi: 10.1212/01.wnl.0000200048.53766.b4

Muller, K., Brenner, D., Weydt, P., Meyer, T., Grehl, T., Kristl, G. C., et al. (2018). Comprehensive analysis of the mutation spectrum in 301 german als families. *J. Neurol. Neurosurg. Psychiatry* 89, 817–827. doi: 10.1136/jnnp-2017-317611

Neuenschwander, A. G., Thai, K. K., Figueroa, K. P., and Pulst, S. M. (2014). Amyotrophic lateral sclerosis risk for spinocerebellar ataxia type 2 atxn2 cag repeat alleles: a meta-analysis. *JAMA Neurol.* 71, 1529–1534.

Neumann, M., Rademakers, R., Roeber, S., Baker, M., Kretzschmar, H. A., and Mackenzie, I. R. (2009). A new subtype of frontotemporal lobar degeneration with fus pathology. *Brain* 132, 2922–2931. doi: 10.1093/brain/awp214

Neumann, M., Sampathu, D. M., Kwong, L. K., Truax, A. C., Micsenyi, M. C., Hans, A. K., et al. (2006). Ubiquitinated tdp-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 314, 130–133.

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, 1268–1283.e6. doi: 10.1016/j.neuron.2018.02.027

Nishimura, A. L., Mitne-Neto, M., Silva, H. C., Richieri-Costa, A., Middleton, S., Cascio, D., et al. (2004). A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. *Am. J. Hum. Genet.* 75, 822–831. doi: 10.1086/425287

Nguyen, D. K. H., Thombre, R., and Wang, J. (2019). Autophagy as a common pathway in amyotrophic lateral sclerosis. *Neurosci. Lett.* 697, 34–48. doi: 10.1016/j.neulet.2018.04.006

Nguyen, H. P., Van Broeckhoven, C., and van der Zee, J. (2018). Als genes in the genomic era and their implications for ftd. *Trends Genet.* 34, 404–423. doi: 10.1016/j.tig.2018.03.001

Nordin, A., Akimoto, C., Wuolikainen, A., Alstermark, H., Jonsson, P., Peter, M. A., et al. (2015). Extensive size variability of the ggggcc expansion in c9orf72 in both neuronal and non-neuronal tissues in 18 patients with als or ftd. *Hum. Mol. Genet.* 24, 3133–3142. doi: 10.1093/hmg/ddv064

Olszewska, D. A., Fallon, E. M., Pastores, G. M., Murphy, K., Blanco, A., Lynch, T., et al. (2019). Autosomal dominant gene negative frontotemporal dementia-think of sca17. *Cerebellum* 18, 654–658. doi: 10.1007/s12311-018-0998-2

Olszewska, D. A., Lonergan, R., Fallon, E. M., and Lynch, T. (2016). Genetics of frontotemporal dementia. *Curr. Neurol. Neurosci. Rep.* 16:107.

O'Rourke, J. G., Bogdanik, L., Muhammad, A., Gendron, T. F., Kim, K. J., Lutz, C. M., et al. (2015). C9orf72 bac transgenic mice display typical pathologic features of als/ftd. *Neuron* 88, 892–901. doi: 10.1016/j.neuron.2015.10.027

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

Osaka, M., Ito, D., Yagi, T., Nihei, Y., and Suzuki, N. (2015). Evidence of a link between ubiquilin 2 and optineurin in amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 24, 1617–1629. doi: 10.1093/hmg/ddu575

Ou, S. H., Wu, F., Harrich, D., Garcia-Martinez, L. F., and Gaynor, R. B. (1995). Cloning and characterization of a novel cellular protein, tdp-43, that binds to human immunodeficiency virus type 1 tar DNA sequence motifs. *J. Virol.* 69, 3584–3596. doi: 10.1128/jvi.69.6.3584-3596.1995

Pan, C., Jiao, B., Xiao, T., Hou, L., Zhang, W., Lu, S., et al. (2017). Mutations of ccnf gene is rare in patients with amyotrophic lateral sclerosis and frontotemporal dementia from mainland china. *Amyotroph. Lateral Scler. Frontotemporal. Degener.* 18, 265–268. doi: 10.1080/21678421.2017.1293111

Parkinson, N., Ince, P. G., Smith, M. O., Highley, R., Skibinski, G., Shaw, P. J., et al. (2006). Als phenotypes with mutations in chmp2b (charged multivesicular body protein 2b). *Neurology* 67, 1074–1077. doi: 10.1212/01.wnl.0000231510.89311.8b

Perrone, F., Nguyen, H. P., Van Mossevelde, S., Moisse, M., Sieben, A., Van, P. D., et al. (2017). Investigating the role of als genes chchd10 and tuba4a in belgian ftd-als spectrum patients. *Neurobiol. Aging* 51:177 e9- 77, e16.

Pesiridis, G. S., Lee, V. M., and Trojanowski, J. Q. (2009). Mutations in tdp-43 link glycine-rich domain functions to amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 18, R156–R162.

Peters, O. M., Cabrera, G. T., Tran, H., Gendron, T. F., McKeon, J. E., Jung, C., et al. (2015). Human c9orf72 hexanucleotide expansion reproduces rna foci and dipeptide repeat proteins but not neurodegeneration in bac transgenic mice. *Neuron* 88, 902–909. doi: 10.1016/j.neuron.2015.11.018

Picher-Martel, V., Dutta, K., Phaneuf, D., Sobue, G., and Julien, J. P. (2015). Ubiquilin-2 drives nf-kappab activity and cytosolic tdp-43 aggregation in neuronal cells. *Mol Brain* 8:71.

Pilli, M., Arko-Mensah, J., Ponpuak, M., Roberts, E., Master, S., Vojo, D., et al. (2012). Tbk-1 promotes autophagy-mediated antimicrobial defense by controlling autophagosome maturation. *Immunity* 37, 223–234. doi: 10.1016/j.immuni.2012.04.015

Polymenidou, M., Lagier-Tourenne, C., Hutt, K. R., Huelga, S. C., Moran, J., Liang, T. Y., et al. (2011). Long pre-mrna depletion and rna missplicing contribute to neuronal vulnerability from loss of tdp-43. *Nat. Neurosci.* 14, 459–468. doi: 10.1038/nn.2779

Pottier, C., Bieniek, K. F., Finch, N., van de Vorst, M., Baker, M., Dennis, W., et al. (2015). Whole-genome sequencing reveals important role for tbk1 and optn mutations in frontotemporal lobar degeneration without motor neuron disease. *Acta Neuropathol.* 130, 77–92. doi: 10.1007/s00401-015-1436-x

Pottier, C., Rampersaud, E., Baker, M., Wu, G., Wuu, J., Jacob, L., et al. (2018). Identification of compound heterozygous variants in optn in an als-ftd patient from the create consortium: a case report. *Amyotroph. Lateral. Scler. Frontotemporal. Degener.* 19, 469–471. doi: 10.1080/21678421.2018.1452947

Pozzi, L., Valenza, F., Mosca, L., Dal Mas, A., Domi, T., Penco, S., et al. (2017). Tbk1 mutations in italian patients with amyotrophic lateral sclerosis: genetic and functional characterisation. *J. Neurol. Neurosurg. Psychiatry* 88, 869–875.

Project MinE ALSSC, (2018). Chchd10 variants in amyotrophic lateral sclerosis: where is the evidence? *Ann. Neurol.* 84, 110–116. doi: 10.1002/ana.25273

Prudencio, M., Belzil, V. V., Batra, R., Ross, C. A., Gendron, T. F., Kevin, B. B., et al. (2015). Distinct brain transcriptome profiles in c9orf72-associated and sporadic als. *Nat. Neurosci.* 18, 1175–1182. doi: 10.1038/nn.4065

Qin, H., Lim, L. Z., Wei, Y., and Song, J. (2014). Tdp-43 n terminus encodes a novel ubiquitin-like fold and its unfolded form in equilibrium that can be shifted by binding to ssdna. *Proc. Natl. Acad. Sci. U.S.A.* 111, 18619–18624. doi: 10.1073/pnas.1413994112

Rabbitts, T. H., Forster, A., Larson, R., and Nathan, P. (1993). Fusion of the dominant negative transcription regulator chop with a novel gene fus by translocation t(12;16) in malignant liposarcoma. *Nat. Genet.* 4, 175–180. doi: 10.1038/ng0693-175

Rappsilber, J., Ryder, U., Lamond, A. I., and Mann, M. (2002). Large-scale proteomic analysis of the human spliceosome. *Genome Res.* 12, 1231–1245. doi: 10.1101/gr.473902

Rea, S. L., Majcher, V., Searle, M. S., and Layfield, R. (2014). Sqstm1 mutations–bridging paget disease of bone and als/ftld. *Exp. Cell Res.* 325, 27–37. doi: 10.1016/j.yexcr.2014.01.020

Reber, S., Stettler, J., Filosa, G., Colombo, M., Jutzi, D., Oliver, M., et al. (2016). Minor intron splicing is regulated by fus and affected by als-associated fus mutants. *EMBO J.* 35, 1504–1521. doi: 10.15252/embj.201593791

Renaud, L., Picher-Martel, V., Codron, P., and Julien, J. P. (2019). Key role of ubqln2 in pathogenesis of amyotrophic lateral sclerosis and frontotemporal dementia. *Acta Neuropathol. Commun.* 7:103.

Renton, A. E., Majounie, E., Waite, A., Simon-Sanchez, J., Rollinson, S., Harmer, D. W., et al. (2011). A hexanucleotide repeat expansion in c9orf72 is the cause of chromosome 9p21-linked als-ftd. *Neuron* 72, 257–268.

Rollinson, S., Bennion, J., Toulson, G., Halliwell, N., Usher, S., Mann, D., et al. (2012). Analysis of optineurin in frontotemporal lobar degeneration. *Neurobiol. Aging* 33, e1–e2.

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. doi: 10.1038/362059a0

Ross, O. A., Rutherford, N. J., Baker, M., Soto-Ortolaza, A. I., Carrasquillo, M. M., Jay, A. V. G., et al. (2011). Ataxin-2 repeat-length variation and neurodegeneration. *Hum. Mol. Genet.* 20, 3207–3212.

Rubino, E., Mancini, C., Boschi, S., Ferrero, P., Ferrone, M., Alfredo, B., et al. (2019). Atxn2 intermediate repeat expansions influence the clinical phenotype in frontotemporal dementia. *Neurobiol. Aging* 73:231.e7-231.e9.

Saracino, D., Clot, F., Camuzat, A., Anquetil, V., Hannequin, D., Daisy, R., et al. (2018). Novel vcp mutations expand the mutational spectrum of frontotemporal dementia. *Neurobiol. Aging* 72:187.e11-187.e14.

Sareen, D., O'Rourke, J. G., Meera, P., Muhammad, A. K., Grant, S., Robert, H. B., et al. (2013). Targeting rna foci in ipsc-derived motor neurons from als patients with a c9orf72 repeat expansion. *Sci. Transl. Med.* 5:208ra149. doi: 10.1126/scitranslmed.3007529

Sarlette, A., Krampfl, K., Grothe, C., Neuhoff, N., Dengler, R., and Petri, S. (2008). Nuclear erythroid 2-related factor 2-antioxidative response element signaling pathway in motor cortex and spinal cord in amyotrophic lateral sclerosis. *J. Neuropathol. Exp. Neurol.* 67, 1055–1062. doi: 10.1097/nen.0b013e31818b4906

Schludi, M. H., Becker, L., Garrett, L., Gendron, T. F., Zhou, Q., Thomas, A., et al. (2017). Spinal poly-ga inclusions in a c9orf72 mouse model trigger motor deficits and inflammation without neuron loss. *Acta Neuropathol.* 134, 241–254. doi: 10.1007/s00401-017-1711-0

Schludi, M. H., May, S., Grasser, F. A., Rentzsch, K., Kremmer, E., Thomas, A., et al. (2015). Distribution of dipeptide repeat proteins in cellular models and c9orf72 mutation cases suggests link to transcriptional silencing. *Acta Neuropathol.* 130, 537–555. doi: 10.1007/s00401-015-1450-z

Scotter, E. L., Chen, H. J., and Shaw, C. E. (2015). Tdp-43 proteinopathy and als: insights into disease mechanisms and therapeutic targets. *Neurotherapeutics* 12, 352–363. doi: 10.1007/s13311-015-0338-x

Sephton, C. F., Cenik, C., Kucukural, A., Dammer, E. B., Cenik, B., Yu, G., et al. (2011). Identification of neuronal rna targets of tdp-43-containing ribonucleoprotein complexes. *J. Biol. Chem.* 286, 1204–1215. doi: 10.1074/jbc.m110.190884

Shahheydari, H., Ragagnin, A., Walker, A. K., Toth, R. P., Vidal, M., Julie, D. A., et al. (2017). Protein quality control and the amyotrophic lateral sclerosis/frontotemporal dementia continuum. *Front. Mol. Neurosci.* 10:119. doi: 10.3389/fnmol.2017.00119

Shatunov, A., Mok, K., Newhouse, S., Weale, M. E., Smith, B., John, E. L., et al. (2010). Chromosome 9p21 in sporadic amyotrophic lateral sclerosis in the uk and seven other countries: a genome-wide association study. *Lancet Neurol.* 9, 986–994.

Shi, K. Y., Mori, E., Nizami, Z. F., Lin, Y., Kato, M., Steven, L. M., et al. (2017). Toxic prn poly-dipeptides encoded by the c9orf72 repeat expansion block nuclear import and export. *Proc. Natl. Acad. Sci. U.S.A.* 114, E1111–E1117.

Shi, Y., Lin, S., Staats, K. A., Li, Y., Chang, W. H., Justin, K. I., et al. (2018). Haploinsufficiency leads to neurodegeneration in c9orf72 als/ftd human induced motor neurons. *Nat. Med.* 24, 313–325.

Sirkis, D. W., Geier, E. G., Bonham, L. W., Karch, C. M., and Yokoyama, J. S. (2019). Recent advances in the genetics of frontotemporal dementia. *Curr. Genet. Med. Rep.* 7, 41–52. doi: 10.1007/s40142-019-0160-6

Skibinski, G., Parkinson, N. J., Brown, J. M., Chakrabarti, L., Lloyd, S. L., John, C., et al. (2005). Mutations in the endosomal escrtiii-complex subunit chmp2b in frontotemporal dementia. *Nat. Genet.* 37, 806–808. doi: 10.1038/ng1609

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

Sproviero, W., Shatunov, A., Stahl, D., Shoai, M., van Rheenen, W., Wim, R., et al. (2017). Atxn2 trinucleotide repeat length correlates with risk of als. *Neurobiol. Aging* 51:178.e1-178.e9.

Sreedharan, J., Blair, I. P., Tripathi, V. B., Hu, X., Vance, C., Shaw, C. E., et al. (2008). Tdp-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* 319, 1668–1672.

Sudria-Lopez, E., Koppers, M., de Wit, M., van der Meer, C., Westeneng, H. J., Jan, H. V., et al. (2016). Full ablation of c9orf72 in mice causes immune system-related pathology and neoplastic events but no motor neuron defects. *Acta Neuropathol.* 132, 145–147. doi: 10.1007/s00401-016-1581-x

Suh, E., Lee, E. B., Neal, D., Wood, E. M., Toledo, J. B., John, Q. T., et al. (2015). Semi-automated quantification of c9orf72 expansion size reveals inverse correlation between hexanucleotide repeat number and disease duration in frontotemporal degeneration. *Acta Neuropathol.* 130, 363–372. doi: 10.1007/s00401-015-1445-9

Takahashi, Y., Fukuda, Y., Yoshimura, J., Toyoda, A., Kurppa, K., Moritoyo, H., et al. (2013). *ERBB4* mutations that disrupt the neuregulin-ErbB4 pathway cause amyotrophic lateral sclerosis type 19. *Am. J. Hum. Genet.* 93, 900–905. doi: 10.1016/j.ajhg.2013.09.008

Takanashi, K., and Yamaguchi, A. (2014). Aggregation of als-linked fus mutant sequesters rna binding proteins and impairs rna granules formation. *Biochem. Biophys. Res. Commun.* 452, 600–607. doi: 10.1016/j.bbrc.2014.08.115

Ticozzi, N., Silani, V., LeClerc, A. L., Keagle, P., Gellera, C., Ratti, A., et al. (2009). Analysis of fus gene mutation in familial amyotrophic lateral sclerosis within an italian cohort. *Neurology* 73, 1180–1185. doi: 10.1212/wnl.0b013e3181bbff05

Torres, P., Ramirez-Nunez, O., Romero-Guevara, R., Bares, G., Granado-Serrano, A. B., Ferrer, I., et al. (2018). Cryptic exon splicing function of tardbp interacts with autophagy in nervous tissue. *Autophagy* 14, 1398–1403. doi: 10.1080/15548627.2018.1474311

Toth, R. P., and Atkin, J. D. (2018). Dysfunction of optineurin in amyotrophic lateral sclerosis and glaucoma. *Front. Immunol.* 9:1017. doi: 10.3389/fimmu.2018.01017

Tran, H., Almeida, S., Moore, J., Gendron, T. F., Chalasani, U., Weng, Z., et al. (2015). Differential toxicity of nuclear rna foci versus dipeptide repeat proteins in a drosophila model of c9orf72 ftd/als. *Neuron* 87, 1207–1214. doi: 10.1016/j.neuron.2015.09.015

Tripolszki, K., Gampawar, P., Schmidt, H., Nagy, Z. F., Nagy, D., Marta, S., et al. (2019). Comprehensive genetic analysis of a hungarian amyotrophic lateral sclerosis cohort. *Front. Genet.* 10:732. doi: 10.3389/fgene.2019.00732

Trojsi, F., Siciliano, M., Femiano, C., Santangelo, G., Lunetta, C., Sonia, M., et al. (2019). Comparative analysis of c9orf72 and sporadic disease in a large multicenter als population: the effect of male sex on survival of c9orf72 positive patients. *Front. Neurosci.* 13:485. doi: 10.3389/fnins.2019.00485

Ugwu, F., Rollinson, S., Harris, J., Gerhard, A., Richardson, A., Stuart, P. B., et al. (2015). Ubqln2 variant of unknown significance in frontotemporal lobar degeneration. *Neurobiol. Aging* 36, e15–e16.

van Blitterswijk, M., Baker, M. C., DeJesus-Hernandez, M., Ghidoni, R., Benussi, L., Rosa, R., et al. (2013a). C9orf72 repeat expansions in cases with previously identified pathogenic mutations. *Neurology* 81, 1332–1341.

van Blitterswijk, M., DeJesus-Hernandez, M., Niemantsverdriet, E., Murray, M. E., Heckman, M. G., Beach, T. G., et al. (2013b). 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 Damme, P., Veldink, J. H., van Blitterswijk, M., Corveleyn, A., van Vught, P. W., Robberecht, W., et al. (2011). Expanded atxn2 cag repeat size in als identifies genetic overlap between als and sca2. *Neurology* 76, 2066–2072. doi: 10.1212/wnl.0b013e31821f445b

van der Zee, J., Gijselinck, I., Van Mossevelde, S., Perrone, F., Dillen, L., Ivailo, T., et al. (2017). Tbk1 mutation spectrum in an extended european patient cohort with frontotemporal dementia and amyotrophic lateral sclerosis. *Hum. Mutat.* 38, 297–309.

van der Zee, J., Urwin, H., Engelborghs, S., Bruyland, M., Vandenberghe, R., Peter, P. D. D., et al. (2008). Chmp2b c-truncating mutations in frontotemporal lobar degeneration are associated with an aberrant endosomal phenotype in vitro. *Hum. Mol. Genet.* 17, 313–322. doi: 10.1093/hmg/ddm309

Van Langenhove, T., van der Zee, J., Sleegers, K., Engelborghs, S., Vandenberghe, R., Cruts, M., et al. (2010). Genetic contribution of fus to frontotemporal lobar degeneration. *Neurology* 74, 366–371. doi: 10.1212/wnl.0b013e3181ccc732

Van Mossevelde, S., van der Zee, J., Cruts, M., and Van Broeckhoven, C. (2017a). Relationship between c9orf72 repeat size and clinical phenotype. *Curr. Opin. Genet. Dev.* 44, 117–124. doi: 10.1016/j.gde.2017.02.008

Van Mossevelde, S., van der Zee, J., Gijselinck, I., Sleegers, K., De Bleecker, J., Van, C. B., et al. (2017b). Clinical evidence of disease anticipation in families segregating a c9orf72 repeat expansion. *JAMA Neurol.* 74, 445–452.

van Zundert, B., and Brown, R. H. Jr. (2017). Silencing strategies for therapy of sod1-mediated als. *Neurosci. Lett.* 636, 32–39. doi: 10.1016/j.neulet.2016.07.059

Vance, C., Al-Chalabi, A., Ruddy, D., Smith, B. N., Hu, X., Shaw, C. E., et al. (2006). Familial amyotrophic lateral sclerosis with frontotemporal dementia is linked to a locus on chromosome 9p13.2-21.3. *Brain* 129, 868–876.

Vance, C., Rogelj, B., Hortobagyi, T., De Vos, K. J., Nishimura, A. L., Shaw, C. E., et al. (2009). Mutations in fus, an rna processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* 323, 1208–1211.

Vandal, S. E., Zheng, X., and Ahmad, S. T. (2018). Molecular genetics of frontotemporal dementia elucidated by drosophila models-defects in endosomal(-)lysosomal pathway. *Int. J. Mol. Sci.* 19:1714. doi: 10.3390/ijms19061714

Vanneste, J., Vercruysse, T., Boeynaems, S., Sicart, A., Van Damme, P., Ludo, V. D. B., et al. (2019). C9orf72-generated poly-gr and poly-pr do not directly interfere with nucleocytoplasmic transport. *Sci. Rep.* 9:15728.

Vatsavayai, S. C., Nana, A. L., Yokoyama, J. S., and Seeley, W. W. (2019). C9orf72-ftd/als pathogenesis: evidence from human neuropathological studies. *Acta Neuropathol.* 137, 1–26. doi: 10.1007/s00401-018-1921-0

Velazquez-Perez, L. C., Rodriguez-Labrada, R., and Fernandez-Ruiz, J. (2017). Spinocerebellar ataxia type 2: clinicogenetic aspects, mechanistic insights, and management approaches. *Front. Neurol.* 8:472. doi: 10.3389/fneur.2017.00472

Wang, T., Jiang, X., Chen, G., and Xu, J. (2015). Interaction of amyotrophic lateral sclerosis/frontotemporal lobar degeneration-associated fused-in-sarcoma with proteins involved in metabolic and protein degradation pathways. *Neurobiol. Aging* 36, 527–535. doi: 10.1016/j.neurobiolaging.2014.07.044

Wang, T., Xu, W., Qin, M., Yang, Y., Bao, P., Jin, X., et al. (2016). Pathogenic mutations in the valosin-containing protein/p97(vcp) n-domain inhibit the sumoylation of vcp and lead to impaired stress response. *J. Biol. Chem.* 291, 14373–14384. doi: 10.1074/jbc.m116.729343

Webster, C. P., Smith, E. F., Bauer, C. S., Moller, A., Hautbergue, G. M., Kurt, J. D. V., et al. (2016). The c9orf72 protein interacts with rab1a and the ulk1 complex to regulate initiation of autophagy. *EMBO J.* 35, 1656–1676. doi: 10.15252/embj.201694401

Wei, Q., Chen, X., Chen, Y., Ou, R., Cao, B., Zhang, L., et al. (2019). Unique characteristics of the genetics epidemiology of amyotrophic lateral sclerosis in china. *Sci. China Life Sci.* 62, 517–525.

Weinreich, M., Shepheard, S. R., Verber, N., Wyles, M., Heath, P. R., Shaw, P. J., et al. (2019). Neuropathological characterization of a novel tank binding kinase (tbk1) gene loss of function mutation associated with amyotrophic lateral sclerosis. *Neuropathol. Appl. Neurobiol.* 46, 279–291. doi: 10.1111/nan.12578

Wild, P., Farhan, H., McEwan, D. G., Wagner, S., Rogov, V. V., Ivan, D., et al. (2011). Phosphorylation of the autophagy receptor optineurin restricts *Salmonella* growth. *Science* 333, 228–233. doi: 10.1126/science.1205405

Williams, K. L., Topp, S., Yang, S., Smith, B., Fifita, J. A., Lee, A., et al. (2016). Ccnf mutations in amyotrophic lateral sclerosis and frontotemporal dementia. *Nat. Commun.* 7:11253.

Williams, K. L., Warraich, S. T., Yang, S., Solski, J. A., Fernando, R., Ian, P.B., et al. (2012). Ubqln2/ubiquilin 2 mutation and pathology in familial amyotrophic lateral sclerosis. *Neurobiol. Aging* 33, e3–e10.

Wong, T. H., Pottier, C., Hondius, D. C., Meeter, L. H. H., van Rooij, J. G. J., John, C. V. S., et al. (2018). Three vcp mutations in patients with frontotemporal dementia. *J. Alzheimers. Dis.* 65, 1139–1146. doi: 10.3233/jad-180301

Woollacott, I. O., and Mead, S. (2014). The c9orf72 expansion mutation: gene structure, phenotypic and diagnostic issues. *Acta Neuropathol.* 127, 319–332. doi: 10.1007/s00401-014-1253-7

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

Xu, Y., Zhang, S., and Zheng, H. (2019). The cargo receptor sqstm1 ameliorates neurofibrillary tangle pathology and spreading through selective targeting of pathological mapt (microtubule associated protein tau). *Autophagy* 15, 583–598. doi: 10.1080/15548627.2018.1532258

Yang, Y., Hentati, A., Deng, H. X., Dabbagh, O., Sasaki, T., Hirano, M., et al. (2001). The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. *Nat. Genet.* 29, 160–165. doi: 10.1038/ng1001-160

Yu, Y., Chi, B., Xia, W., Gangopadhyay, J., Yamazaki, T., Robin, R., et al. (2015). U1 snrnp is mislocalized in als patient fibroblasts bearing nls mutations in fus and is required for motor neuron outgrowth in zebrafish. *Nucleic Acids Res.* 43, 3208–3218. doi: 10.1093/nar/gkv157

Yu, Y., Nakagawa, T., Morohoshi, A., Nakagawa, M., Ishida, N., Keiko, N., et al. (2019). Pathogenic mutations in the als gene ccnf cause cytoplasmic mislocalization of cyclin f and elevated vcp atpase activity. *Hum. Mol. Genet.* 28, 3486–3497. doi: 10.1093/hmg/ddz119

Yu, Y., and Reed, R. (2015). Fus functions in coupling transcription to splicing by mediating an interaction between rnap ii and u1 snrnp. *Proc. Natl. Acad. Sci. U.S.A.* 112, 8608–8613. doi: 10.1073/pnas.1506282112

Zhang, K., Donnelly, C. J., Haeusler, A. R., Grima, J. C., Machamer, J. B., Wang, J., et al. (2015). The c9orf72 repeat expansion disrupts nucleocytoplasmic transport. *Nature* 525, 56–61. doi: 10.1038/nature14973

Zhang, M., Tartaglia, M. C., Moreno, D., Sato, C., McKeever, P., Zinman, L., et al. (2017). DNA methylation age-acceleration is associated with disease duration and age at onset in c9orf72 patients. *Acta Neuropathol.* 134, 271–279. doi: 10.1007/s00401-017-1713-y

Zhang, Y., Schmid, B., Nikolaisen, N. K., Rasmussen, M. A., Aldana, B. I., Xin, L., et al. (2017). Patient ipsc-derived neurons for disease modeling of frontotemporal dementia with mutation in chmp2b. *Stem Cell Rep.* 8, 648–658. doi: 10.1016/j.stemcr.2017.01.012

Zhang, Y. J., Gendron, T. F., Grima, J. C., Sasaguri, H., Jansen-West, K., John, D. F., et al. (2016). C9orf72 poly(ga) aggregates sequester and impair hr23 and nucleocytoplasmic transport proteins. *Nat. Neurosci.* 19, 668–677. doi: 10.1038/nn.4272

Zhou, Y., Liu, S., Liu, G., Ozturk, A., and Hicks, G. G. (2013). Als-associated fus mutations result in compromised fus alternative splicing and autoregulation. *PLoS Genet.* 9:e1003895. doi: 10.1371/journal.pgen.1003895

Zhu, Q., Jiang, J., Gendron, T. F., McAlonis-Downes, M., Jiang, L., Don, W. C., et al. (2020). Reduced c9orf72 function exacerbates gain of toxicity from als/ftd-causing repeat expansion in c9orf72. *Nat. Neurosci.* 23, 615–624. doi: 10.1038/s41593-020-0619-5

Zinszner, H., Sok, J., Immanuel, D., Yin, Y., and Ron, D. (1997). Tls (fus) binds rna in vivo and engages in nucleo-cytoplasmic shuttling. *J. Cell Sci.* 110(Pt 15), 1741–1750.

Zu, T., Liu, Y., Banez-Coronel, M., Reid, T., Pletnikova, O., Juan, C. T., 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.

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REVIEW
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From Multi-Omics Approaches to Precision Medicine in Amyotrophic Lateral Sclerosis

*Giovanna Morello[1,2], Salvatore Salomone[2], Velia D'Agata[3], Francesca Luisa Conforti[4] and Sebastiano Cavallaro[1]**

[1] Institute for Research and Biomedical Innovation (IRIB), Italian National Research Council (CNR), Catania, Italy, [2] Section of Pharmacology, Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy, [3] Human Anatomy and Histology, University of Catania, Catania, Italy, [4] Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Rende, Italy

Amyotrophic lateral sclerosis (ALS) is a devastating and fatal neurodegenerative disorder, caused by the degeneration of upper and lower motor neurons for which there is no truly effective cure. The lack of successful treatments can be well explained by the complex and heterogeneous nature of ALS, with patients displaying widely distinct clinical features and progression patterns, and distinct molecular mechanisms underlying the phenotypic heterogeneity. Thus, stratifying ALS patients into consistent and clinically relevant subgroups can be of great value for the development of new precision diagnostics and targeted therapeutics for ALS patients. In the last years, the use and integration of high-throughput "omics" approaches have dramatically changed our thinking about ALS, improving our understanding of the complex molecular architecture of ALS, distinguishing distinct patient subtypes and providing a rational foundation for the discovery of biomarkers and new individualized treatments. In this review, we discuss the most significant contributions of omics technologies in unraveling the biological heterogeneity of ALS, highlighting how these approaches are revealing diagnostic, prognostic and therapeutic targets for future personalized interventions.

Keywords: amyotrophic lateral sclerosis, ALS-FTD, personalized medicine, molecular taxonomy, multi-omics, systems biology

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***Correspondence:**
Sebastiano Cavallaro
sebastiano.cavallaro@cnr.it

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Abbreviations: ALS, amyotrophic lateral sclerosis; MNs, motor neurons; FDA, Food and Drug Administration; UMN, upper motor neuron; LMN, lower motor neuron; FTD, frontotemporal dementia; FALS, familial ALS; SALS, sporadic ALS; SNPs, single nucleotide polymorphisms; SOD1, Superoxide dismutase 1 [Cu-Zn]; C9orf72, chromosome 9 open reading frame 72; FUS, Fused in Sarcoma RNA binding protein; TDP-43/TARDBP, TAR DNA binding protein; GWAS, genome-wide association studies; WGS, whole-genome sequencing; WES, whole-exome sequencing; KIF5A, kinesin family member 5A; ATXN2, ataxin 2; SPAST, spastin; FIG4, FIG4 phosphoinositide 5-phosphatase; SETX, senataxin; DCTN1, dynactin subunit 1; MATR3, matrin 3; CHCHD10, coiled-coil-helix-coiled-coil-helix domain containing 10; SQSTM1, sequestosome 1; VAPB, VAMP associated protein B and C; HNRNPA1, heterogeneous nuclear ribonucleoprotein A1; VCP, valosin containing protein; OPTN, optineurin; EPHA4, Ephrin type-A receptor 4; KIFAP3, Kinesin Associated Protein 3; UNC13A, Unc-13 Homolog A; CNVs, copy-number variations; SMN, survival motor neuron; PMA, progressive muscular atrophy; mtDNA, mitochondrial DNA; EPHA3, Ephrin type-A receptor 3; iPSC, induced pluripotent stem cells; LCM, laser capture microdissection; MS, Mass Spectrometry; CSF, cerebrospinal fluid; NF-L, neurofilament light chain; pNFH, phosphorylated neurofilament heavy chain; IL-10, interleukin 10; IL-6, interleukin 6; IL-2, interleukin 2; IL-15, interleukin 15; IL-8, interleukin 8; GM-CSF, Granulocyte-Macrophage Colony-Stimulating Factor; MIP-1α, Macrophage Inflammatory Proteins 1-alpha; wrCRP, wide-range C-reactive protein; HMGB, High Mobility Group Box 1; GPNMB, glycoprotein NMB; UCHL1, ubiquitin C-terminal hydrolase L1; bFGF, basic fibroblast growth factor; VGF, Nerve Growth Factor Inducible; PPI, protein-protein interaction; LDL, low-density lipoprotein; HDL, high- density lipoprotein; DNMT, DNA-(cytosine-5)-methyltransferase; miRNA, MicroRNA.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a devastating and fatal neurodegenerative disease, characterized by the progressive deterioration of cortical and spinal motor neurons (MNs), leading invariably to progressive muscle weakness and paralysis. Death, often resulting from respiratory failure due to respiratory muscle weakness, generally occurs after 3–5 years from symptom onset, with only 5–10% of patients' survival beyond 10 years (Brown and Al-Chalabi, 2017). ALS is the most common adult motor neuron disease with a worldwide annual incidence of about 2 per 100,000 persons and with an estimated prevalence of 5.4 per 100,000 individuals (Chiò et al., 2013). In most cases, mean age at onset is 50–60 years, while juvenile (before 25 years of age) and "young-onset" ALS cases (before 45 years), represent between∼1 and ∼10% of all patients, respectively (Artemiadis et al., 2016). No disease-modifying strategies are available so far, and therapies that can effectively stop or reverse the disease progression are urgently needed. The mainstay of treatment for ALS is mainly based on symptom management and respiratory support, with only two Food and Drug Administration (FDA)-approved treatments, riluzole, and edaravone, that appear to mildly slow disease progression and only in some patients (Bhandari et al., 2018; Dash et al., 2018; Jaiswal, 2019). The paucity of effective treatments has been attributed in part to the absence of complete knowledge of ALS pathogenesis, and in part to its heterogeneity with patients displaying widely distinct clinical features and progression patterns, together with a plurality of associated genes.

Over the last few years, the complexity of ALS has led to the concept of a spectrum of different disorders with different pathogenic mechanisms rather than a single disease. From a clinical point of view, in addition to typical or classic ALS (characterized by the simultaneous involvement of upper and lower motor neuron (UMN and LMN) at disease onset), several different phenotypic subtypes can be recognized based on the rate of progression, survival, age of onset, site of onset (bulbar vs. spinal) and prevalence of UMN or LMN motor signs (Brown and Al-Chalabi, 2017). Additionally, while ALS was historically judged as a pure motor neuron disease, it is now recognized that it represents a multi-systemic disorder affecting other brain regions, including frontotemporal, oculomotor, cerebellar, and/or sensory systems, and more rarely the basal ganglia and autonomic nervous system (Abrahams et al., 2014; Fang et al., 2017). To this regard, the most common alternative deficit observed in ALS patients is behavioral dysfunction and/or subtle cognitive impairment, which is also comorbid to ALS in about half of ALS individuals, and where a subset of ∼15% of patients receive the concomitant diagnosis of ALS with a frontotemporal dementia (FTD) syndrome (referred to as ALS-FTD or FTD-ALS patients) (Ferrari et al., 2011; Achi and Rudnicki, 2012; Chiò et al., 2019; Zucchi et al., 2019). The ALS-FTD relationship has been confirmed through genetic studies, suggesting these conditions can be viewed as divergent ends of the spectrum of a single clinically and etiologically heterogeneous condition (Ferrari et al., 2011).

Different clinical profiles are likely to reflect molecular heterogeneity in ALS. In fact, for example, the majority (∼90%) of ALS cases are sporadic (SALS), with unknown cause, while ∼10% of ALS patients show familiarity for the disease, usually transmitted according to an autosomal dominant inheritance (Ryan et al., 2018). However, this distinction is increasingly recognized to be artificial; FALS and SALS are, in fact, phenotypically indistinguishable and seem to show similar patterns of selective MN degeneration and vulnerability, and many mutations in one or more known FALS-associated genes have been found in SALS patients, suggesting the existence of common molecular mechanisms between these two disease forms (Renton et al., 2014; Kirby et al., 2016; Taylor et al., 2016). The complexity and heterogeneity of ALS also emerged from a pathophysiologic point of view, with a series of several biological and molecular pathways differently contributing to its development and progression. Despite the understanding of disease pathogenesis is far from exhaustive, numerous genetic and epidemiological risk factors have been identified, as well as various mechanisms have been suggested, including inflammatory and immune abnormalities, oxidative stress, mitochondrial dysfunction, glutamate excitotoxicity, proteasomal/autophagic impairment, defects in axonal transport and RNA metabolism (Taylor et al., 2016). With this in mind, it is clear that the current diagnostic classification criteria of ALS, primarily based on person's signs and symptoms, are inadequate to characterize the complex and heterogeneous nature of ALS, as well as the use of a single compound to treat the patient population as a whole may hinder the identification of an effective therapy. Defining and stratification of ALS patients into disease subtypes cannot only provide important insights for diagnosis and prognosis but also for clinical trial planning and interpretation, thus achieving better care for ALS patients.

Advances in "omics" technologies (e.g., genome, transcriptome, proteome, epigenome, metabolome) and their correlation with the clinical phenotypes of the individual patient, are enabling medicine to move from a "one-size-fits-all" approach toward a "personalized" model, helping to clarify the molecular mechanisms underlying human disease and to provide both potential biomarkers and pharmacological targets for a more detailed patient stratification and personalized treatments (**Figure 1**). In this review, we discuss advances in the application of "-omics" to further our understanding of ALS, outline the evolving landscape of molecular classifications, and discuss how these techniques are contributing to reveal diagnostic and prognostic biomarkers and molecular targets for future personalized therapeutic interventions.

APPLICATION OF OMICS: A STEP TOWARD A BETTER UNDERSTANDING OF ALS PATHOGENESIS

Applications of omics platforms range from the detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics), epigenomic factors (epigenomics), and metabolites (metabolomics). Thanks to omics technologies, it is now

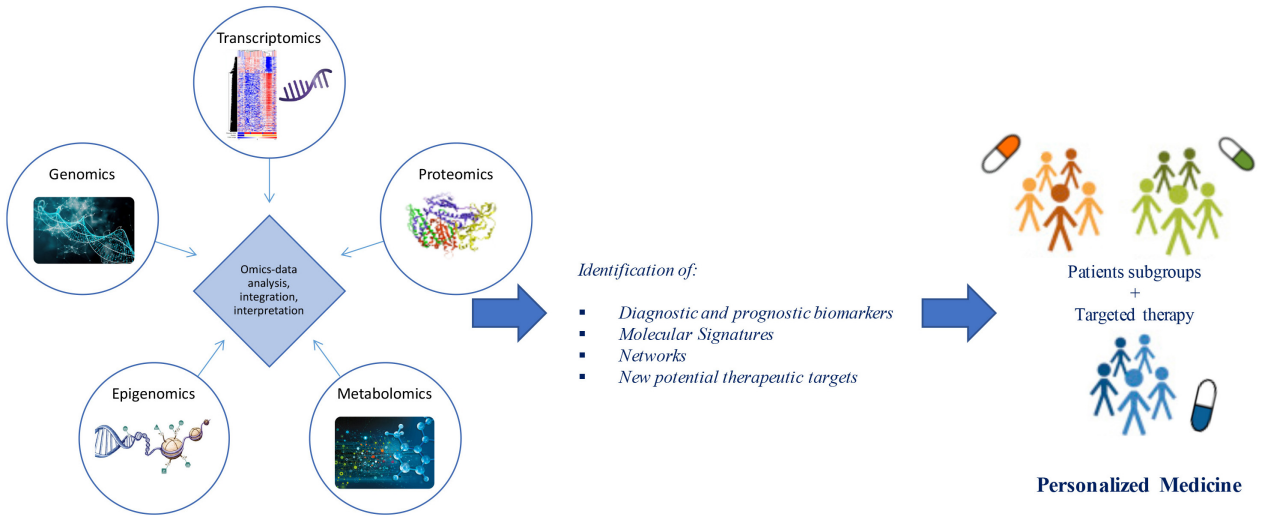

FIGURE 1 | From omics technology to precision medicine in ALS. Multi-omics (e.g., genomics, transcriptomics, proteomics, epigenomics, metabolomics) data analysis and integration may allow patient stratification and targeted therapies. Through a "systems biology" approach, these technologies may move medicine from a "one-size-fits-all" toward a "personalized" model.

possible to quantify the amount of particular molecules (genes, mRNA, protein levels, and metabolites) of a biological system, and observe massive interactomes describing their complex interconnections. For complex and multifactorial pathologies such as ALS, the analysis and integration of different omics layers are crucial for the full knowledge of the disease, opening the way to the development of personalized diagnostic and therapeutic tools. Several omics studies have suggested multiple pathologic mechanisms associated to ALS, providing new insights into molecular signatures/markers and moving toward molecular-based classifications and tailored interventions.

Genomics

The genomic landscape of ALS has been extensively surveyed, contributing to our understanding of ALS biological and clinical complexity. Analysis at this level requires not only the study of DNA sequence variations, including single nucleotide polymorphisms (SNPs) or mutations, but also genomic alterations and chromosomal changes, with consequent protein dysfunction or differences in concentration levels. Detailed information regarding ALS-related genes is available via the Amyotrophic Lateral Sclerosis Online Database (ALSOD)[1]. After the identification of mutations in the *SOD1* gene in 1993 (Rosen et al., 1993), more than 30 genes have been involved in the pathology, with the most common disease-causing variants in *C9orf72, SOD1, FUS, and TARDBP*. However, monogenic forms explain only a fraction of the diagnosed cases, suggesting ALS as a polygenic disease (McCann et al., 2017; Mejzini et al., 2019).

Thanks to the development of genome-wide association studies (GWAS) as well as the advances in massive parallel sequencing approaches, including whole-genome sequencing (WGS) and whole-exome sequencing (WES), enormous progress has been made in understanding genomics of ALS (Ramanan and Saykin, 2013; Cirulli et al., 2015; He et al., 2015; Butchbach, 2016; Van Rheenen et al., 2016; Little et al., 2017; Naruse et al., 2019; Bean et al., 2020). A growing number of causative and susceptibility genes have been identified so far in both familial and sporadic cases, the majority of which encode proteins implicated in cytoskeleton remodeling and axonal transport, mitochondrial metabolism and turnover, autophagy and proteostasis, membrane trafficking, RNA processing and DNA repair (**Table 1**; Ramanan and Saykin, 2013; Robberecht and Eykens, 2015; Maurel et al., 2018; Cook and Petrucelli, 2019; Mejzini et al., 2019; Gall et al., 2020). These genetic findings may guide patient stratification into different subgroups depending on which combination of pathways is deregulated, improving their recruitment for translational research and clinical trials (Vijayakumar et al., 2019; Volonté et al., 2020).

Another important factor increasing the complexity of phenotype-genotype correlations in ALS is the observation of a clinical pleiotropy for ALS genes. Although some mutations associate with very specific ALS clinical profiles (e.g., patients with the Ala4Val mutation in *SOD1* usually have an aggressive form of ALS, whereas those with the homozygous Asp91Ala mutation tend to have a very slowly progressive disease with a generally ascending upper motor neuron phenotype), the majority of disease-causing genes show a high degree of phenotypic heterogeneity, with mutations in the same gene giving rise to different clinical entities, supporting a genetic basis for the observed clinical heterogeneity in ALS. A striking example of pleiotropy is due to *C9orf72* hexanucleotide repeat expansion mutation, which is clearly linked to ALS and FTD but pathogenic expansions have been also observed in a small percentage of patients affected by Alzheimer's (<1%), Huntington's (1–5%), and Parkinson's diseases (1%), as well as atypical parkinsonian syndromes, such as progressive supranuclear palsy (1–8%),

[1]https://alsod.ac.uk

TABLE 1 | Summary of the most known genes linked to ALS, their clinical phenotypes and affected pathway.

Gene symbol	Gene name	Associated phenotype	Oxidative stress	Mito-chondria	Cytoskeleton and axonal dynamics	Protein trafficking and degradation	Autophagy	Vescicle trafficking	DNA repair	RNA processing	Innate immunity and neuro-inflammation
SOD1	Superoxide dismutase 1	ALS, PMA, juvenile ALS	X	X		X	X				
DAO	D-amino acid oxidase	ALS	X								
PPAR-GC1A	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha	ALS	X	X							
OPTN	Optineurin	ALS, FTD		X			X	X			
CHCHD10	Coiled-coil-helix-coiled-coil-helix domain containing 10	ALS, ALS-FTD, FTD, cerebellar ataxia, myophathy	X	X		X					
NEK1	NIMA Related Kinase 1	ALS, ALS-FTD	X	X	X				X		
KIF5A	Kinesin family member 5A	ALS			X						
NEFH	Neurofilament heavy subunit	ALS			X						
TUBA4A	Tubulin Alpha 4a	ALS			X						
DCTN1	Dynactin subunit 1	ALS, ALS-FTD			X			X			
PFN1	Profilin 1	ALS			X	X					
ELP3	Elongator protein 3	ALS, ALS-FTD			X					X	
EPHA4	EPH receptor A4	ALS			X						
C9orf72	Chromosome 9 open reading frame 72	ALS, ALS-FTD, FTD				X	X	X		X	
PRPH	Peripherin	ALS			X						
CHMP2B	Charged multivesicular body protein 2B	ALS, FTD				X	X	X			
VCP	Valosin containing protein	ALS, ALS-FTD, FTD, IBM, PDB				X	X	X			
FIG4	Phosphoinositide 5-Phosphatase	ALS, PLS, CMT					X	X			
VAPB	Vesicle-associated membrane protein-associated protein B/C	ALS, PMA				X		X			
UBQLN2	Ubiquilin 2	ALS, ALS-FTD, juvenile ALS				X	X				
TBK1	TANK binding kinase 1	ALS, FTD				X	X				X
SQSTM1	Sequestosome 1	ALS, ALS-FTD, FTD, IBM, PDB				X	X				

(Continued)

TABLE 1 | Summary of the most known genes linked to ALS, their clinical phenotypes and affected pathway.

Gene symbol	Gene name	Associated phenotype	Oxidative stress	Mito-chondria	Cytoskeleton and axonal dynamics	Protein trafficking and degradation	Autophagy	Vescicle trafficking	DNA repair	RNA processing	Innate immunity and neuro-inflammation
CCNF	Cyclin F	ALS, ALS-FTD				X					
TARDBP	TAR DNA binding protein	ALS, ALS-FTD, FTD							X		
hnRNPA1	Heterogeneous nuclear ribonucleoprotein A1	ALS, ALS-FTD, FTD, IBM, PDB				X				X	
hnRN-PA2B1	Heterogeneous nuclear ribonucleoprotein A2/B1	ALS, ALS-FTD, FTD, IBM, PDB				X				X	
ALS2	Alsin	Juvenile ALS, infantile HSP						X			
SPG11	Spatacsin vescicle trafficking associated	Juvenile ALS, HSP			X	X		X	X		
SIGMAR1	Sigma non-opioid intracellular receptor 1	Juvenile ALS, dHMN					X				
C21orf2	Cilia- and flagella-associated protein 410	ALS							X		
SETX	Senataxin	Juvenile ALS, AOA2, dHMN							X	X	
FUS	Fused in sarcoma	ALS, ALS-FTD, FTD							X	X	
ATXN2	Ataxin 2	ALS, SCA2						X		X	X
ANG	Angiogenin	ALS, ALS-FTD								X	
MATR3	Matrin 3	ALS, ALS-FTD, distal myopathy								X	
EWSR1	EWS RNA binding protein 1	ALS								X	
TAF15	TATA-box binding protein associated factor 15	ALS								X	

The table lists genes thought to be causative or risk factors for ALS sorted on the basis of their functional similarity. ALS, Amyotrophic lateral sclerosis; FTD, Frontotemporal dementia; PMA, Progressive muscular atrophy; IBM, Inclusion-body myositis; PDB, Paget disease of bone; HSP, Hereditary Spastic Paraplegia; dHMN, Distal Hereditary Motor Neuropathy; AOA2, Ataxia with oculomotor apraxia type 2; SCA2, Spinocerebellar ataxia type 2.

corticobasal degeneration (3%), and Lewy body dementia (2%) (van Blitterswijk et al., 2014b; Al-Chalabi et al., 2017; Balendra and Isaacs, 2018; Bourinaris and Houlden, 2018; Foxe et al., 2018). Another interesting example regards a newly identified ALS gene, *KIF5A*. In fact, missense mutations in the N-terminal motor domain of this gene are known to cause hereditary spastic paraplegia and Charcot–Marie–Tooth disease type 2, while ALS-associated mutations are predominantly located at the C-terminal tail domain (Brenner et al., 2018; Nicolas et al., 2018). The possible existence of a common genetic background in neurodegeneration is also supported by the observation that mutations in *ATXN2, SPAST, FIG4, SETX, DCTN1, MATR3, CHCHD10, SQSTM1, VAPB, HNRNPA1, VCP, APOE,* and *OPTN* have been reported both ALS and other multisystem disorders, including FTD, spinocerebellar ataxias, parkinsonism and schizophrenia. Among these, *APOE*, the most prevalent genetic risk factor of AD, has been also studied both as a risk factor for ALS and as a modifier of various phenotypic aspects, including age at onset, site of onset, and duration of the disease. As already found for AD, inheritance of *APOE* alleles is associated with differences in the clinical course of ALS (with a protective role of E2 allele and a deleterious role of E4 allele) suggesting a potential implication of *APOE* genotype as a biomarker to discriminate clinical efficacy in ALS clinical trials (Moulard et al., 1996; Lacomblez et al., 2002; Li et al., 2004). Another genetic determinant of ALS is the trinucleotide repeat expansion occurring in the *ATXN2* gene, with long-expanded repeats that are found to cause spinocerebellar ataxia 2 while intermediate-length polyQ expansion seems to increase the risk of developing ALS, significantly correlate to a spinal phenotype, and associate with shorter survival (Laffita-Mesa et al., 2013; van Blitterswijk et al., 2014a; Borghero et al., 2015; Chiò et al., 2015; Sproviero et al., 2017). As for mutant *C9orf72* and other pathological repeats, *ATXN2*-mediated toxicity seems to involve the creation of small toxic homopolymeric proteins, called dipeptide repeats (DPRs), through a process known as repeat-associated non-ATG-initiated (RAN) translation, leading to an impairment of ribosomal biogenesis, nucleocytoplasmic transport, RNA metabolism and protein sequestration, that can cause neurodegeneration and behavioral deficits (Barker et al., 2017; Hutten and Dormann, 2019; Hergesheimer et al., 2020). Disease-modifying therapies designed or formulated to specifically target the *ATXN2* gene, including the use of antisense oligonucleotides, are currently being studied as a promising therapeutic approach for ALS (Van Den Heuvel et al., 2014; Scoles and Pulst, 2018; Hergesheimer et al., 2020).

Besides clinical diagnosis and identification of risk variants and disease modifiers, the genomic analysis may be helpful for explaining the considerable differences in prognostic profiles of ALS patients, thus providing valuable information for designing new therapeutic strategies (Geyer et al., 2009; Tanaka et al., 2013; Su et al., 2014; Cappella et al., 2019; Chiò et al., 2020). In particular, mutations in *SOD1*, *EPHA4, KIFAP3,* and *UNC13A* seem to affect the progression of ALS disease or the survival of ALS patients (Landers et al., 2009). Loss-of-function mutations in *EPHA4* results in significantly longer survival of ALS patients and pharmacological inhibition of EPHA4 signaling has demonstrated to improve functional performance and motor neuron survival in ALS animal models (Van Hoecke et al., 2012; Rué et al., 2019). Other genetic variants associated with ALS survival include Asp91Ala, one of the most common mutations in *SOD1* that is associated with a long survival when the locus had homozygous genotype, while that of affected heterozygotes varies; and the rs12608932 located in intron 21 of the *UNC13A* gene that is associated with an increased risk and shorter survival of ALS patients (Daoud et al., 2010; Diekstra et al., 2012; Harms and Baloh, 2013; Cady et al., 2015; Gaastra et al., 2016; Yang et al., 2019).

In addition to genetic mutations, the screening of submicroscopic chromosomal changes, known as copy-number variations (CNVs), is potentially informative of genomic alterations related to disease phenotype through the modulation of the expression and function of genes. Several studies have investigated the involvement of these variants in ALS, demonstrating their involvement as risk factors, with multiple rare CNVs more important than common ones (Blauw et al., 2008, 2010; Wain et al., 2009; Uyan et al., 2013; Butchbach, 2016; Morello et al., 2018a; Vadgama et al., 2019). In particular, a large number of rare and novel ALS-specific CNV loci were identified in ALS patients, with the majority of these variants exerting a role in biochemical pathways relevant to ALS pathogenesis, including regulation of synaptic transmission and neuronal action potential, immune response and inflammation, cell adhesion, ion transport, transcriptional regulation and mRNA processing (Wain et al., 2009; Blauw et al., 2010; Morello et al., 2018a). One of the most interesting example is represented by the survival motor neuron (SMN) genes, whose copy number alterations seems to increase risk of developing SALS as well as other neurodegenerative disorders, including progressive muscular atrophy (PMA) (Blauw et al., 2012; Butchbach, 2016; Sangare et al., 2016; Morello et al., 2018a). However, other studies have not found any significant association between the deletion of either SMN1 or SMN2 in ALS, suggesting these conflicting results may be due, in part, to the existence of heterogeneous subgroups of ALS patients. The same ambiguous results are found for copy number changes affecting mitochondrial DNA (mtDNA), with some ALS patients characterized by an accumulation of deletions and other cases showing increased mtDNA copy numbers (Mawrin et al., 2004; Keeney and Bennett, 2010; Morello et al., 2018a). Other examples are heterozygous deletions of *EPHA3*, which seem to confer a protective role against the risk of developing ALS, and deletions in *NEFL* associated with a delayed disease onset and slowed disease progression (Uyan et al., 2013; Morello et al., 2018a).

Notwithstanding the increased knowledge of ALS from a genomic perspective, substantial dilemmas remain from a clinical perspective and large-scale NGS and GWAS projects are currently underway to fully unravel the underlying causes. Among these, of note is Project MinE, an international, large-scale research initiative devoted to discovering genetic causes of ALS by performing whole-genome sequencing of at least 15,000 ALS patients and 7,500 controls, resulting in an open-source genome database, in conjunction with the collection of skin samples to make patient induced

pluripotent stem cell lines (iPSCs) (Van Rheenen et al., 2018; van der Spek et al., 2019). Future follow-up studies will be necessary to shed light on the biological drivers of disease and evaluate the direct effect of newly discovered genes on disease diagnosis and management, also determining if they could form candidates for novel gene therapies.

Transcriptomic

Changes in gene expression are widespread in ALS, as revealed by a large body of work on gene expression profiling of RNA samples from peripheral cells or post-mortem nervous tissue of ALS patients and animal models. These signature patterns of gene expression have started to provide a more detailed picture of molecular events implicated in ALS pathobiology (Dangond et al., 2004; Malaspina and de Belleroche, 2004; Jiang et al., 2005; Kirby et al., 2005; Pasinelli and Brown, 2006; Wang et al., 2006; Lederer et al., 2007; Malaspina et al., 2008; de Oliveira et al., 2013; Saris et al., 2013; Raman et al., 2015; Maria D'erchia et al., 2017; Krokidis and Vlamos, 2018; Recabarren-Leiva and Alarcón, 2018; Dickson et al., 2019; Rahman et al., 2019).

The advent of systems biology and development of high-throughput technologies, including RNA sequencing and high-density microarray platforms, is enabling us not only to discover and define mechanisms of pathogenesis in ALS, but also to differentiate ALS from the "ALS mimic syndromes" and healthy controls and stratify ALS patient into subgroups, facilitating the discovery of biomarkers and new individualized treatments for patients (Cooper-Knock et al., 2012; Heath et al., 2013; Krokidis and Vlamos, 2018; Recabarren-Leiva and Alarcón, 2018; Krokidis, 2020). In this regard, our research group, in the last years, has taken important steps toward the characterization of a biological and molecular heterogeneity of ALS based on transcriptional profiles. In particular, unsupervised hierarchical clustering of genome-wide transcriptomic profiles generated from post-mortem motor cortex samples from SALS patients has led to separate healthy controls and SALS patients and identify two distinct patient groups (SALS1 and SALS2) depending on the combinations of genes and pathways that were deregulated (Aronica et al., 2015). In particular, we observed that cell death, antigen processing and presentation and regulation of chemotaxis were the most representative subgroup-specific pathways in SALS1, while deregulated genes in SALS2 were associated with axonal guidance, oxidative and proteotoxic stress (**Figure 2**; Aronica et al., 2015; Morello et al., 2017a,b). Our analysis also showed that some of the deregulated genes in SALS patients were previously associated with FALS, further supporting the existence of common pathological events between two disease forms. Interestingly, we found the differential expression of a substantial number of genes encoding splicing factors in the motor cortex and spinal cord of the same SALS cohort (La Cognata et al., 2020). In particular, we observed transcriptional deregulation across the tissue types and/or disease states (SALS1, SALS2, controls), with expression changes that were more pronounced for the motor cortex regions than the spinal cord and revealing a significant trend of overexpression for the SALS1 group and a decreased trend in expression for SALS2 (La Cognata et al., 2020). Despite, taken together, our results provided a powerful means for revealing etiopathogenetic mechanisms that were not emerged by considering SALS as a single pathology, it is clear that to successfully translate this knowledge to the real-world clinical contexts, the number of biomarkers should be limited. For this purpose, we next asked if the transcriptome-based classification can be reproduced by utilizing just a list of 203 genes highly associated with an increased ALS susceptibility (Morello et al., 2017a, 2018b). Our results showed that this restricted gene panel was sufficiently representative to separate control from SALS patients, reproducing our previous classification of these patients into molecularly defined and biologically meaningful subtypes and, consequently, facilitating the identification of promising cluster-specific biomarkers. Further studies will be necessary to investigate if peripheral tissues or easily accessible biological fluids (e.g., peripheral blood monocytes, cerebrospinal fluid, or muscle) can reproduce specific molecular patterns observed in brain regions of ALS patients, allowing for an effective mechanism-based selection of patients for clinical trials of molecular-targeted therapies. Emerging molecular heterogeneity of ALS lays the foundations for developing new therapeutic strategies, targeting disease pathogenesis as a complex system rather than at the level of the single protein molecule and that may have greater relevance to distinct sets of patients. In this regard, altered biological pathways emerged from our analysis provided a good number of potential subgroup-specific biomarkers and therapeutic targets, opening the way to the implementation of genomics-based personalized medicine (Morello and Cavallaro, 2015; Morello et al., 2015, 2017c). Of note, some of these target genes exhibit expression profiles similar to those observed in animal models of ALS, thus providing a rationale to ensure their preclinical trial success (Apolloni et al., 2017; Morello et al., 2017c; Apolloni et al., 2019).

Recently, a good number of studies investigated and confirmed the existence of distinct molecular-based clusters of ALS patients, calling attention to the need for better understanding their mechanistic underpinnings and developing treatments based on specific forms of ALS (Jones et al., 2015; Tam et al., 2019; Vijayakumar et al., 2019). In particular, Tam et al. (2019) were able to stratify the transcriptomes by RNA-seq of a largely sporadic set of ALS patients' motor cortex samples into three distinct molecular subgroups, two of which overlapped the molecular signatures observed in our ALS patient samples (Tam et al., 2019). Another study compared brain transcriptome profiles in SALS cases carrying and not carrying the *C9orf72* repeat expansion, revealing both shared and distinct transcriptome changes and pathways associated with these two subsets of ALS cases (Prudencio et al., 2015). A further interesting aspect is the possibility of separating rapid and slow ALS in earlier phases of drug development. To this regard, whole-genome expression analysis conducted by Nardo et al. (2013) in ALS animal models identified specific key genes and molecular pathways associated with fast or slow disease progression, highlighting their role as putative molecular targets for future therapeutic strategies (Prudencio et al., 2015).

The majority of the above-described studies assessed RNA samples from postmortem brain tissues. Although they provide

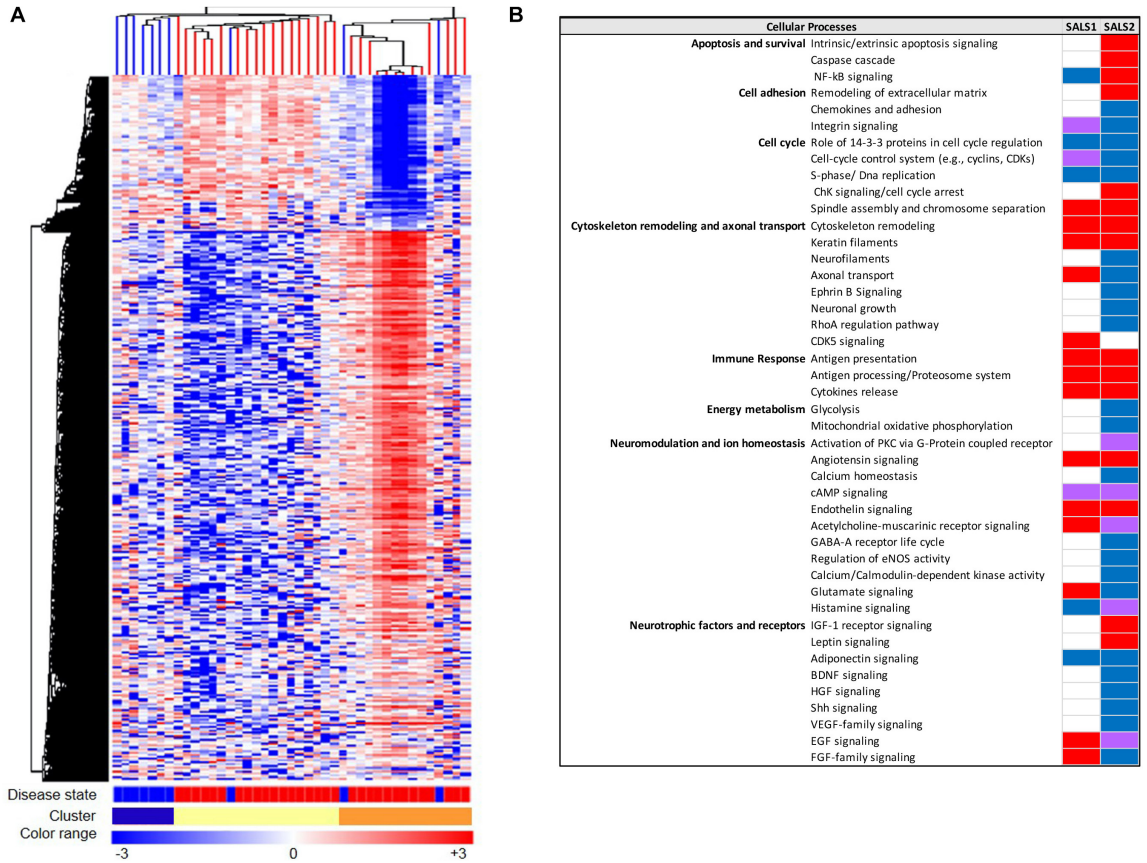

FIGURE 2 | Molecular-based classification of SALS. **(A)** Unsupervised hierarchical clustering (similarity measure: Pearson centered; linkage rule: average) was used to cluster control (10 fresh-frozen motor cortex samples from non-neurological patients) and SALS patients (31 fresh-frozen motor cortex samples) on the basis of the similarity in expression profiles of the most "hypervariable genes" (9.646 genes with a standard deviation >1.5). The same clustering method clearly distinguished two SALS subgroups (SALS1 and SALS2), each associated to differentially expressed genes and pathways. In this two-dimensional presentation, each row represents a single gene and each column a motor cortex from control or SALS patients. As shown in the color bar, highly expressed genes are shown in orange, down-regulated genes in blue, no change in white. In the dendrograms shown (left and top), the length and the subdivision of the branches display the relatedness of the expression of the probes and the motor cortex (top). The disease state is marked as follows: controls patients are indicated by brown rectangles and SALS patients by red rectangles. In the cluster panel, red rectangle refers to control patients, brown to SALS1 and blue to SALS2 patients. For further details, the reader is referred to Aronica et al. (2015). **(B)** Functional pathways deregulated in clustered SALS patients. Orange boxes represent signaling pathways significantly up-regulated, blue bars down-regulated, green bars both up- and down-regulated, white bars indicate no significant change. Figure adapted from Morello and Cavallaro (2015).

essential elements in the pathophysiology of ALS that cannot be otherwise obtained through other approaches used in living patients, these studies reveal end-stage pathogenic mechanisms and do not clarify whether transcriptional differences that separate patient subtypes are a cause or a consequence of the disease process. In that context, the use of iPSC derived from patients suffering from ALS has provided important insights into disease pathophysiology, enabling researchers to explore molecular heterogeneity of ALS and follow the course of degeneration in the dish (Coatti et al., 2015; Bohl et al., 2016; Hedges et al., 2016; Myszczynska and Ferraiuolo, 2016; Sances et al., 2016; Csobonyeiova et al., 2017; Guo et al., 2017; Selvaraj et al., 2017; Centeno et al., 2018; Fujimori et al., 2018; Ghaffari et al., 2018; Lee et al., 2018; Halpern et al., 2019; Ziff and Patani, 2019; Chang et al., 2020; Hawrot et al., 2020). In addition, transcriptome studies on whole tissue (i.e., motor cortex and spinal cord) fail to capture dynamic changes and the complex heterogeneity of the nervous system, making it difficult to determine how gene expression changes disrupt functional interaction between motor neurons and non-neuronal cells (e.g., microglia, oligodendroglia, and astroglia) implicated in ALS pathology. Promising approaches, such as laser capture microdissection (LCM) coupled with RNA sequencing, offer a previously unavailable view of disease progression in ALS, enabling us to explore cell type-specific changes involved in the disease at a particular time point (Namboori et al., 2019; Liu et al., 2020). In a recent paper, Maniatis et al. (2019) used new RNA-seq based technologies, which they called "spatial transcriptomics," for mapping gene expression changes occurring at different disease stages and in different regions of murine models of ALS and human postmortem spinal cords samples, providing important clues

for identifying disease-associated pathways and establishing the key steps in motor neuron degeneration observed in ALS (Maniatis et al., 2019).

Proteomics

Detection of specific protein changes in affected brain tissue samples, cell cultures or body fluids such as CSF represents an important pillar in ALS. The discovery of protein biomarkers for ALS, in fact, may aid earlier diagnosis, measure disease progression, exclude other ALS-mimicking syndromes, discriminate between subtypes of ALS that may theoretically respond to different therapeutic strategies and monitoring drug efficacy during clinical trials (Ruegsegger and Saxena, 2016; Barschke et al., 2017; Webster et al., 2017; Chipika et al., 2019; Hedl et al., 2019; Yerbury et al., 2020). It is well established that the key neuropathological hallmark of the disease is the accumulation of misfolded cytoplasmic proteins in degenerating motor neurons and their non-neuronal neighbors (Perlson et al., 2010; Baloh, 2011; Prell et al., 2013; Tang, 2014; Navone et al., 2015; Parakh and Atkin, 2016; Nguyen et al., 2019; Malik and Wiedau, 2020). One of the main protein components of these protein aggregates is TDP-43, a nuclear RNA binding protein that under stress conditions or when mutated translocates to the cytoplasm where it is hyperphosphorylated and forms insoluble ubiquitin-positive aggregates (Baloh, 2011; Weskamp and Barmada, 2018). Such aggregates are present in almost all cases of ALS, including SALS and FALS patients with pathogenic variants of *C9ORF72* (Mackenzie et al., 2007; Chew et al., 2015), as well as in other neurodegenerative disorders, including FTD, Parkinson's and Alzheimer's disease (Amador-Ortiz et al., 2007; Umoh et al., 2018). It is interesting to note that ALS and FTD have different forms of TDP-43 pathology, suggesting its utility for designing novel diagnostic procedures that could discriminate against these two diseases (Mackenzie and Rademakers, 2008). In addition, although controversial, several results reported that TDP-43 aggregates occur in the vast majority of *SOD1*- and *FUS*-negative FALS patients, but not in *SOD1/FUS* mutation carriers, suggesting that mutant *TDP-43* may cause ALS through specific pathways of inclusion formation that are distinct from those that underlie SALS or other FALS-associated mutations, opening the way to the development of specific therapeutic approaches that take into account these selective modifications (Farrawell et al., 2015; Jeon et al., 2019).

Due to the complex and heterogeneous nature of ALS, it is plausible that a single biomarker could not detect or differentiate between disease subgroups and/or control subjects, sustaining the importance of developing biomarker panels for specific and sensitive diagnostic tests. Recent development of high-throughput Mass Spectrometry-based proteomic (MS) technologies has allowed the simultaneous analysis of multiple proteins, allowing for the definition of comprehensive lists of possible candidate ALS biomarkers (Ekegren et al., 2008; Krüger et al., 2013; Collins et al., 2015; Hedl et al., 2019). In this regard, due to its proximity to the central motor system, the cerebrospinal fluid (CSF) may most probably reflect disease-related alterations, including changes in protein expression, post-translational modification or biochemical turnover than in other body fluids (i.e., blood or urine) (Bowser et al., 2006; Pasinetti et al., 2006; Ryberg and Bowser, 2008; von Neuhoff et al., 2012; Krüger et al., 2013; Lehmer et al., 2017). Analyses of the CSF proteome of ALS patients revealed a panel of candidate biomarkers implicated in synaptic activity, extracellular matrix, inflammatory processes, glial response, axonal damage and apoptosis (Bowser et al., 2006; Pasinetti et al., 2006; Ryberg and Bowser, 2008; von Neuhoff et al., 2012; Krüger et al., 2013; Collins et al., 2015; Collins, 2016; Lehmer et al., 2017; Benatar et al., 2018; Zubiri et al., 2018; Online et al., 2019; Zhu et al., 2019; Oeckl et al., 2020). It is interesting to note that many candidate ALS protein biomarkers show subgroup-specific differential mRNA expression in SALS patients, suggesting their utility in patient stratification and personalized medicine (**Table 2**). Among the most extensively studied fluid biomarkers correlating with the survival of ALS patients, higher levels of neurofilament light chain (NF-L) and the phosphorylated form of neurofilament heavy chain (pNFH) in CSF and plasma samples, as well as their accumulation in brain tissue, have been correlated to shorter life expectancy and a more rapid disease progression and have demonstrated high sensitivity and specificity for separating ALS from ALS-mimic disorders (Gaiottino et al., 2013; Lu et al., 2015; Benatar et al., 2018; De Schaepdryver et al., 2018, 2019; Verde et al., 2019). Recent works also demonstrated the diagnostic utility of CSF pNFH levels in *C9ORF72*-ALS patients, revealing higher pNFH levels in ALS or ALS/FTD patients carrying *C9ORF72* expansion compared with controls and other ALS or ALS/FTD patients (Balendra et al., 2017; Gendron et al., 2017; Floeter and Gendron, 2018). Several other proteins in CSF of ALS patients have demonstrated elevated sensitivity and specificity in distinguishing between ALS patients and neurological disease controls, including IL-10, IL-6, GM-CSF, IL-2, and IL-15 (Mitchell et al., 2009). Proteomic profiling of CSF also identified proteins with a potential prognostic value in ALS, including MIP-1α, wrCRP, HMGB, creatine kinase, granzyme B, and IL-8, whose increased levels have been correlated with more rapidly progressive disease; cystatin C protein levels were positively correlated with survival; increase in GPNMB and UCHL1 were specific for ALS patients showing a short survival time; bFGF increased in ALS patients with longer survival, whereas VGF levels correlated with progressing muscle weakness (Ranganathan et al., 2005; Barschke et al., 2017).

As for genomics studies, systems biology-oriented approaches in proteomics play a crucial role to reveal relevant biological knowledge on pathological mechanisms that trigger the onset and progression of ALS, providing a mechanistic rationale for stratification of ALS patients based on unique molecular profiles, and identification of disease biomarkers and targets for drug efficacy measurements. In this scenario, the analysis of protein-protein interaction (PPI) networks provides the possibility to group proteins that are interacting with each other's in functional complexes and pathways, resulting critically important in helping us to comprehend complex processes, like ALS, and identify key signaling cascades, upstream regulatory components, interactome domains, and novel disease-associated protein candidates suitable for therapeutic intervention (Rao et al., 2014; Snider et al., 2015; Shurte, 2016; Mao et al.,

TABLE 2 | Putative protein biomarkers and their differential expression in distinct SALS patient subgroups.

					Gene expression in SALS motor cortex*	
Biomarker symbol	**Biomarker name**	**CSF/Serum/Plasma**	**Prognostic/Diagnostic value**	**References**	**SALS1**	**SALS2**
Neuron specific						
MAPT	Microtubule-associated protein tau	CSF	Disease progression	(164)	↑	↓
NEFH	Neurofilament, heavy polypeptide	CSF	Diagnosis and progression	Rosengren et al., 2002; Brettschneider et al., 2006	–	↓
NEFM	Neurofilament, medium polypeptide	CSF	Diagnosis and progression	Rosengren et al., 2002	–	↓
NEFL	Neurofilament, light polypeptide	CSF	Diagnosis and progression	Rosengren et al., 2002; Zetterberg et al., 2007	–	↓
Hormones and growth factors						
VEGFA	Vascular endothelial growth factor A	CSF	Diagnosis and progression	Moreau et al., 2006; Pasinetti et al., 2006; Zhao et al., 2008	–	↓
GDNF	Glial cell-line derived neurotrophic factor	CSF	Diagnosis	Tanaka et al., 2006	↓	↑
IGFBP-2	Insulin-like growth factor binding protein 2	Plasma, Serum	Diagnosis and progression	Hosback et al., 2007	–	↓
IGFBP-3	Insulin-like growth factor binding protein 3	Plasma, Serum	Diagnosis and progression	Hosback et al., 2007	↑	↑
IGFBP-5	Insulin-like growth factor binding protein 5	Plasma, Serum	Diagnosis and progression	Hosback et al., 2007	↑	↓
FGF-2	Fibroblast growth factor 2	CSF, Serum	Diagnosis	Johansson et al., 2003	–	↓
HGF	Hepatocyte growth factor	CSF	Diagnosis	Tsuboi et al., 2002	–	↓
Inflammatory system related						
IL2	Interleukin 2	CSF	Diagnosis	Mitchell et al., 2009	–	↑
IL4	Interleukin 4	CSF, Plasma	Diagnosis and progression	Furukawa et al., 2015	–	↑
IL5	Interleukin 5 (colony-stimulating factor, eosinophil)	Plasma	Diagnosis	Lu et al., 2016	–	↑
IL6	Interleukin 6 (interferon, beta 2)	CSF, Plasma	Diagnosis and progression	Bilic et al., 2006; Mitchell et al., 2009	–	↓
IL-10	Interleukin 10	CSF, Plasma	Diagnosis and progression	Mitchell et al., 2009; Furukawa et al., 2015; Andrés-Benito et al., 2017	–	↓
IL-13	Interleukin 13	Plasma	Diagnosis and progression	Shi et al., 2007; Lu et al., 2016	–	↑
IL-15	Interleukin 15	CSF, Plasma	Diagnosis	Mitchell et al., 2009	–	↓
TNF	Tumor necrosis factor-alpha	CSF, Plasma	Diagnosis	Andrés-Benito et al., 2017	↓	–
TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1A	Serum, Plasma	Diagnosis	Andrés-Benito et al., 2017	–	↓

(Continued)

TABLE 2 | Continued

Biomarker symbol	Biomarker name	CSF/Serum/Plasma	Prognostic/Diagnostic value	References	Gene expression in SALS motor cortex*	
					SALS1	SALS2
IFNG	Interferon, gamma	CSF, Plasma	Diagnosis and progression	Guo et al., 2017	↓	↑
TGFB1	Transforming growth factor beta 1	Plasma	Disease progression	Duque et al., 2020	–	↑
GFAP	Glial fibrillary acidic protein	CSF	Diagnosis	Benninger et al., 2016	↑	–
CXCL10	Chemokine (C-X-C motif) ligand 10	CSF	Diagnosis and progression	Tateishi et al., 2010	↓	–
Enzymes and enzyme inhibitors						
CST3	Cystatin C	CSF	Diagnosis	Ranganathan et al., 2005	↑	–
MMP2	Matrix metallopeptidase 2 (gelatinase A, 72 kDa gelatinase, 72 kDa type IV collagenase)	CSF, Plasma	Diagnosis	Niebroj-Dobosz et al., 2010	–	↑
MMP9	Matrix metallopeptidase 9 (gelatinase B, 92 kDa gelatinase, 92 kDa type IV collagenase)	CSF, Serum, Plasma	Diagnosis	Beuche et al., 2000; Lorenzl et al., 2002	–	↓
TIMP1	TIMP metallopeptidase inhibitor 1	CSF, Serum, Plasma	Diagnosis	Lorenzl et al., 2002; Niebroj-Dobosz et al., 2010	↑	↑
SOD1	Superoxide dismutase 1, soluble	CSF, Plasma	Diagnosis	Jacobsson et al., 2001	–	↓
CHIT1	Chitinase 1 (chitotriosidase)	CSF	Diagnosis and progression	Thompson et al., 2018	–	↑
Others						
TARDBP	TAR DNA binding protein	CSF	Diagnosis	Majumder et al., 2018; Kasai et al., 2019	–	↓
S100B	S100 calcium binding protein B	CSF	Disease progression	Süssmuth et al., 2003	–	↓

↑, *Concentration increased in SALS patients compared to controls;* ↓, *Concentration decreased in SALS patients compared to controls.* **Aronica et al. (2015)*.

2017; Vella et al., 2017). In this regard, an interesting example is represented by a recent study investigating modules of co-expressed genes or proteins altered in postmortem cortex samples from patients affected by ALS, FTD, ALS/FTD, and healthy disease controls. In this work, Umoh et al. (2018) identified co-expression modules (i.e., RNA binding proteins, synaptic transmission, inflammation) differing across the ALS-FTD disease spectrum that may be useful for identifying genes associated with different clinical phenotypes along the ALS-FTD disease spectrum (Umoh et al., 2018).

Other Omics (Metabolomics, Epigenomics, miRNomics)

In addition to genomics, transcriptomics and proteomics, the exponential advances in technologies and informatics tools have stimulated an exponential growth of other areas of biomedical science (metabolomics, epigenomics, spliceomics), offering exciting new possibilities for ALS research. In this context, metabolomics, the scientific study of chemical processes involving metabolites (e.g., sugars, lipids, amino acids, organic acids), represents the downstream of systems biology that links the genome, transcriptome and proteome to patient phenotype, providing an important key tool for discovering potential markers in health or disease (Kumar et al., 2013; Blasco et al., 2016; Lanznaster et al., 2018, 2020; Germeys et al., 2019). In the last years, thanks to the development of high-throughput technologies (such as Mass Spectrometry Combined with Liquid and Gas Chromatography), metabolomics studies identified specific metabolic markers and signatures that can discriminate ALS from controls and non-ALS cases, as well as identify distinct subgroups of SALS patients, moving research toward the development of novel targeted personalized treatments (Gross et al., 2018; Lanznaster et al., 2018). In particular, Gross et al. (2018) recently identified two subgroups of SALS case fibroblasts displaying distinct metabotropic patterns that were also observed in plasma samples from the same patients, thus providing a basis for stratify SALS patients for appropriate targeted therapies (Gross et al., 2018). Other metabolite profiling-based studies revealed significantly different metabolic profiles among FALS, SALS and ALS patients carrying different mutations in disease-causing genes (i.e., *C9ORF72, SOD1, TARDBP*, and *FUS*), suggesting the existence of distinct neurodegenerative processes associated with different subtypes of ALS (Wuolikainen et al., 2012; Jääskeläinen et al., 2019; Lanznaster et al., 2020). It is interesting to note that changes in the metabolome as well as alterations in energy metabolism, such as an increase in resting energy expenditure, often precede the development of motor symptoms in ALS and correlates to disease progression. For instance, a lipid-specific metabolic abnormality is present at the pre-symptomatic stage of ALS animal models while increased serum levels of total cholesterol, LDL, LDL/HDL ratio, and triglycerides were associated with longer survival and slower disease progression in ALS patients and animal models (Dorst et al., 2011; De Aguilar, 2019; Germeys et al., 2019). However, the relationship between lipid levels and ALS is still rather controversial and poorly understood, and some follow-up observational studies of ALS did not observe any association between dyslipidemia and the incidence of ALS (Zufiría et al., 2016; De Aguilar, 2019; Zeng and Zhou, 2019). These conflicting results may be partly due to the relatively small sample sizes often employed in these observational studies and to the fact that lipid changes can be affected by a myriad of confounding factors, including genetic, nutritional, physical and pathological factors.

Analysis of metabolite profiles can be also used to identify metabolites and biochemical pathways in ALS patients that are modified before or after treatment exposure, giving rise to a new field called pharmacometabolomics (Rattray and Daouk, 2017; Blasco et al., 2018; Lanznaster et al., 2018). To this regard, an interesting example is represented by a study that analyzed changes in metabolites and lipids composition in the plasma of ALS patients enrolled in a phase III clinical trial for assessing the effects of TRO19622 (olesoxime), a compound with neuroprotective and neurodegenerative properties (Blasco et al., 2018). This study has permitted not only to identify distinct metabolic changes that can distinguish the placebo from the olexosime group but also to reveal metabolic pathways specifically altered after treatment with olesoxime and riluzole in combination in comparison to riluzole therapy alone, supporting the value of blood metabolomic profiles as biomarkers for evaluating the individual response to drug treatments and their side effects (Blasco et al., 2018).

Another layer of complexity to the understanding of complex interactions between the genome and the environment is represented by epigenetic modifications, including DNA methylation, histone post-translational modifications, ATP-dependent chromatin remodeling and RNA-dependent gene silencing (Jirtle, 2009; Dolinar et al., 2018; Douglas, 2018; Ebbert et al., 2018; Bennett et al., 2019; Calió et al., 2020; Klingl et al., 2020; Wang et al., 2020). Several lines of evidence associate epigenome modifications to ALS development, with alterations in DNA methylation and DNA-(cytosine-5)- methyltransferase (DNMT) enzyme activity, as well as alterations to the balance between histone acetylation and deacetylation observed in blood and post-mortem neural tissue from patients with ALS and in different experimental models (Paez-Colasante et al., 2015; Dolinar et al., 2018; Bennett et al., 2019). Of note, variations in epigenetic marks and modifier enzymes, and alterations in the methylation status of some ALS-related genes promoters were also determined, including hypomethylation of *OPTN*, hypermethylation of *C9orf72* expansion CpG islands in the blood of FTD/ALS patients, whereas mutant *SOD1, FUS* and *TDP43* contribute to global epigenome alteration by inducing alterations in histone post-translational modifications and DNA methylation (Masala et al., 2018). While several high-density microarrays or sequencing-based epigenomic technologies are available, particular attention should be paid to EpiSwitch^{TM}, a high-resolution platform, recently developed by Oxford BioDynamics, for analyzing structural-functional epigenetic changes in genomic architecture associated with pathological phenotypes called "chromosome conformation signatures." Using this innovative technological platform, Salter and colleagues performed a comparative interrogation of the genomic architecture from

healthy and ALS-patient blood samples revealing unique chromosomal conformation signatures with the ability to discern between diseased subjects and healthy controls, predict faster versus slower progressing patients at baseline and stratify responsive and non-responsive patients, representing a crucial step toward personalized medicine in ALS (Poesen, 2018; Salter et al., 2018).

MicroRNAs (miRNAs), small non-coding molecules of about 20–22 nucleotides, represent an additional layer of epigenetic regulation that, thanks to their capability to be highly stable in human body fluids, are considered promising biomarkers for neurodegenerative diseases, including ALS (Ricci et al., 2018; Sharma and Lu, 2018). Over the last few years, several whole-genome miRNA profiling studies have identified a panel of a dozen miRNAs that can distinguish ALS from controls with high accuracy in blood cells, serum and CSF, and may be altered in pre-symptomatic ALS mutation carriers even years before the estimated disease onset, representing potentially useful biomarkers of early-stage ALS in coming years (Figueroa-Romero et al., 2016; Rizzuti et al., 2018; Joilin et al., 2019). Despite the heterogeneous nature of ALS may prevent a significant correlation of miRNA levels with clinical disease parameters, down-regulation of two miRNAs, miR-1234-3p and miR-1825, not only is specific for ALS, at least when compared with cohorts of Alzheimer's and Huntington's disease, but also significantly correlated with disease characteristics like age of onset, disease severity and duration (Freischmidt et al., 2015; Takahashi et al., 2015). In particular, while the downregulation of miR-1825 is a general early feature in both FALS and SALS, miR-1234-3p is significantly downregulated only in SALS patients. Of note, a large proportion of SALS patients showed miRNA signatures resembling those of FALS patients and mutation carriers, suggesting alteration of common pathways and a high contribution of genetic factors also in SALS (Freischmidt et al., 2015; Takahashi et al., 2015). Other examples include down-regulation of miR-206, a specific modulator of skeletal muscle growth involved in nerve regeneration after injury, which accelerates disease progression in ALS mice, whereas up-regulation of miR-208B and miR-499 is found in the skeletal muscles of patients with slower disease progression, suggesting the potential utility of these microRNAs as promising candidate biomarkers and targets for this motor neuron disease (Toivonen et al., 2014; Ma et al., 2015; Di Pietro et al., 2017; De Luna et al., 2020).

FROM SINGLE LEVEL TO MULTI-OMICS INTEGRATIVE ANALYSES: TOWARD PRECISION MEDICINE IN ALS

As detailed in the previous paragraphs, omics technologies have been used to identify and/or provide functional supporting information for deciphering important players and pathways involved in ALS pathogenesis and identifying a panel of candidate therapeutic targets and biomarkers that will assist in the rapid diagnosis and prognosis assessment of the disease, and in the stratification of patients into different subgroups for specific targeted therapies. However, if considered individually, these technologies are insufficient to clarify the intricate disease mechanisms implicated in ALS. Taking a holistic molecular approach, based on the integration of multiple types of omics data with existing biological knowledge, has the potential role in improving the knowledge of the molecular basis underlying complex and heterogeneous diseases, establishing different molecular subtypes and patient stratification, thus providing a rational foundation for designing new studies to identify novel targets and clinical trials (**Figure 1**; Mitropoulos et al., 2018; Yu and Zeng, 2018; Mirza et al., 2019; Nguyen and Wang, 2020). Numerous studies have demonstrated the utility of whole- and multi-omics strategies for deciphering the molecular landscape of neurodegenerative diseases, including ALS, providing a feasible opportunity to develop an efficient and effective personalized diagnostics and patient-guided therapies (Bu et al., 2016; Santiago et al., 2017; Castrillo et al., 2018; Hampel et al., 2018a,b; Mitropoulos et al., 2018; Olivier et al., 2019; Vijayakumar et al., 2019; Lam et al., 2020).

An interesting example of applying integrated omics approaches to define an individual's molecular profile useful for the development and application of personalized medicine in ALS, is represented by recent studies carried out by our research groups. As previously described, transcriptional profiling of post-mortem motor cortex samples from SALS patients has allowed to differentiate two distinct patient subgroups characterized by different deregulated genes and pathways (Aronica et al., 2015; Morello and Cavallaro, 2015; Morello et al., 2017a,b,c). To investigate whether these transcriptional alterations may be related to genomic DNA alterations, and thus represent potential markers for a molecular-based stratification of SALS patients, we integrated gene expression profiling with the analysis of genomic structural aberrations occurring in the motor cortex of the same set of SALS samples (Morello et al., 2019). This comprehensive molecular characterization at the genomic and transcriptomic levels revealed subtype-specific genomic alterations positively correlating with transcriptional signature profiles, further confirming the existence of molecular and functional heterogeneity in SALS and suggesting that genomic and transcriptomic events complement each other in driving disease pathogenesis (Morello et al., 2019). Beyond refining ALS molecular architecture, our results also pinpointed candidate driver genes potentially useful as therapeutic targets and biomarkers for genomic-based patient stratification and individualized treatment (Morello and Cavallaro, 2015; Morello et al., 2017c, 2019; Maugeri et al., 2019). Among these, numerous genes involved in histamine receptors, metabolism, transport, secretion and signal transduction, were differentially expressed in the motor cortex as well as in the spinal cord of two molecular-based subgroups of SALS patients and, of note, some of these genes are located within genomic regions disrupted by DNA copy number occurring in SALS patients (Apolloni et al., 2019). By integrating our data with the known pathogenic variants of ALS-related gene reported in the ALSOD database, we identified a good number of coding variants in these genes, supporting the hypothesis that histamine-related genes might represent candidate biomarkers and targets for patient-oriented ALS care (Apolloni et al., 2019). In this regard, pharmacological modulation of the histamine-related pathway has already proved

broad efficacy in ameliorating ALS features, improving motor performance and survival in ALS mice and increasing motor neurons survival *in vivo* and *in vitro* ALS models (Apolloni et al., 2017, 2019).

CONCLUSION

In the past decade, advanced omics technologies have fostered our understanding of the complex molecular architecture of ALS, contributing in part to explain its clinical heterogeneity, and providing a basis for a molecular taxonomy that may radically change our medical approach to ALS. The identification of relevant classifiers and subgroup-specific diagnostic, prognostic and predictive biomarkers is in fact urgently needed for accelerating the development of effective and personalized treatment approaches in ALS. In this review, we discuss the most significant contributions of omics approaches in unraveling the biological complexity of ALS, highlight how holistic systems biology approaches and multi-omics data integration are ideal to provide a comprehensive characterization of patient-specific molecular signatures that could potentially guide therapeutic decisions. We strongly believe that the future research in ALS, as well as in other neurodegenerative diseases, calls a multidisciplinary holistic approach, integrating multi-layer omics data with multimodal neuroimaging and clinical data. This approach will provide a clear understanding of disease prognosis and progression and accelerate development of innovative, effective and personalized strategies for ALS.

AUTHOR CONTRIBUTIONS

GM wrote the manuscript. SS, VD'A, and FC participated in revising the manuscript. SC conceived, directed, and supervised the project. All authors contributed to the article and approved the submitted version.

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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

Achi, E. Y., and Rudnicki, S. A. (2012). ALS and frontotemporal dysfunction: a review. *Neurol. Res. Int.* 2012:806306. doi: 10.1155/2012/806306

Al-Chalabi, A., Van Den Berg, L. H., and Veldink, J. (2017). Gene discovery in amyotrophic lateral sclerosis: implications for clinical management. *Nat. Rev. Neurol.* 13, 96–104. doi: 10.1038/nrneurol.2016.182

Amador-Ortiz, C., Lin, W. L., Ahmed, Z., Personett, D., Davies, P., Duara, R., et al. (2007). TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. *Ann. Neurol.* 61, 435–445. doi: 10.1002/ana.21154

Andrés-Benito, P., Moreno, J., Domínguez, R., Aso, E., Povedano, M., and Ferrer, I. (2017). Inflammatory gene expression in whole peripheral blood at early stages of sporadic amyotrophic lateral sclerosis. *Front. Neurol.* 8:546. doi: 10.3389/fneur.2017.00546

Apolloni, S., Amadio, S., Fabbrizio, P., Morello, G., Spampinato, A. G., Latagliata, E. C., et al. (2019). Histaminergic transmission slows progression of amyotrophic lateral sclerosis. *J. Chachexia Sarcopenia Muscle.* 10, 872–893. doi: 10.1002/jcsm.12422

Apolloni, S., Fabbrizio, P., Amadio, S., Napoli, G., Verdile, V., Morello, G., et al. (2017). Histamine regulates the inflammatory profile of SOD1-G93A microglia and the histaminergic system is dysregulated in amyotrophic lateral sclerosis. *Front. Immunol.* 8:1689. doi: 10.3389/fimmu.2017.01689

Aronica, E., Baas, F., Iyer, A., ten Asbroek, A. L. M. A., Morello, G., and Cavallaro, S. (2015). Molecular classification of amyotrophic lateral sclerosis by unsupervised clustering of gene expression in motor cortex. *Neurobiol. Dis.* 74, 359–376. doi: 10.1016/j.nbd.2014.12.002

Artemiadis, A. K., Peppas, C., Giannopoulos, S., Zouvelou, V., and Triantafyllou, N. (2016). Case of young-onset sporadic amyotrophic lateral sclerosis. *J. Clin. Neuromuscul. Dis.* 17, 220–222. doi: 10.1097/CND.0000000000000107

Balendra, R., and Isaacs, A. M. (2018). C9orf72-mediated ALS and FTD: multiple pathways to disease. *Nat. Rev. Neurol.* 14, 544–558. doi: 10.1038/s41582-018-0047-2

Balendra, R., Moens, T. G., and Isaacs, A. M. (2017). Specific biomarkers for C9orf72 FTD / ALS could expedite the journey towards effective therapies. *EMBO Mol. Med.* 9, 853–855. doi: 10.15252/emmm.201707848

Baloh, R. H. (2011). TDP-43: the relationship between protein aggregation and neurodegeneration in amyotrophic lateral sclerosis and frontotemporal lobar degeneration. *FEBS J.* 278, 3539–3549. doi: 10.1111/j.1742-4658.2011.08256.x

Barker, H. V., Niblock, M., Lee, Y. B., Shaw, C. E., and Gallo, J. M. (2017). RNA misprocessing in C9orf72-linked neurodegeneration. *Front. Cell. Neurosci.* 11:195. doi: 10.3389/fncel.2017.00195

Barschke, P., Oeckl, P., Steinacker, P., Ludolph, A., and Otto, M. (2017). Proteomic studies in the discovery of cerebrospinal fluid biomarkers for amyotrophic lateral sclerosis. *Expert Rev. Proteomics* 14, 769–777. doi: 10.1080/14789450.2017.1365602

Bean, D. M., Al-Chalabi, A., Dobson, R. J. B., and Iacoangeli, A. (2020). A knowledge-based machine learning approach to gene prioritisation in amyotrophic lateral sclerosis. *Genes (Basel)* 11:668. doi: 10.3390/genes11060668

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

Bennett, S. A., Tanaz, R., Cobos, S. N., and Torrente, M. P. (2019). Epigenetics in amyotrophic lateral sclerosis: a role for histone post-translational modifications in neurodegenerative disease. *Transl. Res.* 204, 19–30. doi: 10.1016/j.trsl.2018.10.002

Benninger, F., Glat, M. J., Offen, D., and Steiner, I. (2016). Glial fibrillary acidic protein as a marker of astrocytic activation in the cerebrospinal fluid of patients with amyotrophic lateral sclerosis. *J. Clin. Neurosci.* 26, 75–78. doi: 10.1016/j.jocn.2015.10.008

Beuche, W., Yushchenko, M., Mäder, M., Maliszewska, M., Felgenhauer, K., and Weber, F. (2000). Matrix metalloproteinase-9 is elevated in serum of patients with amyotrophic lateral sclerosis. *Neuroreport* 11, 3419–3422. doi: 10.1097/00001756-200011090-00003

Bhandari, R., Kuhad, A., and Kuhad, A. (2018). Edaravone: a new hope for deadly amyotrophic lateral sclerosis. *Drugs Today* 54, 349–360. doi: 10.1358/dot.2018.54.6.2828189

Bilic, E., Bilic, E., Rudan, I., Kusec, V., Zurak, N., Delimar, D., et al. (2006). Comparison of the growth hormone, IGF-1 and insulin in cerebrospinal fluid and serum between patients with motor neuron disease and healthy controls. *Eur. J. Neurol.* 13, 1340–1345. doi: 10.1111/j.1468-1331.2006.01503.x

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

Blasco, H., Patin, F., Madji Hounoum, B., Gordon, P. H., Vourc'h, P., Andres, C. R., et al. (2016). Metabolomics in amyotrophic lateral sclerosis: how far can it take us? *Eur. J. Neurol.* 23, 447–454. doi: 10.1111/ene.12956

Blauw, H. M., Al-Chalabi, A., Andersen, P. M., van Vught, P. W. J., Diekstra, F. P., van Es, M. A., et al. (2010). A large genome scan for rare CNVs in amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 19, 4091–4099. doi: 10.1093/hmg/ddq323

Blauw, H. M., Barnes, C. P., Van Vught, P. W. J., Van Rheenen, W., Verheul, M., Cuppen, E., et al. (2012). SMN1 gene duplications are associated with sporadic ALS. *Neurology* 78, 776–780. doi: 10.1212/WNL.0b013e318249f697

Blauw, H. M., Veldink, J. H., van Es, M. A., van Vught, P. W., Saris, C. G., van der Zwaag, B., et al. (2008). Copy-number variation in sporadic amyotrophic lateral sclerosis: a genome-wide screen. *Lancet Neurol.* 7, 319–326. doi: 10.1016/S1474-4422(08)70048-6

Bohl, D., Pochet, R., Mitrecic, D., and Nicaise, C. (2016). Modelling and treating amyotrophic lateral sclerosis through induced- pluripotent stem cells technology. *Curr. Stem Cell Res. Ther.* 11, 301–312. doi: 10.2174/1574888x10666150528144303

Borghero, G., Pugliatti, M., Marrosu, F., Marrosu, M. G., Murru, M. R., Floris, G., et al. (2015). ATXN2 is a modifier of phenotype in ALS patients of Sardinian ancestry. *Neurobiol. Aging* 36, 2906.e1–2906.e5. doi: 10.1016/j.neurobiolaging.2015.06.013

Bourinaris, T., and Houlden, H. (2018). C9orf72 and its relevance in parkinsonism and movement disorders: a comprehensive review of the literature. *Mov. Disord. Clin. Pract.* 5, 575–585. doi: 10.1002/mdc3.12677

Bowser, R., Cudkowicz, M., and Kaddurah-Daouk, R. (2006). Biomarkers for amyotrophic lateral sclerosis. *Expert Rev. Mol. Diagn.* 6, 387–398. doi: 10.1586/14737159.6.3.387

Brenner, D., Yilmaz, R., Müller, 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., Petzold, A., Süßmuth, S. D., Landwehrmeyer, G. B., Ludolph, A. C., Kassubek, J., et al. (2006). Neurofilament heavy-chain NfHSMI35 in cerebrospinal fluid supports the differential diagnosis of Parkinsonian syndromes. *Mov. Disord.* 21, 2224–2227. doi: 10.1002/mds.21124

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

Bu, L. L., Yang, K., Xiong, W. X., Liu, F. T., Anderson, B., Wang, Y., et al. (2016). Toward precision medicine in Parkinson's disease. *Ann. Transl. Med.* 4:26. doi: 10.3978/j.issn.2305-5839.2016.01.21

Butchbach, M. E. R. (2016). Copy number variations in the survival motor neuron genes: implications for spinal muscular atrophy and other neurodegenerative diseases. *Front. Mol. Biosci.* 3:7. doi: 10.3389/fmolb.2016.00007

Cady, J., Allred, P., Bali, T., Pestronk, A., Goate, A., Miller, T. M., et al. (2015). Amyotrophic lateral sclerosis onset is influenced by the burden of rare variants in known amyotrophic lateral sclerosis genes. *Ann. Neurol.* 77, 100–113. doi: 10.1002/ana.24306

Calió, M. L., Henriques, E., Siena, A., Bertoncini, C. R. A., Gil-Mohapel, J., and Rosenstock, T. R. (2020). Mitochondrial dysfunction, neurogenesis, and epigenetics: putative implications for amyotrophic lateral sclerosis neurodegeneration and treatment. *Front. Neurosci.* 14:679. doi: 10.3389/fnins.2020.00679

Cappella, M., Ciotti, C., Cohen-Tannoudji, M., and Biferi, M. G. (2019). Gene therapy for ALS-A perspective. *Int. J. Mol. Sci.* 20:4388. doi: 10.3390/ijms20184388

Castrillo, J. I., Lista, S., Hampel, H., and Ritchie, C. W. (2018). Systems biology methods for Alzheimer's disease research toward molecular signatures, subtypes, and stages and precision medicine: application in cohort studies and trials. *Methods Mol. Biol.* 1750, 31–66. doi: 10.1007/978-1-4939-7704-8_3

Centeno, E. G. Z., Cimarosti, H., and Bithell, A. (2018). 2D versus 3D human induced pluripotent stem cell-derived cultures for neurodegenerative disease modelling. *Mol. Neurodegener.* 13:27. doi: 10.1186/s13024-018-0258-4

Chang, C. Y., Ting, H. C., Liu, C. A., Su, H. L., Chiou, T. W., Lin, S. Z., et al. (2020). Induced pluripotent stem cell (iPSC)-based neurodegenerative disease models for phenotype recapitulation and drug screening. *Molecules* 25:2000. doi: 10.3390/molecules25082000

Chew, J., Gendron, T. F., Prudencio, M., Sasaguri, H., Zhang, Y. J., Castanedes-Casey, M., et al. (2015). C9ORF72 repeat expansions in mice cause TDP-43 pathology, neuronal loss, and behavioral deficits. *Science* 348, 1151–1154. doi: 10.1126/science.aaa9344

Chiò, A., Calvo, A., Moglia, C., Canosa, A., Brunetti, M., Barberis, M., et al. (2015). ATXN2 polyQ intermediate repeats are a modifier of ALS survival. *Neurology* 84, 251–258. doi: 10.1212/WNL.0000000000001159

Chiò, A., Logroscino, G., Traynor, B. J., Collins, J., Simeone, J. C., Goldstein, L. A., et al. (2013). Global epidemiology of amyotrophic lateral sclerosis: a systematic review of the published literature. *Neuroepidemiology* 41, 118–130. doi: 10.1159/000351153

Chiò, A., Mazzini, L., and Mora, G. (2020). Disease-modifying therapies in amyotrophic lateral sclerosis. *Neuropharmacology* 167:107986. doi: 10.1016/j.neuropharm.2020.107986

Chiò, A., Moglia, C., Canosa, A., Manera, U., Vasta, R., Brunetti, M., et al. (2019). Cognitive impairment across ALS clinical stages in a population-based cohort. *Neurology* 93, E984–E994. doi: 10.1212/WNL.0000000000008063

Chipika, R. H., Finegan, E., Li Hi Shing, S., Hardiman, O., and Bede, P. (2019). Tracking a fast-moving disease: longitudinal markers, monitoring, and clinical trial endpoints in ALS. *Front. Neurol.* 10:229. doi: 10.3389/fneur.2019.00229

Cirulli, E. T., Lasseigne, B. N., Petrovski, S., Sapp, P. C., Dion, P. A., Leblond, C. S., et al. (2015). Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. *Science* 347, 1436–1441. doi: 10.1126/science.aaa3650

Coatti, G. C., Beccari, M. S., Olávio, T. R., Mitne-Neto, M., Okamoto, O. K., and Zatz, M. (2015). Stem cells for amyotrophic lateral sclerosis modeling and therapy: myth or fact? *Cytom. Part A* 87, 197–211. doi: 10.1002/cyto.a.22630

Collins, M. A. (2016). *Identification of Amyotrophic Lateral Sclerosis Disease Mechanisms by Cerebrospinal Fluid Proteomic Profiling*. Doctoral Dissertation, University of Pittsburgh, Pittsburgh, PA.

Collins, M. A., An, J., Hood, B. L., Conrads, T. P., and Bowser, R. P. (2015). Label-free LC-MS/MS proteomic analysis of cerebrospinal fluid identifies protein/pathway alterations and candidate biomarkers for amyotrophic lateral sclerosis. *J. Proteome Res.* 14, 4486–4501. doi: 10.1021/acs.jproteome.5b00804

Cook, C., and Petrucelli, L. (2019). Genetic convergence brings clarity to the enigmatic red line in ALS. *Neuron* 101, 1057–1069. doi: 10.1016/j.neuron.2019.02.032

Cooper-Knock, J., Kirby, J., Ferraiuolo, L., Heath, P. R., Rattray, M., and Shaw, P. J. (2012). Gene expression profiling in human neurodegenerative disease. *Nat. Rev. Neurol.* 8, 518–530. doi: 10.1038/nrneurol.2012.156

Csobonyeiova, M., Polak, S., Nicodemou, A., and Danisovic, L. (2017). Induced pluripotent stem cells in modeling and cell-based therapy of amyotrophic lateral sclerosis. *J. Physiol. Pharmacol.* 68, 649–657.

Dangond, F., Hwang, D., Camelo, S., Pasinelli, P., Frosch, M. P., Stephanopoulos, G., et al. (2004). Molecular signature of late-stage human ALS revealed by expression profiling of postmortem spinal cord gray matter. *Physiol. Genomics* 16, 229–239. doi: 10.1152/physiolgenomics.00087.2001

Daoud, H., Belzil, V., Desjarlais, A., Camu, W., Dion, P. A., and Rouleau, G. A. (2010). Analysis of the UNC13A gene as a risk factor for sporadic amyotrophic lateral sclerosis. *Arch. Neurol.* 67, 516–517. doi: 10.1001/archneurol.2010.46

Dash, R. P., Babu, R. J., and Srinivas, N. R. (2018). Two decades-long journey from riluzole to edaravone: revisiting the clinical pharmacokinetics of the only two amyotrophic lateral sclerosis therapeutics. *Clin. Pharmacokinet.* 57, 1385–1398. doi: 10.1007/s40262-018-0655-4

De Aguilar, J. L. G. (2019). Lipid biomarkers for amyotrophic lateral sclerosis. *Front. Neurol.* 10:284. doi: 10.3389/fneur.2019.00284

De Luna, N., Turon-Sans, J., Cortes-Vicente, E., Carrasco-Rozas, A., Illán-Gala, I., Dols-Icardo, O., et al. (2020). Downregulation of miR-335-5P in amyotrophic lateral sclerosis can contribute to neuronal mitochondrial dysfunction and apoptosis. *Sci. Rep.* 10, 1–12. doi: 10.1038/s41598-020-61246-1

de Oliveira, G. P., Alves, C. J., and Chadi, G. (2013). Early gene expression changes in spinal cord from SOD1G93A amyotrophic lateral sclerosis animal model. *Front. Cell. Neurosci.* 6:216. doi: 10.3389/fncel.2013.00216

De Schaepdryver, M., Goossens, J., De Meyer, S., Jeromin, A., Masrori, P., Brix, B., et al. (2019). Serum neurofilament heavy chains as early marker of motor neuron degeneration. *Ann. Clin. Transl. Neurol.* 6, 1971–1979. doi: 10.1002/acn3.50890

De Schaepdryver, M., Jeromin, A., Gille, B., Claeys, K. G., Herbst, V., Brix, B., et al. (2018). Comparison of elevated phosphorylated neurofilament heavy chains in serum and cerebrospinal fluid of patients with amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* 89, 367–373. doi: 10.1136/jnnp-2017-316605

Di Pietro, L., Baranzini, M., Berardinelli, M. G., Lattanzi, W., Monforte, M., Tasca, G., et al. (2017). Potential therapeutic targets for ALS: MIR206, MIR208b and MIR499 are modulated during disease progression in the skeletal muscle of patients. *Sci. Rep.* 7, 1–11. doi: 10.1038/s41598-017-10161-z

Dickson, D. W., Baker, M. C., Jackson, J. L., Dejesus-Hernandez, M., Finch, N. C. A., Tian, S., et al. (2019). Extensive transcriptomic study emphasizes importance of vesicular transport in C9orf72 expansion carriers. *Acta Neuropathol. Commun.* 7:150. doi: 10.1186/s40478-019-0797-0

Diekstra, F. P., van Vught, P. W. J., van Rheenen, W., Koppers, M., Pasterkamp, R. J., van Es, M. A., et al. (2012). UNC13A is a modifier of survival in amyotrophic lateral sclerosis. *Neurobiol. Aging* 33, 630.e3–8. doi: 10.1016/j.neurobiolaging.2011.10.029

Dolinar, A., Ravnik-Glavač, M., and Glavač, D. (2018). Epigenetic mechanisms in amyotrophic lateral sclerosis: a short review. *Mech. Ageing Dev.* 174, 103–110. doi: 10.1016/j.mad.2018.03.005

Dorst, J., Kühnlein, P., Hendrich, C., Kassubek, J., Sperfeld, A. D., and Ludolph, A. C. (2011). Patients with elevated triglyceride and cholesterol serum levels have a prolonged survival in amyotrophic lateral sclerosis. *J. Neurol.* 258, 613–617. doi: 10.1007/s00415-010-5805-z

Douglas, A. G. L. (2018). Non-coding RNA in C9orf72-related amyotrophic lateral sclerosis and frontotemporal dementia: a perfect storm of dysfunction. *Non Coding RNA Res.* 3, 178–187. doi: 10.1016/j.ncrna.2018.09.001

Duque, T., Gromicho, M., Pronto-Laborinho, A. C., and de Carvalho, M. (2020). Transforming growth factor-β plasma levels and its role in amyotrophic lateral sclerosis. *Med. Hypotheses* 139:109632. doi: 10.1016/j.mehy.2020.109632

Ebbert, M. T. W., Lank, R. J., and Belzil, V. V. (2018). An epigenetic spin to ALS and FTD. *Adv. Neurobiol.* 20, 1–29. doi: 10.1007/978-3-319-89689-2_1

Ekegren, T., Hanrieder, J., and Bergquist, J. (2008). Clinical perspectives of high-resolution mass spectrometry-based proteomics in neuroscience: exemplified in amyotrophic lateral sclerosis biomarker discovery research. *J. Mass Spectrom.* 43, 559–571. doi: 10.1002/jms.1409

Fang, T., Jozsa, F., and Al-Chalabi, A. (2017). Nonmotor symptoms in amyotrophic lateral sclerosis: a systematic review. *Int. Rev. Neurobiol.* 134, 1409–1441. doi: 10.1016/bs.irn.2017.04.009

Farrawell, N. E., Lambert-Smith, I. A., Warraich, S. T., Blair, I. P., Saunders, D. N., Hatters, D. M., et al. (2015). Distinct partitioning of ALS associated TDP-43, FUS and SOD1 mutants into cellular inclusions. *Sci. Rep.* 5:13416. doi: 10.1038/srep13416

Ferrari, R., Kapogiannis, D., Huey, E. D., and Momeni, P. (2011). FTD and ALS: a tale of two diseases. *Curr. Alzheimer Res.* 8, 273–294. doi: 10.2174/156720511795563700

Figueroa-Romero, C., Hur, J., Lunn, J. S., Paez-Colasante, X., Bender, D. E., Yung, R., et al. (2016). Expression of microRNAs in human post-mortem amyotrophic lateral sclerosis spinal cords provides insight into disease mechanisms. *Mol. Cell Neurosci.* 71, 34–45. doi: 10.1016/j.mcn.2015.12.008

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

Foxe, D., Elan, E., Burrell, J. R., Felicity, F. V., Devenney, E., Kwok, J. B., et al. (2018). Intrafamilial phenotypic variability in the C9orf72 gene expansion: 2 case studies. *Front. Psychol.* 9:1615. doi: 10.3389/fpsyg.2018.01615

Freischmidt, A., Müller, K., Zondler, L., Weydt, P., Mayer, B., von Arnim, C. A. F., et al. (2015). Serum microRNAs in sporadic amyotrophic lateral sclerosis. *Neurobiol. Aging* 36, 2660.e15–20. doi: 10.1016/j.neurobiolaging.2015.06.003

Fujimori, K., Ishikawa, M., Otomo, A., Atsuta, N., Nakamura, R., Akiyama, T., et al. (2018). Modeling sporadic ALS in iPSC-derived motor neurons identifies a potential therapeutic agent. *Nat. Med.* 24, 1579–1589. doi: 10.1038/s41591-018-0140-5

Furukawa, T., Matsui, N., Fujita, K., Nodera, H., Shimizu, F., Miyamoto, K., et al. (2015). CSF cytokine profile distinguishes multifocal motor neuropathy from progressive muscular atrophy. *Neurol. Neuroimmunol. NeuroInflammation* 2:e138. doi: 10.1212/NXI.0000000000000138

Gaastra, B., Shatunov, A., Pulit, S., Jones, A. R., Sproviero, W., Gillett, A., et al. (2016). Rare genetic variation in UNC13A may modify survival in amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Front. Degener.* 17, 593–599. doi: 10.1080/21678421.2016.1213852

Gaiottino, J., Norgren, N., Dobson, R., Topping, J., Nissim, A., Malaspina, A., et al. (2013). Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. *PLoS One* 8:e0075091. doi: 10.1371/journal.pone.0075091

Gall, L., Anakor, E., Connolly, O., Vijayakumar, U. G., Duddy, W. J., and Duguez, S. (2020). Molecular and cellular mechanisms affected in ALS. *J. Pers. Med.* 10, E101. doi: 10.3390/JPM10030101

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

Germeys, C., Vandoorne, T., Bercier, V., and Van Den Bosch, L. (2019). Existing and emerging metabolomic tools for ALS research. *Genes (Basel)* 10:1011. doi: 10.3390/genes10121011

Geyer, F. C., Decker, T., and Reis-Filho, J. S. (2009). Genomweite Expressionsprofile als klinische Entscheidungshilfe: bereit für die Praxis? *Pathologe* 30, 141–146. doi: 10.1007/s00292-008-1104-1

Ghaffari, L. T., Starr, A., Nelson, A. T., and Sattler, R. (2018). Representing diversity in the dish: using patient-derived in vitro models to recreate the heterogeneity of neurological disease. *Front. Neurosci.* 12:56. doi: 10.3389/fnins.2018.00056

Gross, S., Chen, Q., Sandhu, D., Konrad, C., Roychoudhury, D., Schwartz, B. I., et al. (2018). Identification of a distinct metabolomic subtype of sporadic ALS Patients. *bioRxiv*[Preprint] doi: 10.1101/416396

Guo, J., Yang, X., Gao, L., and Zang, D. (2017). Evaluating the levels of CSF and serum factors in ALS. *Brain Behav.* 7:e00637. doi: 10.1002/brb3.637

Guo, W., Fumagalli, L., Prior, R., and van den Bosch, L. (2017). Current advances and limitations in modeling ALS/FTD in a dish using induced pluripotent stem cells. *Front. Neurosci.* 11:671. doi: 10.3389/fnins.2017.00671

Halpern, M., Brennand, K. J., and Gregory, J. (2019). Examining the relationship between astrocyte dysfunction and neurodegeneration in ALS using hiPSCs. *Neurobiol. Dis.* 132:104562. doi: 10.1016/j.nbd.2019.104562

Hampel, H., Toschi, N., Babiloni, C., Baldacci, F., Black, K. L., Bokde, A. L. W., et al. (2018a). Revolution of Alzheimer precision neurology. Passageway of systems biology and neurophysiology. *J. Alzheimers Dis.* 64, S47–S105. doi: 10.3233/JAD-179932

Hampel, H., Vergallo, A., Aguilar, L. F., Benda, N., Broich, K., Cuello, A. C., et al. (2018b). Precision pharmacology for Alzheimer's disease. *Pharmacol. Res.* 130, 331–365. doi: 10.1016/j.phrs.2018.02.014

Harms, M. B., and Baloh, R. H. (2013). Clinical neurogenetics: amyotrophic lateral sclerosis. *Neurol. Clin.* 31, 929–950. doi: 10.1016/j.ncl.2013.05.003

Hawrot, J., Imhof, S., and Wainger, B. J. (2020). Modeling cell-autonomous motor neuron phenotypes in ALS using iPSCs. *Neurobiol. Dis.* 134:104680. doi: 10.1016/j.nbd.2019.104680

He, J., Mangelsdorf, M., Fan, D., Bartlett, P., and Brown, M. A. (2015). Amyotrophic lateral sclerosis genetic studies: from genome-wide association mapping to genome sequencing. *Neuroscientist* 21, 599–615. doi: 10.1177/1073858414555404

Heath, P. R., Kirby, J., and Shaw, P. J. (2013). Investigating cell death mechanisms in amyotrophic lateral sclerosis using transcriptomics. *Front. Cell. Neurosci.* 7:259. doi: 10.3389/fncel.2013.00259

Hedges, E. C., Mehler, V. J., and Nishimura, A. L. (2016). The use of stem cells to model amyotrophic lateral sclerosis and frontotemporal dementia: from basic research to regenerative medicine. *Stem Cells Int.* 2016:9279516. doi: 10.1155/2016/9279516

Hedl, T. J., Gil, R. S., Cheng, F., Rayner, S. L., Davidson, J. M., De Luca, A., et al. (2019). Proteomics approaches for biomarker and drug target discovery in ALS and FTD. *Front. Neurosci.* 13:548. doi: 10.3389/fnins.2019.00548

Hergesheimer, R., Lanznaster, D., Vourc'h, P., Andres, C., Bakkouche, S., Beltran, S., et al. (2020). Advances in disease-modifying pharmacotherapies for the treatment of amyotrophic lateral sclerosis. *Expert Opin. Pharmacother.* 21, 1103–1110. doi: 10.1080/14656566.2020.1746270

Hosback, S., Hardiman, O., Nolan, C. M., Doyle, M. A. C., Gorman, G., Lynch, C., et al. (2007). Circulating insulin-like growth factors and related binding proteins are selectively altered in amyotrophic lateral sclerosis and multiple sclerosis. *Growth Horm. IGF Res.* 17, 472–479. doi: 10.1016/j.ghir.2007.06.002

Hutten, S., and Dormann, D. (2019). RAN translation down. *Nat. Neurosci.* 22, 1379–1380. doi: 10.1038/s41593-019-0482-4

Jääskeläinen, O., Solje, E., Hall, A., Katisko, K., Korhonen, V., Tiainen, M., et al. (2019). Low serum high-density lipoprotein cholesterol levels associate with the C9orf72 repeat expansion in frontotemporal lobar degeneration patients. *J. Alzheimers Dis.* 72, 127–137. doi: 10.3233/JAD-190132

Jacobsson, J., Jonsson, P. A., Andersen, P. M., Forsgren, L., and Marklund, S. L. (2001). Superoxide dismutase in CSF from amyotrophic lateral sclerosis patients with and without CuZn-superoxide dismutase mutations. *Brain* 124, 1461–1466. doi: 10.1093/brain/124.7.1461

Jaiswal, M. K. (2019). Riluzole and edaravone: a tale of two amyotrophic lateral sclerosis drugs. *Med. Res. Rev.* 39, 733–748. doi: 10.1002/med.21528

Jeon, G. S., Shim, Y. M., Lee, D. Y., Kim, J. S., Kang, M. J., Ahn, S. H., et al. (2019). Pathological modification of TDP-43 in amyotrophic lateral sclerosis with SOD1 Mutations. *Mol. Neurobiol.* 56, 2007–2021. doi: 10.1007/s12035-018-1218-2

Jiang, Y. M., Yamamoto, M., Kobayashi, Y., Yoshihara, T., Liang, Y., Terao, S., et al. (2005). Gene expression profile of spinal motor neurons in sporadic amyotrophic lateral sclerosis. *Ann. Neurol.* 57, 236–251. doi: 10.1002/ana.20379

Jirtle, R. L. (2009). Epigenome: the program for human health and disease. *Epigenomics* 1, 13–16. doi: 10.2217/epi.09.16

Johansson, A., Larsson, A., Nygren, I., Blennow, K., and Askmark, H. (2003). Increased serum and cerebrospinal fluid FGF-2 levels in amyotrophic lateral sclerosis. *Neuroreport* 14, 1867–1869. doi: 10.1097/00001756-200310060-00022

Joilin, G., Leigh, P. N., Newbury, S. F., and Hafezparast, M. (2019). An overview of microRNAs as biomarkers of ALS. *Front. Neurol.* 10:186. doi: 10.3389/fneur.2019.00186

Jones, A. R., Troakes, C., King, A., Sahni, V., De Jong, S., Bossers, K., et al. (2015). Stratified gene expression analysis identifies major amyotrophic lateral sclerosis genes. *Neurobiol. Aging* 36, 2006.e1–9. doi: 10.1016/j.neurobiolaging.2015.02.017

Kasai, T., Kojima, Y., Ohmichi, T., Tatebe, H., Tsuji, Y., Noto, Y. I., et al. (2019). Combined use of CSF NfL and CSF TDP-43 improves diagnostic performance in ALS. *Ann. Clin. Transl. Neurol.* 6, 2489–2502. doi: 10.1002/acn3.50943

Keeney, P. M., and Bennett, J. P. (2010). ALS spinal neurons show varied and reduced mtDNA gene copy numbers and increased mtDNA gene deletions. *Mol. Neurodegener.* 5:21. doi: 10.1186/1750-1326-5-21

Kirby, J., Al Sultan, A., Waller, R., and Heath, P. (2016). The genetics of amyotrophic lateral sclerosis: current insights. *Degener. Neurol. Neuromuscul. Dis.* 6:49. doi: 10.2147/dnnd.s84956

Kirby, J., Halligan, E., Baptista, M. J., Allen, S., Heath, P. R., Holden, H., et al. (2005). Mutant SOD1 alters the motor neuronal transcriptome: implications for familial ALS. *Brain* 128, 1686–1706. doi: 10.1093/brain/awh503

Klingl, Y. E., Pakravan, D., and Van Den Bosch, L. (2020). Opportunities for histone deacetylase inhibition in amyotrophic lateral sclerosis. *Br. J. Pharmacol.* doi: 10.1111/bph.15217 [Epub ahead of print].

Krokidis, M. G. (2020). Transcriptomics and metabolomics in amyotrophic lateral sclerosis. *Adv. Exp. Med. Biol.* 1195, 205–212. doi: 10.1007/978-3-030-32633-3_29

Krokidis, M. G., and Vlamos, P. (2018). Transcriptomics in amyotrophic lateral sclerosis. *Front. Biosci.* 10:103–121. doi: 10.2741/e811

Krüger, T., Lautenschläger, J., Grosskreutz, J., and Rhode, H. (2013). Proteome analysis of body fluids for amyotrophic lateral sclerosis biomarker discovery. *Proteomics Clin. Appl.* 7, 123–135. doi: 10.1002/prca.201200067

Kumar, A., Ghosh, D., and Singh, R. L. (2013). Amyotrophic lateral sclerosis and metabolomics: clinical implication and therapeutic approach. *J. Biomarkers* 2013, 1–15. doi: 10.1155/2013/538765

La Cognata, V., Gentile, G., Aronica, E., and Cavallaro, S. (2020). Splicing players are differently expressed in sporadic amyotrophic lateral sclerosis molecular clusters and brain regions. *Cells* 9:159. doi: 10.3390/cells9010159

Lacomblez, L., Doppler, V., Beucler, I., Costes, G., Salachas, F., Raisonnier, A., et al. (2002). APOE: a potential marker of disease progression in ALS. *Neurology* 58, 1112–1114. doi: 10.1212/WNL.58.7.1112

Laffita-Mesa, J. M., Rodríguez Pupo, J. M., Moreno Sera, R., Vázquez Mojena, Y., Kourí, V., Laguna-Salvia, L., et al. (2013). De novo mutations in Ataxin-2 Gene and ALS Risk. *PLoS One* 8:e70560. doi: 10.1371/journal.pone.0070560

Lam, S., Bayraktar, A., Zhang, C., Turkez, H., Nielsen, J., Boren, J., et al. (2020). A systems biology approach for studying neurodegenerative diseases. *Drug Discov. Today* 25, 1146–1159. doi: 10.1016/j.drudis.2020.05.010

Landers, J. E., Melki, J., Meininger, V., Glass, J. D., Van Den Berg, L. H., Van Es, M. A., et al. (2009). Reduced expression of the Kinesin-Associated Protein 3 (KIFAP3) gene increases survival in sporadic amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 106, 9004–9009. doi: 10.1073/pnas.0812937106

Lanznaster, D., de Assis, D. R., Corcia, P., Pradat, P.-F., and Blasco, H. (2018). Metabolomics biomarkers: a strategy toward therapeutics improvement in ALS. *Front. Neurol.* 9:1126. doi: 10.3389/fneur.2018.01126

Lanznaster, D., Veyrat-Durebex, C., Vourc'h, P., Andres, C. R., Blasco, H., and Corcia, P. (2020). Metabolomics: a tool to understand the impact of genetic mutations in amyotrophic lateral sclerosis. *Genes (Basel)* 11:537. doi: 10.3390/genes11050537

Lederer, C. W., Torrisi, A., Pantelidou, M., Santama, N., and Cavallaro, S. (2007). Pathways and genes differentially expressed in the motor cortex of patients with sporadic amyotrophic lateral sclerosis. *BMC Genomics* 8:26. doi: 10.1186/1471-2164-8-26

Lee, J. H., Liu, J. W., Lin, S. Z., Harn, H. J., and Chiou, T. W. (2018). Advances in patient-specific induced pluripotent stem cells shed light on drug discovery for amyotrophic lateral sclerosis. *Cell Transplant.* 27, 1301–1312. doi: 10.1177/0963689718785154

Lehmer, C., Oeckl, P., Weishaupt, J. H., Volk, A. E., Diehl-Schmid, J., Schroeter, M. L., et al. (2017). Poly-GP in cerebrospinal fluid links C9orf72-associated dipeptide repeat expression to the asymptomatic phase of ALS/FTD. *EMBO Mol. Med.* 9, 859–868. doi: 10.15252/emmm.201607486

Li, Y. J., Pericak-Vance, M. A., Haines, J. L., Siddique, N., McKenna-Yasek, D., Hung, W. Y., et al. (2004). Apolipoprotein E is associated with age at onset of amyotrophic lateral sclerosis. *Neurogenetics* 5, 209–213. doi: 10.1007/s10048-004-0193-0

Little, J., Barakat-Haddad, C., Martino, R., Pringsheim, T., Tremlett, H., McKay, K. A., et al. (2017). Genetic variation associated with the occurrence and progression of neurological disorders. *Neurotoxicology* 61, 243–264. doi: 10.1016/j.neuro.2016.09.018

Liu, W., Venugopal, S., Majid, S., Ahn, I. S., Diamante, G., Hong, J., et al. (2020). Single-cell RNA-seq analysis of the brainstem of mutant SOD1 mice reveals perturbed cell types and pathways of amyotrophic lateral sclerosis. *Neurobiol. Dis.* 141:104877. doi: 10.1016/j.nbd.2020.104877

Lorenzl, S., Albers, D. S., Narr, S., Chirichigno, J., and Beal, M. F. (2002). Expression of MMP-2, MMP-9, and MMP-1 and their endogenous counterregulators TIMP-1 and TIMP-2 in postmortem brain tissue of Parkinson's disease. *Exp. Neurol.* 178, 13–20. doi: 10.1006/exnr.2002.8019

Lu, C.-H., Allen, K., Oei, F., Leoni, E., Kuhle, J., Tree, T., et al. (2016). Systemic inflammatory response and neuromuscular involvement in amyotrophic lateral sclerosis. *Neurol. Neuroimmunol. Neuroinflamm.* 3:e244. doi: 10.1212/nxi.0000000000000244

Lu, C. H., Macdonald-Wallis, C., Gray, E., Pearce, N., Petzold, A., Norgren, N., et al. (2015). Neurofilament light chain: a prognostic biomarker in amyotrophic lateral sclerosis. *Neurology* 84, 2247–2257. doi: 10.1212/WNL.0000000000001642

Ma, G., Wang, Y., Li, Y., Cui, L., Zhao, Y., Zhao, B., et al. (2015). MiR-206, a key modulator of skeletal muscle development and disease. *Int. J. Biol. Sci.* 11, 345–352. doi: 10.7150/ijbs.10921

Mackenzie, I. R. A., Bigio, E. H., Ince, P. G., Geser, F., Neumann, M., Cairns, N. J., et al. (2007). Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations. *Ann. Neurol.* 61, 427–434. doi: 10.1002/ana.21147

Mackenzie, I. R. A., and Rademakers, R. (2008). The role of transactive response DNA-binding protein-43 in amyotrophic lateral sclerosis and frontotemporal dementia. *Curr. Opin. Neurol.* 21, 693–700. doi: 10.1097/WCO.0b013e3283168d1d

Majumder, V., Gregory, J. M., Barria, M. A., Green, A., and Pal, S. (2018). TDP-43 as a potential biomarker for amyotrophic lateral sclerosis: a systematic review and meta-analysis. *BMC Neurol.* 18:90. doi: 10.1186/s12883-018-1091-7

Malaspina, A., and de Belleroche, J. (2004). Spinal cord molecular profiling provides a better understanding of amyotrophic lateral sclerosis pathogenesis. *Brain Res. Brain Res. Rev.* 45, 213–229. doi: 10.1016/j.brainresrev.2004.04.002

Malaspina, A., Kaushik, N., and De Belleroche, J. (2008). Differential expression of 14 genes in amyotrophic lateral sclerosis spinal cord detected using gridded cDNA arrays. *J. Neurochem.* 77, 132–145. doi: 10.1046/j.1471-4159.2001.00231.x

Malik, R., and Wiedau, M. (2020). Therapeutic approaches targeting protein aggregation in amyotrophic lateral sclerosis. *Front. Mol. Neurosci.* 13:98. doi: 10.3389/fnmol.2020.00098

Maniatis, S., Äijö, T., Vickovic, S., Braine, C., Kang, K., Mollbrink, A., et al. (2019). Spatiotemporal dynamics of molecular pathology in amyotrophic lateral sclerosis. *Science* 364, 89–93. doi: 10.1126/science.aav9776

Mao, Y., Kuo, S.-W., Chen, L., Heckman, C. J., and Jiang, M. C. (2017). The essential and downstream common proteins of amyotrophic lateral sclerosis: a protein-protein interaction network analysis. *PLoS One* 12:e0172246. doi: 10.1371/journal.pone.0172246

Maria D'erchia, A., Gallo, A., Manzari, C., Raho, S., Horner, D. S., Chiara, M., et al. (2017). Massive transcriptome sequencing of human spinal cord tissues provides new insights into motor neuron degeneration in ALS. *Sci. Rep.* 7:10046. doi: 10.1038/s41598-017-10488-7

Masala, A., Sanna, S., Esposito, S., Rassu, M., Galioto, M., Zinellu, A., et al. (2018). Epigenetic changes associated with the expression of Amyotrophic Lateral Sclerosis (ALS) causing genes. *Neuroscience* 390, 1–11. doi: 10.1016/j.neuroscience.2018.08.009

Maugeri, G., D'Amico, A. G., Rasà, D. M., Federico, C., Saccone, S., Morello, G., et al. (2019). Molecular mechanisms involved in the protective effect of pituitary adenylate cyclase-activating polypeptide in an in vitro model of amyotrophic lateral sclerosis. *J. Cell. Physiol.* 234, 5203–5214. doi: 10.1002/jcp.27328

Maurel, C., Dangoumau, A., Marouillat, S., Brulard, C., Chami, A., Hergesheimer, R., et al. (2018). Causative genes in amyotrophic lateral sclerosis and protein degradation pathways: a link to neurodegeneration. *Mol. Neurobiol.* 55, 6480–6499. doi: 10.1007/s12035-017-0856-0

Mawrin, C., Kirches, E., Krause, G., Wiedemann, F. R., Vorwerk, C. K., Bogerts, B., et al. (2004). Single-cell analysis of mtDNA deletion levels in sporadic amyotrophic lateral sclerosis. *Neuroreport* 15, 939–943. doi: 10.1097/00001756-200404290-00002

McCann, E. P., Williams, K. L., Fifita, J. A., Tarr, I. S., O'Connor, J., Rowe, D. B., et al. (2017). The genotype-phenotype landscape of familial amyotrophic lateral sclerosis in Australia. *Clin. Genet.* 92, 259–266. doi: 10.1111/cge.12973

Mejzini, R., Flynn, L. L., Pitout, I. L., Fletcher, S., Wilton, S. D., and Akkari, P. A. (2019). ALS genetics, mechanisms, and therapeutics: where are we now? *Front. Neurosci.* 13:1310. doi: 10.3389/fnins.2019.01310

Mirza, B., Wang, W., Wang, J., Choi, H., Chung, N. C., and Ping, P. (2019). Machine learning and integrative analysis of biomedical big data. *Genes (Basel)* 10:87. doi: 10.3390/genes10020087

Mitchell, R. M., Freeman, W. M., Randazzo, W. T., Stephens, H. E., Beard, J. L., Simmons, Z., et al. (2009). A CSF biomarker panel for identification of patients with amyotrophic lateral sclerosis. *Neurology* 72, 14–19. doi: 10.1212/01.wnl.0000333251.36681.a5

Mitropoulos, K., Katsila, T., Patrinos, G. P., and Pampalakis, G. (2018). Multi-omics for biomarker discovery and target validation in biofluids for amyotrophic lateral sclerosis diagnosis. *OMICS* 22, 52–64. doi: 10.1089/omi.2017.0183

Moreau, C., Devos, D., Brunaud-Danel, V., Defebvre, L., Perez, T., Destée, A., et al. (2006). Paradoxical response of VEGF expression to hypoxia in CSF of patients with ALS. *J. Neurol. Neurosurg. Psychiatry* 77, 255–257. doi: 10.1136/jnnp.2005.070904

Morello, G., and Cavallaro, S. (2015). Transcriptional analysis reveals distinct subtypes in amyotrophic lateral sclerosis: implications for personalized therapy. *Future Med. Chem.* 7, 1335–1359. doi: 10.4155/fmc.15.60

Morello, G., Conforti, F. L., Parenti, R., D'Agata, V., and Cavallaro, S. (2015). Selection of potential pharmacological targets in ALS based on whole-genome expression profiling. *Curr. Med. Chem.* 22, 2004–2021. doi: 10.2174/0929867322666150408112135

Morello, G., Guarnaccia, M., Spampinato, A. G., La Cognata, V., D'Agata, V., and Cavallaro, S. (2018a). Copy number variations in amyotrophic lateral sclerosis: piecing the mosaic tiles together through a systems biology approach. *Mol. Neurobiol.* 55, 1299–1322. doi: 10.1007/s12035-017-0393-x

Morello, G., Guarnaccia, M., Spampinato, A. G., Salomone, S., D'Agata, V., Conforti, F. L., et al. (2019). Integrative multi-omic analysis identifies new drivers and pathways in molecularly distinct subtypes of ALS. *Sci. Rep.* 9:9968. doi: 10.1038/s41598-019-46355-w

Morello, G., Spampinato, A. G., and Cavallaro, S. (2017a). Molecular taxonomy of sporadic amyotrophic lateral sclerosis using disease-associated genes. *Front. Neurol.* 8:152. doi: 10.3389/fneur.2017.00152

Morello, G., Spampinato, A. G., and Cavallaro, S. (2017b). Neuroinflammation and ALS: transcriptomic insights into molecular disease mechanisms and therapeutic targets. *Mediat. Inflamm.* 2017:7070469. doi: 10.1155/2017/7070469

Morello, G., Spampinato, A. G., Conforti, F. L., and Cavallaro, S. (2018b). Taxonomy meets neurology, the case of amyotrophic lateral sclerosis. *Front. Neurosci.* 12:673. doi: 10.3389/fnins.2018.00673

Morello, G., Spampinato, A. G., Conforti, F. L., D'Agata, V., and Cavallaro, S. (2017c). Selection and prioritization of candidate drug targets for amyotrophic lateral sclerosis through a meta-analysis approach. *J. Mol. Neurosci.* 61, 563–580. doi: 10.1007/s12031-017-0898-9

Moulard, B., Sefiani, A., Laamri, A., Malafosse, A., and Camu, W. (1996). Apolipoprotein E genotyping in sporadic amyotrophic lateral sclerosis, evidence for a major influence on the clinical presentation and prognosis. *J. Neurol. Sci.* 139, 34–37. doi: 10.1016/0022-510X(96)00085-8

Myszczynska, M., and Ferraiuolo, L. (2016). New in vitro models to study amyotrophic lateral sclerosis. *Brain Pathol.* 26, 258–265. doi: 10.1111/bpa.12353

Namboori, S. C., Thomas, P., Ames, R., Garrett, L. O., Willis, C. R. G., Stanton, L. W., et al. (2019). Single cell transcriptomics identifies master regulators of dysfunctional pathways in SOD1 ALS motor neurons. *bioRxiv*[Preprint] doi: 10.1101/593129

Naruse, H., Ishiura, H., Mitsui, J., Takahashi, Y., Matsukawa, T., Tanaka, M., et al. (2019). Burden of rare variants in causative genes for amyotrophic lateral sclerosis (ALS) accelerates age at onset of ALS. *J. Neurol. Neurosurg. Psychiatry* 90, 537–542. doi: 10.1136/jnnp-2018-318568

Nardo, G., Iennaco, R., Fusi, N., Heath, P. R., Marino, M., Trolese, M. C., et al. (2013). Transcriptomic indices of fast and slow disease progression in two mouse models of amyotrophic lateral sclerosis. *Brain* 136, 3305–3332. doi: 10.1093/brain/awt250

Navone, F., Genevini, P., and Borgese, N. (2015). Autophagy and neurodegeneration: insights from a cultured cell model of ALS. *Cells* 4, 354–386. doi: 10.3390/cells4030354

Nguyen, D. K. H., Thombre, R., and Wang, J. (2019). Autophagy as a common pathway in amyotrophic lateral sclerosis. *Neurosci. Lett.* 697, 34–48. doi: 10.1016/j.neulet.2018.04.006

Nguyen, N. D., and Wang, D. (2020). Multiview learning for understanding functional multiomics. *PLoS Comput. Biol.* 16:e1007677. doi: 10.1371/journal.pcbi.1007677

Nicolas, A., Kenna, K., Renton, A. E., Ticozzi, N., Faghri, F., Chia, R., et al. (2018). Genome-wide Analyses Identify KIF5A as a Novel ALS Gene. *Neuron* 97, 1268–1283.e6. doi: 10.1016/j.neuron.2018.02.027

Niebroj-Dobosz, I., Janik, P., Sokołowska, B., and Kwiecinski, H. (2010). Matrix metalloproteinases and their tissue inhibitors in serum and cerebrospinal fluid of patients with amyotrophic lateral sclerosis. *Eur. J. Neurol.* 17, 226–231. doi: 10.1111/j.1468-1331.2009.02775.x

Oeckl, P., Weydt, P., Thal, D. R., Weishaupt, J. H., Ludolph, A. C., and Otto, M. (2020). Proteomics in cerebrospinal fluid and spinal cord suggests UCHL1,

MAP2 and GPNMB as biomarkers and underpins importance of transcriptional pathways in amyotrophic lateral sclerosis. *Acta Neuropathol.* 139, 119–134. doi: 10.1007/s00401-019-02093-x

Olivier, M., Asmis, R., Hawkins, G. A., Howard, T. D., and Cox, L. A. (2019). The need for multi-omics biomarker signatures in precision medicine. *Int. J. Mol. Sci.* 20:4781. doi: 10.3390/ijms20194781

Online, R., Hedl, T., Gil, R. S., Cheng, F., Rayner, S. L., Davidson, J., et al. (2019). Proteomics approaches for biomarker and drug target discovery in ALS and FTD publication details. *Front Neurosci.* 13:548.

Paez-Colasante, X., Figueroa-Romero, C., Sakowski, S. A., Goutman, S. A., and Feldman, E. L. (2015). Amyotrophic lateral sclerosis: mechanisms and therapeutics in the epigenomic era. *Nat. Rev. Neurol.* 11, 266–279. doi: 10.1038/nrneurol.2015.57

Parakh, S., and Atkin, J. D. (2016). Protein folding alterations in amyotrophic lateral sclerosis. *Brain Res.* 1648, 633–649. doi: 10.1016/j.brainres.2016.04.010

Pasinelli, P., and Brown, R. H. (2006). Molecular biology of amyotrophic lateral sclerosis: insights from genetics. *Nat. Rev. Neurosci.* 7, 710–723. doi: 10.1038/nrn1971

Pasinetti, G. M., Ungar, L. H., Lange, D. J., Yemul, S., Deng, H., Yuan, X., et al. (2006). Identification of potential CSF biomarkers in ALS. *Neurology* 66, 1218–1222. doi: 10.1212/01.wnl.0000203129.82104.07

Perlson, E., Maday, S., Fu, M. M., Moughamian, A. J., and Holzbaur, E. L. F. (2010). Retrograde axonal transport: pathways to cell death? *Trends Neurosci.* 33, 335–344. doi: 10.1016/j.tins.2010.03.006

Poesen, K. (2018). The chromosomal conformation signature: a new kid on the block in ALS biomarker research? *EBioMedicine* 33, 6–7. doi: 10.1016/j.ebiom.2018.07.003

Prell, T., Lautenschläger, J., and Grosskreutz, J. (2013). Calcium-dependent protein folding in amyotrophic lateral sclerosis. *Cell Calcium* 54, 132–143. doi: 10.1016/j.ceca.2013.05.007

Prudencio, M., Belzil, V. V., Batra, R., Ross, C. A., Gendron, T. F., Pregent, L. J., et al. (2015). Distinct brain transcriptome profiles in C9orf72-associated and sporadic ALS. *Nat. Neurosci.* 18, 1175–1182. doi: 10.1038/nn.4065

Rahman, M. R., Islam, T., Huq, F., Quinn, J. M. W., and Moni, M. A. (2019). Identification of molecular signatures and pathways common to blood cells and brain tissue of amyotrophic lateral sclerosis patients. *Inform. Med. Unlock.* 16:100193. doi: 10.1016/j.imu.2019.100193

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

Ramanan, V. K., and Saykin, A. J. (2013). Pathways to neurodegeneration: mechanistic insights from GWAS in Alzheimer's disease, Parkinson's disease, and related disorders. *Am. J. Neurodegener. Dis.* 2, 145–175.

Ranganathan, S., Williams, E., Ganchev, P., Gopalakrishnan, V., Lacomis, D., Urbinelli, L., et al. (2005). Proteomic profiling of cerebrospinal fluid identifies biomarkers for amyotrophic lateral sclerosis. *J. Neurochem.* 95, 1461–1471. doi: 10.1111/j.1471-4159.2005.03478.x

Rao, V. S., Srinivas, K., Sujini, G. N., and Kumar, G. N. S. (2014). Protein-protein interaction detection: methods and analysis. *Int. J. Proteomics* 2014:147648. doi: 10.1155/2014/147648

Rattray, N. J. W., and Daouk, R. K. (2017). Pharmacometabolomics and precision medicine special issue editorial. *Metabolomics* 13, 1–4. doi: 10.1007/s11306-017-1191-1

Recabarren-Leiva, D., and Alarcón, M. (2018). New insights into the gene expression associated to amyotrophic lateral sclerosis. *Life Sci.* 193, 110–123. doi: 10.1016/j.lfs.2017.12.016

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

Ricci, C., Marzocchi, C., and Battistini, S. (2018). MicroRNAs as biomarkers in amyotrophic lateral sclerosis. *Cells* 7:219. doi: 10.3390/cells7110219

Rizzuti, M., Filosa, G., Melzi, V., Calandriello, L., Dioni, L., Bollati, V., et al. (2018). MicroRNA expression analysis identifies a subset of downregulated miRNAs in ALS motor neuron progenitors. *Sci. Rep.* 8, 1–12. doi: 10.1038/s41598-018-28366-1

Robberecht, W., and Eykens, C. (2015). The genetic basis of amyotrophic lateral sclerosis: recent breakthroughs. *Adv. Genomics Genet.* 5:327. doi: 10.2147/agg.s57397

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. doi: 10.1038/362059a0

Rosengren, L. E., Karlsson, J.-E., Karlsson, J.-O., Persson, L. I., and Wikkelsø, C. (2002). Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. *J. Neurochem.* 67, 2013–2018. doi: 10.1046/j.1471-4159.1996.67052013.x

Rué, L., Timmers, M., Lenaerts, A., Smolders, S., Poppe, L., de Boer, A., et al. (2019). Reducing EphA4 before disease onset does not affect survival in a mouse model of Amyotrophic Lateral Sclerosis. *Sci. Rep.* 9, 1–9. doi: 10.1038/s41598-019-50615-0

Ruegsegger, C., and Saxena, S. (2016). Proteostasis impairment in ALS. *Brain Res.* 1648, 571–579. doi: 10.1016/j.brainres.2016.03.032

Ryan, M., Heverin, M., Doherty, M. A., Davis, N., Corr, E. M., Vajda, A., et al. (2018). Determining the incidence of familiality in ALS. *Neurol. Genet.* 4:e239. doi: 10.1212/NXG.0000000000000239

Ryberg, H., and Bowser, R. (2008). Protein biomarkers for amyotrophic lateral sclerosis. *Expert Rev. Proteomics* 5, 249–262. doi: 10.1586/14789450.5.2.249

Salter, M., Corfield, E., Ramadass, A., Grand, F., Green, J., Westra, J., et al. (2018). Initial identification of a blood-based chromosome conformation signature for aiding in the diagnosis of amyotrophic lateral sclerosis. *EBioMedicine* 33, 169–184. doi: 10.1016/j.ebiom.2018.06.015

Sances, S., Bruijn, L. I., Chandran, S., Eggan, K., Ho, R., Klim, J. R., et al. (2016). Modeling ALS with motor neurons derived from human induced pluripotent stem cells. *Nat. Neurosci.* 19, 542–553. doi: 10.1038/nn.4273

Sangare, M., Dicko, I., Guinto, C. O., Sissoko, A., Dembele, K., Coulibaly, Y., et al. (2016). Does the survival motor neuron copy number variation play a role in the onset and severity of sporadic amyotrophic lateral sclerosis in Malians? *eNeurologicalSci* 3, 17–20. doi: 10.1016/j.ensci.2015.12.001

Santiago, J. A., Bottero, V., and Potashkin, J. A. (2017). Dissecting the molecular mechanisms of neurodegenerative diseases through network biology. *Front. Aging Neurosci.* 9:166. doi: 10.3389/fnagi.2017.00166

Saris, C. G. J., Groen, E. J. N., Koekkoek, J. A. F., Veldink, J. H., and Van Den Berg, L. H. (2013). Meta-analysis of gene expression profiling in amyotrophic lateral sclerosis: a comparison between transgenic mouse models and human patients. *Amyotroph. Lateral Scler. Front. Degener.* 14, 177–189. doi: 10.3109/21678421.2012.729842

Scoles, D. R., and Pulst, S. M. (2018). Oligonucleotide therapeutics in neurodegenerative diseases. *RNA Biol.* 15, 707–714. doi: 10.1080/15476286.2018.1454812

Selvaraj, B. T., Livesey, M. R., and Chandran, S. (2017). Modeling the C9ORF72 repeat expansion mutation using human induced pluripotent stem cells. *Brain Pathol.* 27, 518–524. doi: 10.1111/bpa.12520

Sharma, S., and Lu, H. C. (2018). microRNAs in neurodegeneration: current findings and potential impacts. *J. Alzheimers Dis. Park.* 9, 166. doi: 10.4172/2161-0460.1000420

Shi, N., Kawano, Y., Tateishi, T., Kikuchi, H., Osoegawa, M., Ohyagi, Y., et al. (2007). Increased IL-13-producing T cells in ALS: positive correlations with disease severity and progression rate. *J. Neuroimmunol.* 182, 232–235. doi: 10.1016/j.jneuroim.2006.10.001

Shurte, L. (2016). *Determining Protein-Protein Interactions of ALS-Associated SOD1. Brows. all Theses Diss.* Available online at: https://corescholar.libraries.wright.edu/etd_all/2043 (accessed June 3, 2020).

Snider, J., Kotlyar, M., Saraon, P., Yao, Z., Jurisica, I., and Stagljar, I. (2015). Fundamentals of protein interaction network mapping. *Mol. Syst. Biol.* 11:848. doi: 10.15252/msb.20156351

Sproviero, W., Shatunov, A., Stahl, D., Shoai, M., van Rheenen, W., Jones, A. R., et al. (2017). ATXN2 trinucleotide repeat length correlates with risk of ALS. *Neurobiol. Aging* 51, 178.e1–178.e9. doi: 10.1016/j.neurobiolaging.2016.11.010

Su, X. W., Broach, J. R., Connor, J. R., Gerhard, G. S., and Simmons, Z. (2014). Genetic heterogeneity of amyotrophic lateral sclerosis: implications for clinical practice and research. *Muscle and Nerve* 49, 786–803. doi: 10.1002/mus.24198

Süssmuth, S. D., Tumani, H., Ecker, D., and Ludolph, A. C. (2003). Amyotrophic lateral sclerosis: disease stage related changes of tau protein and S100 beta in cerebrospinal fluid and creatine kinase in serum. *Neurosci. Lett.* 353, 57–60. doi: 10.1016/j.neulet.2003.09.018

Takahashi, I., Hama, Y., Matsushima, M., Hirotani, M., Kano, T., Hohzen, H., et al. (2015). Identification of plasma microRNAs as a biomarker of sporadic

Amyotrophic Lateral Sclerosis. *Mol. Brain* 8:67. doi: 10.1186/s13041-015-0161-7

Tam, O. H., Rozhkov, N. V., Shaw, R., Ravits, J., Dubnau, J., Gale, M., et al. (2019). Postmortem cortex samples identify distinct molecular subtypes of ALS: retrotransposon activation, oxidative stress, and activated glia. *CellReports* 29, 1164–1177.e5. doi: 10.1016/j.celrep.2019.09.066

Tanaka, F., Sone, J., Atsuta, N., Nakamura, R., Doi, H., Koyano, S., et al. (2013). Personal genome analysis in amyotrophic lateral sclerosis. *Brain Nerve* 65, 257–265.

Tanaka, M., Kikuchi, H., Ishizu, T., Minohara, M., Osoegawa, M., Motomura, K., et al. (2006). Intrathecal upregulation of granulocyte colony stimulating factor and its neuroprotective actions on motor neurons in amyotrophic lateral sclerosis. *J. Neuropathol. Exp. Neurol.* 65, 816–825. doi: 10.1097/01.jnen.0000232025.84238.e1

Tang, A. Y. (2014). RNA-binding proteins associated molecular mechanisms of motor neuron degeneration pathogenesis. *Mol. Biotechnol.* 56, 779–786. doi: 10.1007/s12033-014-9785-6

Tateishi, T., Yamasaki, R., Tanaka, M., Matsushita, T., Kikuchi, H., Isobe, N., et al. (2010). CSF chemokine alterations related to the clinical course of amyotrophic lateral sclerosis. *J. Neuroimmunol.* 222, 76–81. doi: 10.1016/j.jneuroim.2010.03.004

Taylor, J. P., Brown, R. H., and Cleveland, D. W. (2016). Decoding ALS: from genes to mechanism. *Nature* 539, 197–206. doi: 10.1038/nature20413

Thompson, A. G., Gray, E., Thézénas, M. L., Charles, P. D., Evetts, S., Hu, M. T., et al. (2018). Cerebrospinal fluid macrophage biomarkers in amyotrophic lateral sclerosis. *Ann. Neurol.* 83, 258–268. doi: 10.1002/ana.25143

Toivonen, J. M., Manzano, R., Oliván, S., Zaragoza, P., García-Redondo, A., and Osta, R. (2014). MicroRNA-206: a potential circulating biomarker candidate for amyotrophic lateral sclerosis. *PLoS One* 9:e89065. doi: 10.1371/journal.pone.0089065

Tsuboi, Y., Kakimoto, K., Akatsu, H., Daikuhara, Y., and Yamada, T. (2002). Hepatocyte growth factor in cerebrospinal fluid in neurologic disease. *Acta Neurol. Scand.* 106, 99–103. doi: 10.1034/j.1600-0404.2002.01125.x

Umoh, M. E., Dammer, E. B., Dai, J., Duong, D. M., Lah, J. J., Levey, A. I., et al. (2018). A proteomic network approach across the ALS – FTD disease spectrum resolves clinical phenotypes and genetic vulnerability in human brain. *EMBO Mol. Med.* 10, 48–62. doi: 10.15252/emmm.201708202

Uyan, Ö, Ömür, Ö, Ağım, Z. S., Özoğuz, A., Li, H., Parman, Y., et al. (2013). Genome-wide copy number variation in sporadic amyotrophic lateral sclerosis in the Turkish population: deletion of EPHA3 is a possible protective factor. *PLoS One* 8:e72381. doi: 10.1371/journal.pone.0072381

Vadgama, N., Pittman, A., Simpson, M., Nirmalananthan, N., Murray, R., Yoshikawa, T., et al. (2019). De novo single-nucleotide and copy number variation in discordant monozygotic twins reveals disease-related genes. *Eur. J. Hum. Genet.* 27, 1121–1133. doi: 10.1038/s41431-019-0376-7

van Blitterswijk, M., Mullen, B., Heckman, M. G., Baker, M. C., DeJesus-Hernandez, M., Brown, P. H., et al. (2014a). Ataxin-2 as potential disease modifier in C9ORF72 expansion carriers. *Neurobiol. Aging* 35, 2421.e13–7. doi: 10.1016/j.neurobiolaging.2014.04.016

van Blitterswijk, M., Mullen, B., Wojtas, A., Heckman, M. G., Diehl, N. N., Baker, M. C., et al. (2014b). Genetic modifiers in carriers of repeat expansions in the C9ORF72 gene. *Mol. Neurodegener.* 9:38. doi: 10.1186/1750-1326-9-38

Van Den Heuvel, D. M. A., Harschnitz, O., Van Den Berg, L. H., and Pasterkamp, R. J. (2014). Taking a risk: a therapeutic focus on ataxin-2 in amyotrophic lateral sclerosis? *Trends Mol. Med.* 20, 25–35. doi: 10.1016/j.molmed.2013.09.001

van der Spek, R. A. A., van Rheenen, W., Pulit, S. L., Kenna, K. P., van den Berg, L. H., and Veldink, J. H. (2019). The project MinE databrowser: bringing large-scale whole-genome sequencing in ALS to researchers and the public. *Amyotroph. Lateral Scler. Front. Degener.* 20, 432–440. doi: 10.1080/21678421.2019.1606244

Van Hoecke, A., Schoonaert, L., Lemmens, R., Timmers, M., Staats, K. A., Laird, A. S., et al. (2012). EPHA4 is a disease modifier of amyotrophic lateral sclerosis in animal models and in humans. *Nat. Med.* 18, 1418–1422. doi: 10.1038/nm.2901

Van Rheenen, W., Pulit, S. L., Dekker, A. M., Al Khleifat, A., Brands, W. J., Iacoangeli, A., et al. (2018). Project MinE: study design and pilot analyses of a large-scale whole-genome sequencing study in amyotrophic lateral sclerosis. *Eur. J. Hum. Genet.* 26, 1537–1546. doi: 10.1038/s41431-018-0177-4

Van Rheenen, W., Shatunov, A., Dekker, A. M., McLaughlin, R. L., Diekstra, F. P., Pulit, S. L., et al. (2016). Genome-wide association analyses identify new risk variants and the genetic architecture of amyotrophic lateral sclerosis. *Nat. Genet.* 48, 1043–1048. doi: 10.1038/ng.3622

Vella, D., Zoppis, I., Mauri, G., Mauri, P., and Di Silvestre, D. (2017). From protein-protein interactions to protein co-expression networks: a new perspective to evaluate large-scale proteomic data. *Eurasip J. Bioinforma. Syst. Biol.* 2017, 1–16. doi: 10.1186/s13637-017-0059-z

Verde, F., Steinacker, P., Weishaupt, J. H., Kassubek, J., Oeckl, P., Halbgebauer, S., et al. (2019). Neurofilament light chain in serum for the diagnosis of amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* 90, 157–164. doi: 10.1136/jnnp-2018-318704

Vijayakumar, U. G., Milla, V., Stafford, M. Y. C., Bjourson, A. J., Duddy, W., and Duguez, S. M. R. (2019). A systematic review of suggested molecular strata, biomarkers and their tissue sources in ALS. *Front. Neurol.* 10:400. doi: 10.3389/fneur.2019.00400

Volonté, C., Morello, G., Spampinato, A. G., Amadio, S., Apolloni, S., D'Agata, V., et al. (2020). Omics-based exploration and functional validation of neurotrophic factors and histamine as therapeutic targets in ALS. *Ageing Res. Rev.* 62, 101121. doi: 10.1016/j.arr.2020.101121

von Neuhoff, N., Oumeraci, T., Wolf, T., Kollewe, K., Bewerunge, P., Neumann, B., et al. (2012). Monitoring CSF proteome alterations in amyotrophic lateral sclerosis: obstacles and perspectives in translating a novel marker panel to the clinic. *PLoS One* 7:e44401. doi: 10.1371/journal.pone.0044401

Wain, L. V., Pedroso, I., Landers, J. E., Breen, G., Shaw, C. E., Leigh, P. N., et al. (2009). The role of copy number variation in susceptibility to amyotrophic lateral sclerosis: genome-wide association study and comparison with published loci. *PLoS One* 4:e8175. doi: 10.1371/journal.pone.0008175

Wang, T., Zhang, J., and Xu, Y. (2020). Epigenetic basis of lead-induced neurological disorders. *Int. J. Environ. Res. Public Health* 17, 1–23. doi: 10.3390/ijerph17134878

Wang, X. S., Simmons, Z., Liu, W., Boyer, P., and Connor, J. (2006). Differential expression of genes in amyotrophic lateral sclerosis revealed by profiling the post mortem cortex. *Amyotroph. Lateral Scler.* 7, 201–216. doi: 10.1080/17482960600947689

Webster, C. P., Smith, E. F., Shaw, P. J., and De Vos, K. J. (2017). Protein homeostasis in amyotrophic lateral sclerosis: therapeutic opportunities? *Front. Mol. Neurosci.* 10:123. doi: 10.3389/fnmol.2017.00123

Weskamp, K., and Barmada, S. J. (2018). TDP43 and RNA instability in amyotrophic lateral sclerosis. *Brain Res.* 1693, 67–74. doi: 10.1016/j.brainres.2018.01.015

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

Yang, B., Jiang, H., Wang, F., Li, S., Wu, C., Bao, J., et al. (2019). UNC13A variant rs12608932 is associated with increased risk of amyotrophic lateral sclerosis and reduced patient survival: a meta-analysis. *Neurol. Sci.* 40, 2293–2302. doi: 10.1007/s10072-019-03951-y

Yerbury, J. J., Farrawell, N. E., and McAlary, L. (2020). Proteome homeostasis dysfunction: a unifying principle in ALS pathogenesis. *Trends Neurosci.* 43, 274–284. doi: 10.1016/j.tins.2020.03.002

Yu, X. T., and Zeng, T. (2018). Integrative analysis of omics big data. *Methods Mol. Biol.* 1754, 109–135. doi: 10.1007/978-1-4939-7717-8_7

Zeng, P., and Zhou, X. (2019). Causal effects of blood lipids on amyotrophic lateral sclerosis: a Mendelian randomization study. *Hum. Mol. Genet.* 28, 688–697. doi: 10.1093/hmg/ddy384

Zetterberg, H., Jacobsson, J., Rosengren, L., Blennow, K., and Andersen, P. M. (2007). Cerebrospinal fluid neurofilament light levels in amyotrophic lateral sclerosis: impact of SOD1 genotype. *Eur. J. Neurol.* 14, 1329–1333. doi: 10.1111/j.1468-1331.2007.01972.x

Zhao, Z., Lange, D. J., Ho, L., Bonini, S., Shao, B., Salton, S. R., et al. (2008). Vgf is a novel biomarker associated with muscle weakness in amyotrophic lateral

sclerosis (ALS), with a potential role in disease pathogenesis. *Int. J. Med. Sci.* 5, 92–99. doi: 10.7150/ijms.5.92

Zhu, S., Wuolikainen, A., Wu, J., Öhman, A., Wingsle, G., Moritz, T., et al. (2019). Targeted multiple reaction monitoring analysis of CSF identifies UCHL1 and GPNMB as candidate biomarkers for ALS. *J. Mol. Neurosci.* 69, 643–657. doi: 10.1007/s12031-019-01411-y

Ziff, O. J., and Patani, R. (2019). Harnessing cellular aging in human stem cell models of amyotrophic lateral sclerosis. *Aging Cell* 18:e12862. doi: 10.1111/acel.12862

Zubiri, I., Lombardi, V., Bremang, M., Mitra, V., Nardo, G., Adiutori, R., et al. (2018). Tissue-enhanced plasma proteomic analysis for disease stratification in amyotrophic lateral sclerosis. *Mol. Neurodegener.* 13:60. doi: 10.1186/s13024-018-0292-2

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

Zufiría, M., Gil-Bea, F. J., Fernández-Torrón, R., Poza, J. J., Muñoz-Blanco, J. L., Rojas-García, R., et al. (2016). ALS: a bucket of genes, environment, metabolism and unknown ingredients. *Prog. Neurobiol.* 142, 104–129. doi: 10.1016/j.pneurobio.2016.05.004

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Key Molecules and Pathways Underlying Sporadic Amyotrophic Lateral Sclerosis: Integrated Analysis on Gene Expression Profiles of Motor Neurons

*Jianing Lin[1], Pian Huang[1], Weineng Chen[1], Chenghui Ye[2], Huanxing Su[3] and Xiaoli Yao[1]**

[1] Department of Neurology, The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Diagnosis and Treatment of Major Neurological Diseases, National Key Clinical Department and Key Discipline of Neurology, Guangzhou, China, [2] Department of Neurology, The Seventh Affiliated Hospital of Sun Yat-sen University, Shenzhen, China, [3] State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by progressive loss of motor neurons. The complex mechanisms underlying ALS are yet to be elucidated, while the lack of disease biomarkers and therapeutic options are associated with the poor prognosis of ALS patients. In this study, we performed bioinformatics analysis to clarify potential mechanisms in sporadic ALS (sALS). We compared three gene expression profiles (GSE18920, GSE56500, and GSE68605) of motor neurons obtained from sALS patients and healthy controls to discover differentially expressed genes (DEGs), and then performed integrated bioinformatics analyses to identify key molecules and pathways underlying sALS. We found that these DEGs were mainly enriched in the structure and functions of extracellular matrix (ECM), while functional enrichment in blood vessel morphogenesis was less correlated with motor neurons. The clustered subnetworks of the constructed protein-protein interaction network for DEGs and the group of selected hub genes were more significantly involved in the organization of collagen-containing ECM. The transcriptional factors database proposed RelA and NF-κB1 from NF-κB family as the key regulators of these hub genes. These results mainly demonstrated the alternations in ECM-related gene expression in motor neurons and suggested the role of NF-κB regulatory pathway in the pathogenesis of sALS.

Keywords: sporadic amyotrophic lateral sclerosis, gene expression profiles, motor neurons, bioinformatics analysis, *FN1* gene

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MicroPharm Ltd., United Kingdom

****Correspondence:***
Xiaoli Yao
yaoxiaol@mail.sysu.edu.cn

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by notable degeneration in upper and lower motor neurons (Oskarsson et al., 2018). The clinical features of ALS vary in upper and lower motor neuron signs in different regions, and some patients may develop cognitive and behavioral symptoms (van Es et al., 2017; Oskarsson et al., 2018). The incidence of ALS rises in midlife and reaches the peak at the age of 70 years or above, while the prevalence has been estimated

at about 4–8 in 100,000 people in most populations (Marin et al., 2018; Longinetti and Fang, 2019). Progressive weakness in muscles will lead to death eventually within 2–3 years after disease onset, mainly due to respiratory failure (Oskarsson et al., 2018). Apart from riluzole and edaravone as two widely FDA-approved drugs that can prolong the survival time for several months, ALS is still an incurable disease with limited therapeutic options (Brown and Al-Chalabi, 2017).

It is essential to explore the underlying mechanisms in ALS, but the etiology of ALS remains largely unknown. Both genetic and environmental factors play crucial roles in the pathogenesis of ALS. Since the first mutation in Cu/Zn superoxide dismutase (SOD1) gene was discovered, more than 25 ALS-related genes have been identified to date, due to the development of high-throughput DNA sequencing (Chia et al., 2018; Nguyen et al., 2018). More than 90% of ALS cases are sporadic ALS (sALS) without reported family history, while two-thirds of familial ALS (fALS) are hereditary (Renton et al., 2014). Mutations in common ALS-related genes, including SOD1, TARDBP, C9ORF72, and FUS, were found in less than 50% fALS and about 5% sALS, and genetic factors were found in only two thirds of fALS and about 10% of sALS, indicating that genetic factors cannot fully explain the pathogenesis of ALS, especially the sporadic form of ALS (Renton et al., 2014; Zou et al., 2017). Vigorous physical activity, smoking and exposure to pesticides, heavy metals, and electromagnetic fields were suspected risk factors for ALS (Zufiria et al., 2016).

The development in microarray and sequencing technology makes it possible for researchers to study diseases at DNA and RNA levels, and comprehensive analyses of multiple studies are more reliable and effective in searching for common gene targets (Kotni et al., 2016; Al-Chalabi et al., 2017). Hence, we performed integrated bioinformatics analysis among three gene expression series (GSE) from the Gene Expression Omnibus (GEO) to identify differentially expressed genes (DEGs) in motor neurons between sALS cases and healthy controls (HC). Subsequently, we conducted functional and pathway enrichment for DEGs and explored their protein-protein interactions (PPI), followed by identification of hub genes and clustered subnetworks from the PPI network, and the discovery of regulatory transcriptional factors (TFs). Our study aims to provide new insights into the pathogenesis of sALS and clues in discovering novel biomarkers and therapeutic options.

MATERIALS AND METHODS

Data Collection and Processing

According to the main purpose of our study, we searched relevant gene expression profiles from the GEO database[1], a public functional genomics data repository. Datasets focusing on familial ALS, *in vitro* studies, or studies concerning samples of motor cortex and spinal cord without the isolation of motor neurons were excluded in our analysis. At last, we collected three gene expression profiles: GSE18920, GSE56500, and GSE68605. Among them, GSE18920 and GSE56500 were based on GPL5188 platform ([HuEx-1_0-st] Affymetrix Human Exon 1.0 ST Array), while GSE68605 was based on GPL570 platform ([HG-133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array). The original researchers obtained motor neurons through laser capture microdissection in human spinal cords, followed by extraction of total RNA and gene expression profiling with Affymatrix microarrays. Gene expression profiles of 19 neurologically HC and 19 sALS patients were included, and we excluded fALS patients in our analysis. As all gene expression profiles from GEO were public online, we did not perform any experiments on human tissues in our study. The overview of detailed information of three gene expression series selected in our study was shown in **Table 1**.

Identification and Analysis of DEGs

GEO2R[2] was an online analysis tool to compare two or more groups of samples for DEGs by employing GEOquery and limma R packages from the Bioconductor project[3]. Here we utilized GEO2R to discover DEGs between sALS and HC, and Graphpad Prism 8 (GraphPad Software Inc., San Diego, CA, United States) was used for volcano plot visualization. The cut-off criteria were set at $P < 0.05$ and $|\log_2\text{Fold Change (FC)}| > 1$ for DEGs. An online Venn diagram was applied to discover the overlapped DEGs from all gene expression profiles[4].

Functional and Pathway Enrichment Analysis of DEGs

We performed function and pathway enrichment analysis based on Metascape[5], an integrated online tool for gene list annotation and biological analysis (Zhou et al., 2019). Gene ontology (GO) analysis is a common method in functional enrichment analysis, aiming to provide gene annotations in biological processes (BP), molecular functions (MF), and cellular components (CC). Kyoto Encyclopedia of Genes and Genomes (KEGG) database is crucial in understanding high-level functions and utilities of biological systems, which is especially famous for its pathway enrichment analysis. The overlapped DEGs was analyzed and visualized by Metascape with the criteria of minimum overlap > 3, P-value cut-off < 0.01, and minimum enrichment score > 1.5.

PPI Network Construction and Identification of Hub Genes

The Search Tool for the Retrieval of Interacting Genes (STRING) online database (version 11.0)[6] is a common tool for predicting PPI networks with increased coverage in genome-wide experimental datasets (Szklarczyk et al., 2019). The predicted PPIs of DEGs was under the criteria at a medium confidence (minimum required interaction score > 0.4) and subsequently visualized by Cytoscape software (version 3.7.2)[7], an open

[1]https://www.ncbi.nlm.nih.gov/geo/

[2]https://www.ncbi.nlm.nih.gov/geo/geo2r/

[3]http://www.bioconductor.org/

[4]http://bioinformatics.psb.ugent.be/webtools/Venn/

[5]https://metascape.org/

[6]https://string-db.org/

[7]https://cytoscape.org/index.html

TABLE 1 | Details of three gene expression profiles derived from GEO database.

Series	GSE18920	GSE56500	GSE68605
Platform	GPL5188	GPL5188	GPL570
Array	HuEx-1_0-st; Affymetrix Human Exon 1.0 ST Array	HuEx-1_0-st; Affymetrix Human Exon 1.0 ST Array	HG-133_Plus_2; Affymetrix Human Genome U133 Plus 2.0 Array
Tissue	Motor neurons from lumber spinal cord	Motor neurons from cervical spinal cord	Motor neurons from cervical spinal cord
Healthy control	10	6	3
Male: Female	8:2	5:1	1:2
Mean age (years)	72.8	61.7	60.0
Sporadic ALS	12	3	4
Male: Female	6:6	2:1	1:3
Mean Age (years)	66.4	65.7	60.5
Mean ALS duration (years)	2.88	Not available	2.27
Bulbar onset: Spinal onset	6:6	Not available	2:2

source software for visualization and integration of biomolecular interaction networks (Shannon et al., 2003). The Molecular Complex Detection (MCODE) plugin (version 1.6.1) was applied to discover highly connected clusters in a given network, with the analysis criteria as degree cutoff = 2, node score cutoff = 0.2, K-core = 2, and maximum depth = 100. The key nodes were selected according to the scoring of maximum correlation criterion (MCC) by using the cytoHubba plugin, which explores important nodes and subnetworks by topological algorithms. Twenty genes scoring the highest were identified as hub genes in our study.

Analysis of Hub Genes and the Regulatory Transcriptional Network

The identified clusters with more than five nodes and the selected hub genes in our study were analyzed by Metascape, respectively, for enrichment analysis. The ClueGO (version 2.5.7) and CluePedia (version 1.5.7) plugins in Cytoscape were applied for visualization of the functionally grouped networks of the hub genes. The Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining (TRRUST) (version 2)[8] is a manual database of human and mouse transcriptional regulatory networks, which was utilized to discover key regulators for the hub genes in our study (Han et al., 2018). Depending on the recorded TFs-targeted genes regulatory relationships in TRRUST, we identified TFs that regulated the expression of hub genes and then visualized the regulatory network by STRING.

RESULTS

Identification of DEGs in ALS

After the exclusion of fALS samples, three selected gene expression profiles (GSE18920, GSE56500, and GSE68605) were analyzed in our study. With the standardization of the value of gene expression and calculation by GEO2R tools, the DEGs were identified between sALS patients and HC of each series according to the filtering criteria, which were displayed in the volcano plots respectively (**Figures 1A–C**). A total of 3,184 DEGs were found in GSE18920, with 2,428 upregulated and 756 downregulated. In GSE56500, 4018 DEGs were found, including 2,100 upregulated and 1,918 downregulated genes. Of 2,061 DEGs found in GSE68605, 971 were upregulated and 1,090 were downregulated. In total, 206 overlapping DEGs were identified by Venn diagram and then processed for further analysis (**Figure 1D**).

Function and Pathway Enrichment Analysis of DEGs

GO and pathway enrichment analysis of DEGs was performed by Metascape, which is useful in identifying crucial biological functions of a specific gene group. The results showed that DEGs were significantly enriched in various biological processes, including blood vessel morphogenesis, extracellular structure organization, positive regulation of hydrolase activity, and cell-substrate adhesion. DEGs were more likely to be enriched in collagen-containing extracellular matrix (ECM), platelet alpha granule, lytic vacuole, apical part of cell, and adherens junction in cellular components subgroup. In terms of molecular functions, DEGs were involved in several functions, including ECM structural constituent, enzyme activator activity, glycosaminoglycan binding, phospholipid binding, and cell adhesion molecule binding. Referring to the KEGG database, the analysis revealed enrichment mainly in pathways of complement and coagulation cascades, and ECM-receptor interaction (**Figure 2**). Considering the object of this study as motor neurons, the comprehensive enrichment analysis indicated crucial molecular alternation in ECM structural organization and relevant functions in sALS motor neurons.

PPI Network Construction and Identification of Subnetworks

The protein interactions among all 206 DEGs was analyzed by STRING online database. With the confidence at medium level, the PPI network was constructed with an average node degree of 4.03 and the PPI enrichment $P < 1.0 \times 10^{-16}$ (**Figure 3A**). The obtained files were subsequently managed by Cytoscape for

[8]https://www.grnpedia.org/trrust/

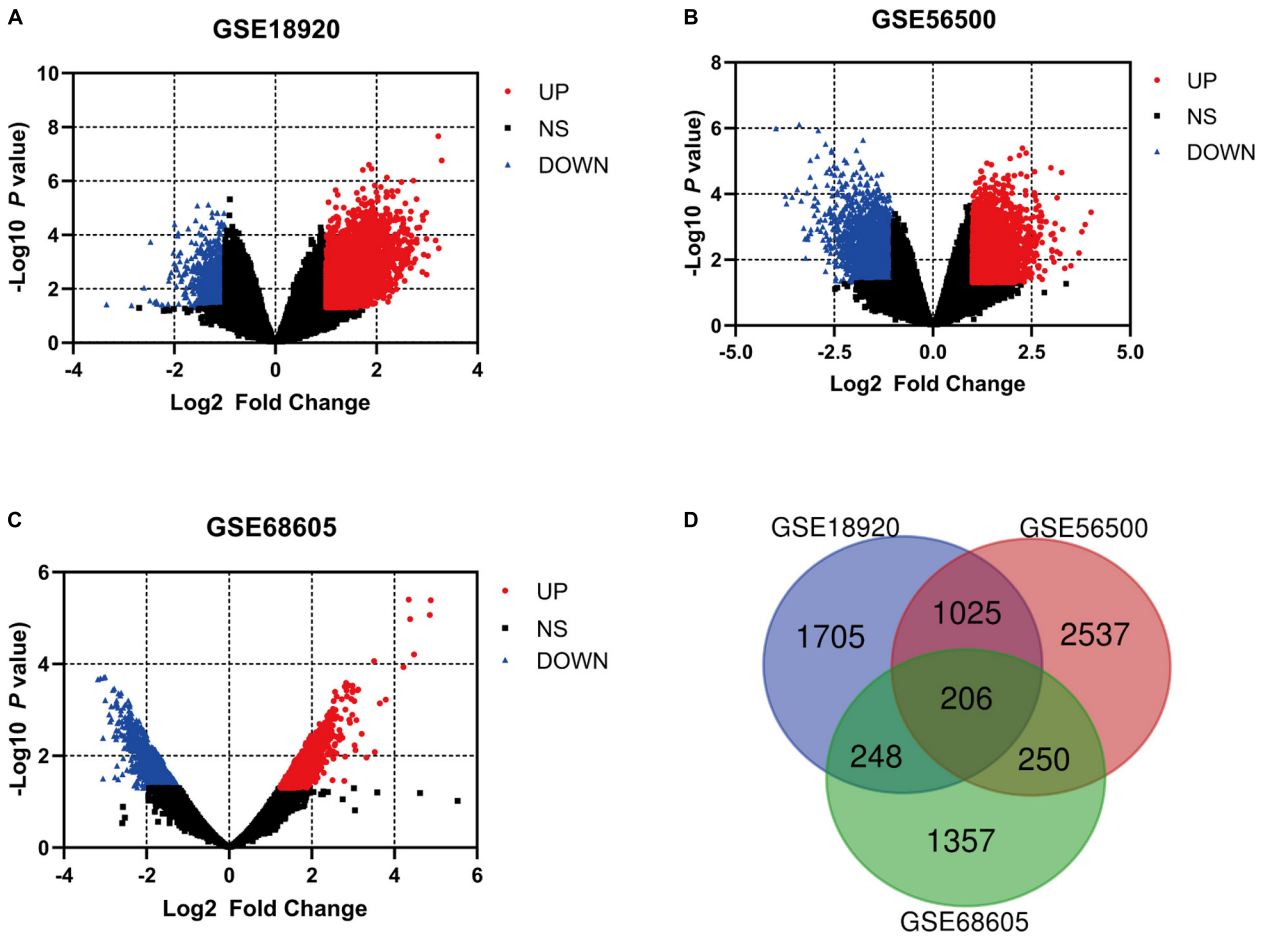

FIGURE 1 | The analysis of differentially expressed genes (DEGs) of multiple gene expression profiles by GEO2R. **(A–C)** The volcano plots of the distribution of multiple gene expression series, with red dots and blue dots indicating upregulated and downregulated genes, respectively. **(D)** Online Venn diagram tool was applied to discover the overlapping DEGs of three datasets.

visualization. The analysis by MCODE plugin revealed several subnetworks in the whole DEGs network. However, only two subnetworks containing more than five nodes were identified (**Figures 3B,D**). The subnetwork with the highest MCODE score was significantly enriched in ECM organization, collagen-containing ECM, collagen binding, and basement membrane, while the other one was enriched in blood vessel morphogenesis, positive regulation of endocytosis, and negative regulation of epithelial cell proliferation (**Figures 3C,E**).

Identification of Hub Genes and Enrichment Analysis

The top 20 nodes scoring the highest in MCC by cytoHubba plugin were identified as hub genes in the network (**Figure 4A**). Besides, the degrees of these nodes were calculated. These hub genes were mostly upregulated in motor neurons of sALS patients in three gene expression profiles (**Figure 4B**). The detailed descriptions of these hub genes were shown in **Table 2**.

Gene ontology analysis of hub genes demonstrated significant enrichment in extracellular structure organization, response to wounding, glycosaminoglycan binding, and ECM-receptor interaction. But these hub genes were also associated with blood vessel morphogenesis (**Figure 4C**). The enrichment analysis of hub genes was similar to previous analysis of DEGs. **Figure 4D** visualized the association between most of these hub genes and the main enrichment GO terms by establishing a functional group network. Notably, most genes were relevant to ECM-related functions or processes, such as collagen, glycosaminoglycan, and proteoglycan binding and ECM-receptor interaction.

Regulatory Transcriptional Factors Associated With Hub Genes

Subsequently, the online TRRUST database was applied to demonstrate the regulators of the selected hub genes. The top 10 identified TFs were displayed in **Table 3**. We identified RelA (transcription factor p65) and NF-κB1 (transcription factor p105/p50) from NF-κB family markedly overlapped with seven genes, including *BGN, CCL2, F3, FN1*, *GFAP*, *TIMP1*, and *VWF*. The overall network of target hub genes and these two significant

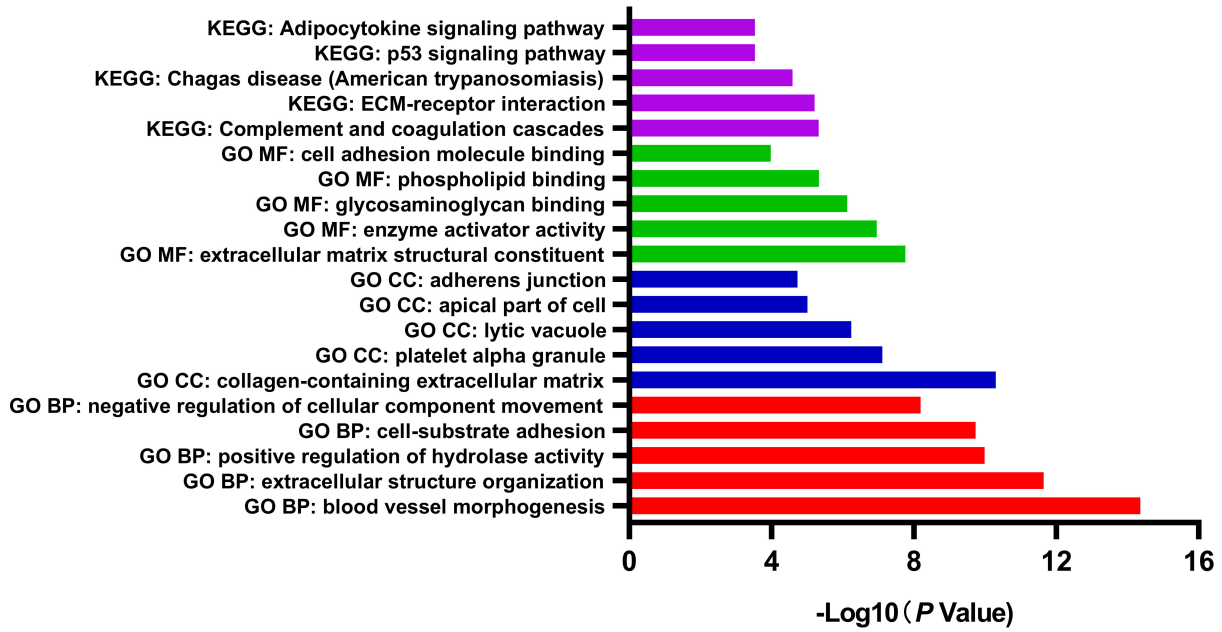

FIGURE 2 | Gene Ontology (GO) function and pathway enrichment analysis of all differentially expressed genes by Metascape. The results were colored by different GO terms.

TFs were visualized in **Figure 4E**. Other TFs such as NFIC, SP1, and a pair of homologous TWIST1 and TWIST2 of the Twist family were also identified.

DISCUSSION

Integrated Analysis Identifies ECM as the Key Regulator in sALS Motor Neurons

Bioinformatics methods directly performed on ALS motor neurons have been applied in former studies. Rabin et al. (2010) demonstrated the altered cell-matrix adhesion as well as transmembrane and secreted protein in sALS motor neurons in a single microarray analysis. The comparison of ALS patients with *C9ORF72* mutation and sporadic ALS exhibited similar gene expression profiles, and a recent study highlighted the gene expression changes relevant to excitotoxicity between human oculomotor neurons and spinal motor neurons (Wong and Venkatachalam, 2019; Patel and Mathew, 2020). Although bioinformatics analysis provided insights into in-depth molecular studies, higher false positive rate and biased results were inevitable in single microarray analysis, which were due to the heterogeneity of the disease, specimen, sample size, and the platform of different microarrays to be analyzed (Wei et al., 2020). Hence, in the present study, we obtained three gene expression profiles from the public database and performed integrated bioinformatics analysis to discover genes that are differentially expressed in motor neurons from sALS patients and healthy controls. A total of 206 DEGs that are intersected in three microarray series were identified. These DEGs are significantly enriched in two parts of functions or biological processes, blood vessel morphogenesis, and extracellular structure organization. The application of STRING and Cytoscape enabled the exploration and visualization of protein-protein interactive networks, where 20 hub genes and two clustered subnetworks were identified. The two subnetworks were associated with the organization of collagen-containing ECM and blood vessel morphogenesis, respectively. These hub genes were mostly upregulated in motor neurons from sALS patients. The subsequent TF prediction proposed RelA-NF-κB1 (p65-p105/p50) as the main regulatory pathway in the hub genes network.

It was suggested by the above integrated analysis that genes relevant to both blood vessel morphogenesis and ECM were differentially expressed in sALS. But considering the study objects of our research, blood vessel morphogenesis was less likely associated with the biological processes and molecular functions of motor neurons in the spinal cord, as motor neurons were not involved in the composition of blood vessels directly. Hence, we would like to discuss more about the role of ECM and related molecules. FN1, CD44, COL4A1, and VWF were related to ECM-receptor interaction, while FN1, CD44, VWF, DCN, SPARC, and TGFBI were relevant to collagen binding process in the extracellular space. Proteoglycan binding and glycosaminoglycan catabolic process were also ECM related processes. Accounting for over 10% of the total volume of the brain between neurons and glia cells, ECM is essential in maintaining the normal functions of the central nervous system (CNS) (Benarroch, 2015). The basement membrane rich in collagen type IV, fibronectin, and laminin contributes to the integrity of brain-blood barrier (BBB), while other ECM molecules, including hyaluronic acid and multiple proteoglycans, were secreted to form ECM and

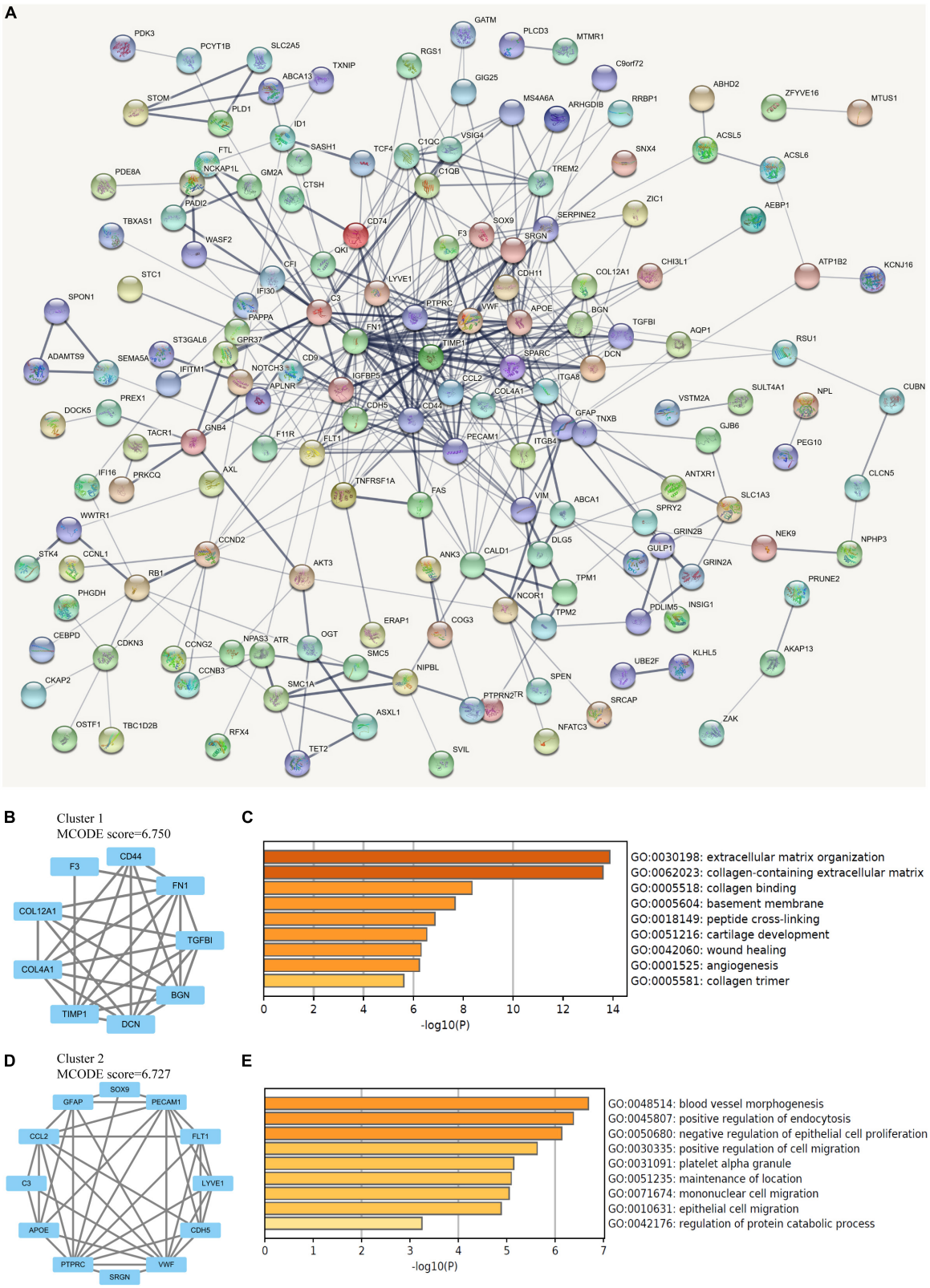

FIGURE 3 | Network analysis of differentially expressed genes (DEGs). **(A)** The protein-protein interaction (PPI) network of overlapping DEGs, in which thicker line indicates stronger data support. **(B)** The clustered subnetwork 1 identified from the whole PPI network. **(C)** Enrichment analysis of the cluster 1 by Metascape. **(D)** The clustered subnetwork 2 identified from the whole PPI network. **(E)** Enrichment analysis of the cluster 2 by Metascape.

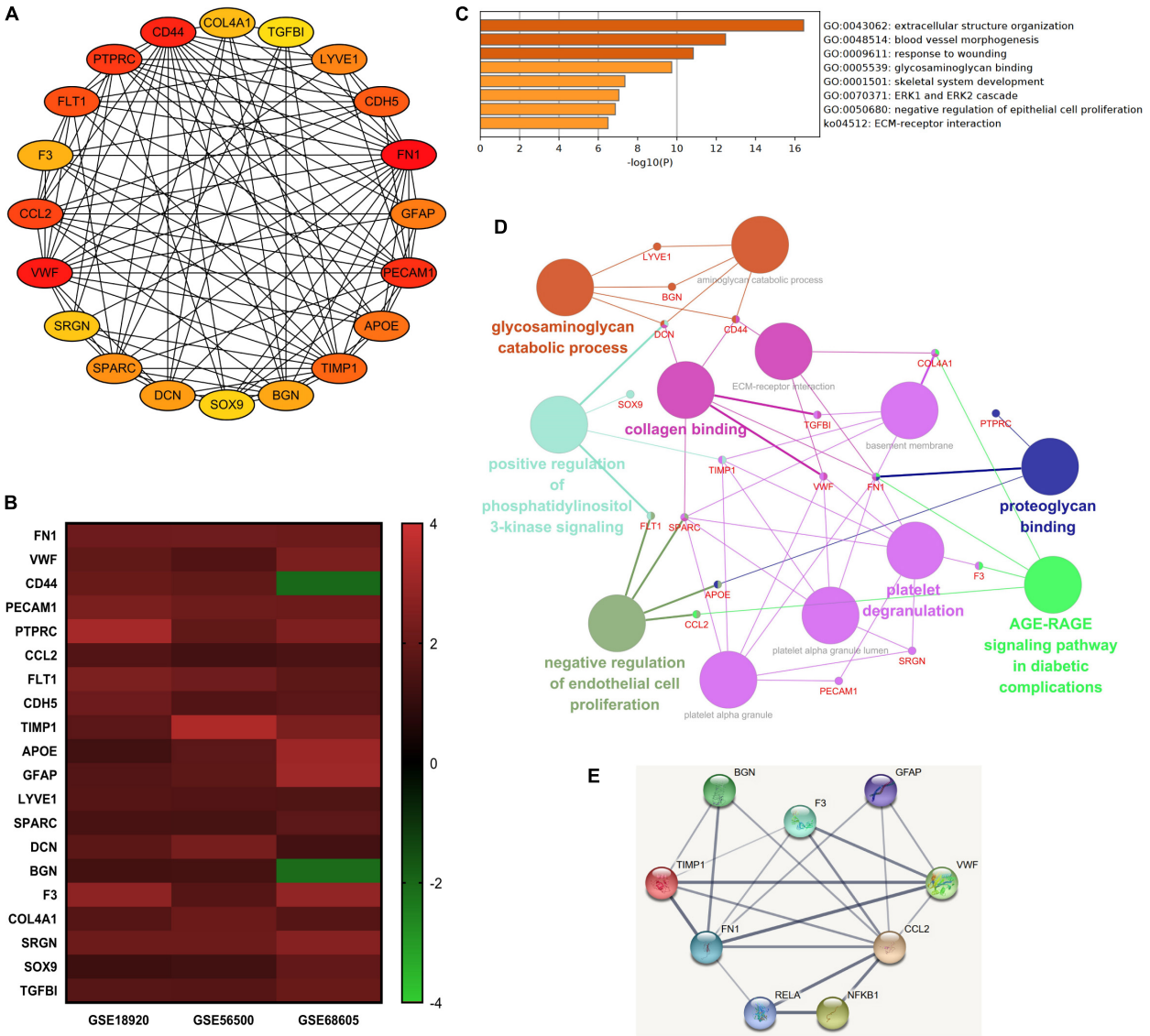

FIGURE 4 | The integrated bioinformatics analysis on hub genes. **(A)** Twenty hub genes were identified by the maximum correlation criterion scoring in Cytoscape. **(B)** The heat map of expression levels of 20 hub genes in the original gene expression series. Red indicated higher expression level in motor neurons in sALS compared with healthy controls, while green indicated lower expression level. **(C)** Enrichment analysis of the identified hub genes by Metascape. **(D)** The visualization of the hub genes and the functional group network constructed by the ClueGO and CluePedia plugin of Cytoscape. **(E)** Interactions between the predicted regulatory transcriptional factors and target genes.

perineuronal and perisynaptic nets in the extracellular space (Dityatev et al., 2010; Benarroch, 2015). Functional research has also revealed the roles of ECM in neural stem cell behavior, axonal growth, and myelination and synaptogenesis (Barros et al., 2011). Moreover, the cell adhesive network constructed by cell adhesion molecules (CAMs) provides connections between ECM molecules and intracellular components, which offers elaborate structural supports for force transmission, cytoskeletal regulation, and intercellular signaling (Kanchanawong et al., 2010; Edwards and Bix, 2019). It remained unclear whether the upregulation of these ECM related genes was the fundamental pathology of degenerated motor neurons or the secondary alternation due to other factors, but the alternation of gene expression affected the homeostasis of ECM to a certain extent.

Altered Expression of Various ECM-Related Genes in the PPI Network

Of all the upregulated hub genes enriched in ECM in the cellular component subgroup, *FN1* gene scored the highest in the MCC score and node degrees, indicating strong correlations with other genes in the network. Encoded by *FN1* gene, fibronectin is a kind of glycoprotein abundant in the basement membrane during embryogenesis and pathological angiogenesis in a wide

TABLE 2 | Top 20 hub genes of PPI network ranked by MCC method in Cytoscape.

Rank	Name	MCC score	Degree	Description
1	FN1	25,099	42	Fibronectin 1
2	VWF	21,065	26	von Willebrand factor
3	CD44	21,032	28	CD44 molecule (Indian blood group)
4	PECAM1	20,082	18	Platelet and endothelial cell adhesion molecule 1
5	PTPRC	15,701	23	Protein tyrosine phosphatase receptor type C
6	CCL2	14,630	19	C-C motif chemokine ligand 2
7	FLT1	12,964	14	fms related receptor tyrosine kinase 1
8	CDH5	10,935	14	Cadherin 5
9	TIMP1	8,436	21	TIMP metallopeptidase inhibitor 1
10	APOE	7,474	22	Apolipoprotein E
11	GFAP	5,322	18	Glial fibrillary acidic protein
12	LYVE1	5,066	9	Lymphatic vessel endothelial hyaluronan receptor 1
13	SPARC	4,633	17	Secreted protein acidic and cysteine rich
14	DCN	3,770	13	Decorin
15	BGN	3,219	16	Biglycan
16	F3	2,906	11	Coagulation factor III, tissue factor
17	COL4A1	1,624	13	Collagen type IV alpha 1 chain
18	SRGN	1,468	11	Serglycin
19	SOX9	968	11	SRY-box transcription factor 9
20	TGFBI	889	9	Transforming growth factor beta induced

TABLE 3 | Top 10 predicted transcription factors of hub genes by TRRUST.

Rank	Key TF	Description	Target genes	*P*-value	FDR
1	RELA	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	BGN, CCL2, F3, FN1, GFAP, TIMP1, VWF	1.59E-08	1.41E-07
2	NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	BGN, CCL2, F3, FN1, GFAP, TIMP1, VWF	1.66E-08	1.41E-07
3	NFIC	Nuclear factor I/C (CCAAT- binding transcription factor)	CCL2, GFAP, VWF	2.93E-06	1.66E-05
4	TWIST1	Twist basic helix-loop-helix transcription factor 1	CD44, FN1, TIMP1	6.52E-06	2.32E-05
5	SP1	Sp1 transcription factor	APOE, CCL2, CD44, F3, SOX9, TIMP1	6.81E-06	2.32E-05
6	EGR1	Early growth response 1	F3, FLT1, FN1	0.000105	0.000299
7	REL	v-rel reticuloendotheliosis viral oncogene homolog (avian)	CCL2, F3	0.000243	0.000591
8	TWIST2	Twist basic helix-loop-helix transcription factor 2	CD44, FN1	0.000316	0.000671
9	ERG	v-ets erythroblastosis virus E26 oncogene homolog (avian)	CDH5,VWF	0.000369	0.000696
10	STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)	CCL2, GFAP, TIMP1	0.000433	0.000736

range of tissues (Mongiat et al., 2016; Edwards and Bix, 2019). Fibronectin secreted by proliferating neurons and astrocytes is notably responsive in multiple neurologic disorders (Benarroch, 2015). The upregulation of fibronectin and its receptors, α5β1 and αvβ3 integrins in cerebral ischemia suggested the positive role of angiogenesis (Li et al., 2012). In multiple sclerosis (MS), the secretion of fibronectin was mediated by pro-inflammatory cytokines in a strong inflammatory process, while the aggregation of fibronectin in the white matter impaired the function of oligodendrocytes and remyelination of the lesion in MS (Stoffels et al., 2013; Werkman et al., 2020). In peripheral neuropathies, fibronectin was highly expressed in neuron regenerating sites necessary for Schwann cell differentiation and axonal regrowth, and the decreased amount of fibronectin in non-regenerating neurons was accounted for ECM degradation and leakage from disrupted basement membrane (Previtali et al., 2008). Specifically, the axon regeneration and neurite outgrowth of neurons was supported by the α5β1 integrin of fibronectin (Tonge et al., 2012). These results suggested the potential positive role of fibronectin by secreted into ECM in response to injuries. However, the role of fibronectin in the pathogenesis of ALS was still ambiguous. Fibronectin together with collagen I and III were accumulated in skeletal muscle in symptomatic hSOD1G93A mice, indicating the fibrotic process induced by the transforming growth factor-β (TGF-β) signaling pathway (Gonzalez et al., 2017). In human motor cortex, the upregulated TGF-β system also led to the enhanced expression level of FN1 and collagen IV (Peters et al., 2017). On the contrary, plasma fibronectin was found significantly lower in ALS patients and in negative correlation with duration of the disease (Ono et al., 2000). Nevertheless, the expression of fibronectin in plasma did not directly reflect the neurodegeneration in motor neurons.

Apart from FN1, the upregulated COL4A1 and COL12A1 identified in the cluster network were both genes that encode collagen related substances. COL4A1 encoding collagen IV is large molecules as the main components in the basement membrane scaffold (Yurchenco, 2011). Collagen IV deposition occurred in the perivascular space and the basement membrane in ALS patients (Garbuzova-Davis et al., 2012). Such compensatory process as response to the injury of BBB could probably hinder the drug transport into the CNS (Garbuzova-Davis et al., 2016). Collagen IV, together with laminin and vimentin, were the main components of the glial scar after injuries in the spinal cord (Saxena et al., 2012). The upregulation of collagen IV in the basal laminae correlated with the softening of the tissue, a negative factor for neuronal growth (Moeendarbary et al., 2017). On the other hand, the fibril-associated collagen XII is encoded by COL12A1 gene with two variants (Koch et al., 1995). It interacts with multiple of ECM proteins, mainly collagen I fibrils, and other components such as tenascin-X (Chiquet et al., 2014; Delbaere et al., 2020). Remarkably, the collagen XII functioned as the pro-regenerated factor in navigating the axons through non-neuronal lesion sites through the Wnt/β-catenin pathway (Wehner et al., 2017). The altered collagens expression played dual roles in the pathogenesis of ALS. In the present study, we also found the upregulation of TGFBI protein associated with collagen binding in the pathway analysis. TGFBI protein has high affinity in binding with fibronectin and functions in cell adhesion, migration, inflammation, and wound healing (Lakshminarayanan et al., 2015). Multiple studies have linked the activation of TGF-β system and other proinflammatory cytokines to the overexpression of TGFBI protein (Thapa et al., 2007). Taken together, the upregulation of *FN1*, *COL4A1*, and *TGFBI* genes in our study suggested that abundantly expressed fibronectin and other collagen components were associated with the degeneration of motor neurons and the fibrotic process surrounding motor neurons in the spinal cord, partly through the activation of proinflammatory TGF-β pathway.

Moreover, the upregulation of TIMP1 expression in motor neurons suggested changes in the vital dynamic balance of degradation and remodeling of ECM. This was mainly maintained by the interactions between metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) (Jackson et al., 2017). MMPs are crucial in the development of CNS and many physiological processes of the adult brain by degrading ECM, but the overactivation of MMPs associated with inflammatory response was discovered in multiple neurologic disorders (Singh et al., 2015; De Stefano and Herrero, 2017). As the most studied member, MMP9 was involved in the muscle degeneration process by enhancing ER stress, and its reduction rescued TDP-43 triggered death of motor neurons (Kaplan et al., 2014; Spiller et al., 2019). The upregulation of MMP9 resulted in the destruction of neurovascular unit in the presymptomatic period in ALS-G93A mice, even prior to the degeneration of motor neurons (Miyazaki et al., 2011). The proteolytic activity of MMPs was inhibited by four types of TIMPs, namely TIMP1–TIMP4 (Lukaszewicz-Zajac et al., 2014; Jackson et al., 2017). TIMP1 is secreted in the soluble form in ECM and plays the role as a strong inhibitor for MMP1, MMP3, MMP7, and MMP9 (Lukaszewicz-Zajac et al., 2014). The elevated TIMP-1 level in acute ischemic stroke patients correlated with poor prognosis, suggesting the possible compensatory mechanism to inhibit excessive MMPs activity in maintaining ECM integrity (Zhong et al., 2019). However, the specific role of TIMP-1 in ALS is still unknown, even though the upregulation of TIMP1 was found in serum, cerebrospinal fluid (CSF), and the spinal cord of ALS patients in several studies (Lim et al., 1996; Fang et al., 2009; Niebroj-Dobosz et al., 2010). It seems that the elevated level of TIMP-1 was insufficient to suppress the degradation of ECM or in a malfunction state, suggesting the role of other MMPs or TIMPs in maintaining the balance. The pathological alternation of homeostasis in ECM was hazardous to the integrity of BBB, and the mechanism is worth further exploration.

Identification of the Potential Regulatory Pathways in Motor Neurons

According to the list of hub genes, TRRUST suggested the key role of p65 and p105/p50 as the most significant prediction of the transcriptional factors, while the STRING online database demonstrated their direct interactions with seven target genes. The p65 and p105/p50 (including precursor proteins p105 and its mature form p50) are both members of NF-κB family, which is often activated in the presence of DNA damage, reactive oxygen species, and intracellular pathogens (Hayden and Ghosh, 2012). The transactivation domains (TADs) in the C-terminal enables the ability of initiating transcription in p65, while p105/p50 without TADs regulates the transcription processes by binding to TADs-containing members or other subunits (Hayden and Ghosh, 2012). The p65/p50 heterodimer is commonly regulated by IκB kinase in the NF-κB pathway (Siggers et al., 2011; Hayden and Ghosh, 2012). Recent studies revealed the complex roles of the activation of NF-κB pathway in the pathogenesis of ALS. Astrocyte-derived NF-κB activation was neuroprotective in presymptomatic stage by delaying disease onset, but it facilitated the inflammatory process by the activation of microglial, which induced motor neuron death in ALS models (Frakes et al., 2014; Ouali Alami et al., 2018). NF-κB activation in motor neurons resulted in the pathological TDP-43 aggregation in cytoplasm and nucleus as the landmark pathology in most ALS cases (Swarup et al., 2011; Picher-Martel et al., 2015). Recent studies demonstrated the fibrosis mediated by NF-κB activation in multiple diseases including cardiac hypertrophy and idiopathic pulmonary fibrosis, and the inhibitions of such pathways prevented excessive ECM deposition and remodeling (Kumar et al., 2011; Song et al., 2016). In spinal cord injury, the fibrotic scarring produced by the chronic fibrotic response limited the axons recovery in the lesion site and motor functional recovery of the mouse model, which can be reversed by eliminating the isoform of fibronectin containing the Extra Domain A (FnEDA) (Cooper et al., 2018). Similarly, FnEDA also exacerbated stroke outcome through initiating inflammation (Dhanesha et al., 2019).

On the other hand, C-C motif chemokine ligand 2 (CCL2) was also correlated with the NF-κB pathway directly in the interaction

network. Also known as monocyte chemoattractant proteins 1 (MCP1), CCL2 is widely expressed in CNS and associated with the neuroinflammation process by binding to its receptor CCR2 (Conductier et al., 2010). It was previously confirmed that CCL2 (MCP1) was upregulated in serum, CSF, and peripheral blood mononuclear cell in ALS patients (Kuhle et al., 2009; Gupta et al., 2011a,b). Specifically, an early innate immune response represented by MCP1-CCR2 interaction was identified in motor cortex in mouse model, where MCP-1 were found in microglia-like cells (Jara et al., 2017). In a further research concerning motor cortex of ALS with TDP-43 pathology, the CCL2 (MCP1) expression increased in Betz cells, which was even prior to the infiltration of CCR2 positive monocytes (Jara et al., 2019). These studies were significant in proposing direct evidence for MCP1-CCR2 interaction in neuroinflammation in the motor cortex. CCL2 (MCP1) was also abundantly expressed in motor neurons in the spinal cord, and its level elevated during the disease progression in ALS mice (Kawaguchi-Niida et al., 2013). Considering the overexpression of CCL2 among the three analyzed gene expression profiles included in our study, it represented the upregulated neuroinflammation process surrounding motor neurons in sALS.

Taken together, we proposed an interaction network with several hub genes and the indicated regulatory pathways, and suggested that the dysregulation of NF-κB pathway induced by neuroinflammation contributes to the overexpression of fibronectin and a series of fibrotic processes as the major changes in the spinal cord, which could be the potential target for ALS therapy.

Limitations of the Study and Conclusion

To avoid biased results in bioinformatics analysis, we included multiple gene expression profiles of motor neurons from sALS patients and healthy controls for analysis, aiming to provide insights of the genetic patterns of the motor neurons in the advanced disease state. Nevertheless, there are still some limitations in our study. First, as the analysis on gene expression profiles in advanced state of motor neurons did not adequately explain the progression of the disease, it is still necessary to elucidate whether the upregulation of the hub genes are primary or secondary changes in the pathogenesis of sALS. Second, due to the lack of clinical data of these sALS patients, we are not able to perform survival analysis or demonstrate the association of hub genes and disease progression in sALS patients. More importantly, although we pointed out multiple upregulated molecules and related pathways in motor neurons in terminal-stage sALS, we suggest that validation from cellular, animal experiments, and even larger cohort are needed in demonstrating the overall alternation in ECM molecules during the disease progression.

In this study, the integrated bioinformatics analyses revealed the upregulation of multiple genes related to biological processes of ECM components and proposed Rela/NF-κB1 as the regulatory transcriptional factors in the pathogenesis of sALS. The bioinformatics analysis on alternation of gene expression profiles and their protein interaction networks will not only enhance our understanding of sALS but also provide insights in searching for distinct biomarkers and therapies.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

JL and XY contributed to the study design and management. JL and PH contributed to the data collection. PH, WC, CY, and HS contributed to the data analysis and visualization. JL contributed to writing the manuscript. HS and XY contributed to the manuscript review and editing. All authors reviewed and approved the final manuscript.

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REFERENCES

Al-Chalabi, A., van den Berg, L. H., and Veldink, J. (2017). Gene discovery in amyotrophic lateral sclerosis: implications for clinical management. *Nat. Rev. Neurol.* 13, 96–104. doi: 10.1038/nrneurol.2016.182

Barros, C. S., Franco, S. J., and Muller, U. (2011). Extracellular matrix: functions in the nervous system. *Cold Spring Harb. Perspect. Biol.* 3:a005108. doi: 10.1101/cshperspect.a005108

Benarroch, E. E. (2015). Extracellular matrix in the CNS: dynamic structure and clinical correlations. *Neurology* 85, 1417–1427. doi: 10.1212/wnl.0000000000002044

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

Chia, R., Chiò, 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

Chiquet, M., Birk, D. E., Bonnemann, C. G., and Koch, M. (2014). Collagen XII: protecting bone and muscle integrity by organizing collagen fibrils. *Int. J. Biochem. Cell Biol.* 53, 51–54. doi: 10.1016/j.biocel.2014.04.020

Conductier, G., Blondeau, N., Guyon, A., Nahon, J. L., and Rovere, C. (2010). The role of monocyte chemoattractant protein MCP1/CCL2 in neuroinflammatory diseases. *J. Neuroimmunol.* 224, 93–100. doi: 10.1016/j.jneuroim.2010.05.010

Cooper, J. G., Jeong, S. J., McGuire, T. L., Sharma, S., Wang, W., Bhattacharyya, S., et al. (2018). Fibronectin EDA forms the chronic fibrotic scar after contusive spinal cord injury. *Neurobiol. Dis.* 116, 60–68. doi: 10.1016/j.nbd.2018.04.014

De Stefano, M. E., and Herrero, M. T. (2017). The multifaceted role of metalloproteinases in physiological and pathological conditions in embryonic and adult brains. *Prog. Neurobiol.* 155, 36–56. doi: 10.1016/j.pneurobio.2016.08.002

Delbaere, S., Dhooge, T., Syx, D., Petit, F., Goemans, N., Destree, A., et al. (2020). Novel defects in collagen XII and VI expand the mixed myopathy/Ehlers-Danlos syndrome spectrum and lead to variant-specific alterations in the extracellular matrix. *Genet. Med.* 22, 112–123. doi: 10.1038/s41436-019-0599-6

Dhanesha, N., Chorawala, M. R., Jain, M., Bhalla, A., Thedens, D., Nayak, M., et al. (2019). Fn-EDA (fibronectin containing extra domain A) in the plasma, but not endothelial cells, exacerbates stroke outcome by promoting thrombo-inflammation. *Stroke* 50, 1201–1209. doi: 10.1161/strokeaha.118.023697

Dityatev, A., Seidenbecher, C. I., and Schachner, M. (2010). Compartmentalization from the outside: the extracellular matrix and functional microdomains in the brain. *Trends Neurosci.* 33, 503–512. doi: 10.1016/j.tins.2010.08.003

Edwards, D. N., and Bix, G. J. (2019). Roles of blood-brain barrier integrins and extracellular matrix in stroke. *Am. J. Physiol. Cell Physiol.* 316, C252–C263.

Fang, L., Huber-Abel, F., Teuchert, M., Hendrich, C., Dorst, J., Schattauer, D., et al. (2009). Linking neuron and skin: matrix metalloproteinases in amyotrophic lateral sclerosis (ALS). *J. Neurol. Sci.* 285, 62–66. doi: 10.1016/j.jns.2009.05.025

Frakes, A. E., Ferraiuolo, L., Haidet-Phillips, A. M., Schmelzer, L., Braun, L., Miranda, C. J., et al. (2014). Microglia induce motor neuron death via the classical NF-kappaB pathway in amyotrophic lateral sclerosis. *Neuron* 81, 1009–1023. doi: 10.1016/j.neuron.2014.01.013

Garbuzova-Davis, S., Hernandez-Ontiveros, D. G., Rodrigues, M. C., Haller, E., Frisina-Deyo, A., Mirtyl, S., et al. (2012). Impaired blood-brain/spinal cord barrier in ALS patients. *Brain Res.* 1469, 114–128. doi: 10.1016/j.brainres.2012.05.056

Garbuzova-Davis, S., Thomson, A., Kurien, C., Shytle, R. D., and Sanberg, P. R. (2016). Potential new complication in drug therapy development for amyotrophic lateral sclerosis. *Expert Rev. Neurother.* 16, 1397–1405. doi: 10.1080/14737175.2016.1207530

Gonzalez, D., Contreras, O., Rebolledo, D. L., Espinoza, J. P., van Zundert, B., and Brandan, E. (2017). ALS skeletal muscle shows enhanced TGF-beta signaling, fibrosis and induction of fibro/adipogenic progenitor markers. *PLoS One* 12:e0177649. doi: 10.1371/journal.pone.0177649

Gupta, P. K., Prabhakar, S., Abburi, C., Sharma, N. K., and Anand, A. (2011a). Vascular endothelial growth factor-A and chemokine ligand (CCL2) genes are upregulated in peripheral blood mononuclear cells in Indian amyotrophic lateral sclerosis patients. *J. Neuroinflammation* 8:114. doi: 10.1186/1742-2094-8-114

Gupta, P. K., Prabhakar, S., Sharma, S., and Anand, A. (2011b). Vascular endothelial growth factor-A (VEGF-A) and chemokine ligand-2 (CCL2) in amyotrophic lateral sclerosis (ALS) patients. *J. Neuroinflammation* 8:47. doi: 10.1186/1742-2094-8-47

Han, H., Cho, J.-W., Lee, S., Yun, A., Kim, H., Bae, D., et al. (2018). TRRUST v2: an expanded reference database of human and mouse transcriptional regulatory interactions. *Nucleic Acids Res.* 46, D380–D386.

Hayden, M. S., and Ghosh, S. (2012). NF-κB, the first quarter-century: remarkable progress and outstanding questions. *Genes Dev.* 26, 203–234. doi: 10.1101/gad.183434.111

Jackson, H. W., Defamie, V., Waterhouse, P., and Khokha, R. (2017). TIMPs: versatile extracellular regulators in cancer. *Nat. Rev. Cancer* 17, 38–53. doi: 10.1038/nrc.2016.115

Jara, J. H., Gautam, M., Kocak, N., Xie, E. F., Mao, Q., Bigio, E. H., et al. (2019). MCP1-CCR2 and neuroinflammation in the ALS motor cortex with TDP-43 pathology. *J. Neuroinflammation* 16:196.

Jara, J. H., Genc, B., Stanford, M. J., Pytel, P., Roos, R. P., Weintraub, S., et al. (2017). Evidence for an early innate immune response in the motor cortex of ALS. *J. Neuroinflammation* 14:129.

Kanchanawong, P., Shtengel, G., Pasapera, A. M., Ramko, E. B., Davidson, M. W., Hess, H. F., et al. (2010). Nanoscale architecture of integrin-based cell adhesions. *Nature* 468, 580–584. doi: 10.1038/nature09621

Kaplan, A., Spiller, K. J., Towne, C., Kanning, K. C., Choe, G. T., Geber, A., et al. (2014). Neuronal matrix metalloproteinase-9 is a determinant of selective neurodegeneration. *Neuron* 81, 333–348. doi: 10.1016/j.neuron.2013.12.009

Kawaguchi-Niida, M., Yamamoto, T., Kato, Y., Inose, Y., and Shibata, N. (2013). MCP-1/CCR2 signaling-mediated astrocytosis is accelerated in a transgenic mouse model of SOD1-mutated familial ALS. *Acta Neuropathol. Commun.* 1:21.

Koch, M., Bohrmann, B., Matthison, M., Hagios, C., Trueb, B., and Chiquet, M. (1995). Large and small splice variants of collagen XII: differential expression and ligand binding. *J. Cell Biol.* 130, 1005–1014. doi: 10.1083/jcb.130.4.1005

Kotni, M. K., Zhao, M., and Wei, D. Q. (2016). Gene expression profiles and protein-protein interaction networks in amyotrophic lateral sclerosis patients with C9orf72 mutation. *Orphanet J. Rare Dis.* 11:148.

Kuhle, J., Lindberg, R. L. P., Regeniter, A., Mehling, M., Steck, A. J., Kappos, L., et al. (2009). Increased levels of inflammatory chemokines in amyotrophic lateral sclerosis. *Eur. J. Neurol.* 16, 771–774. doi: 10.1111/j.1468-1331.2009.02560.x

Kumar, S., Seqqat, R., Chigurupati, S., Kumar, R., Baker, K. M., Young, D., et al. (2011). Inhibition of nuclear factor kappaB regresses cardiac hypertrophy by modulating the expression of extracellular matrix and adhesion molecules. *Free Radic. Biol. Med.* 50, 206–215. doi: 10.1016/j.freeradbiomed.2010.10.711

Lakshminarayanan, R., Chaurasia, S. S., Murugan, E., Venkatraman, A., Chai, S. M., Vithana, E. N., et al. (2015). Biochemical properties and aggregation propensity of transforming growth factor-induced protein (TGFBIp) and the amyloid forming mutants. *Ocul. Surf.* 13, 9–25. doi: 10.1016/j.jtos.2014.04.003

Li, L., Liu, F., Welser-Alves, J. V., McCullough, L. D., and Milner, R. (2012). Upregulation of fibronectin and the α5β1 and αvβ3 integrins on blood vessels within the cerebral ischemic penumbra. *Exp. Neurol.* 233, 283–291. doi: 10.1016/j.expneurol.2011.10.017

Lim, G. P., Backstrom, J. R., Cullen, M. J., Miller, C. A., Atkinson, R. D., and Tökés, Z. A. (1996). Matrix metalloproteinases in the neocortex and spinal cord of amyotrophic lateral sclerosis patients. *J. Neurochem.* 67, 251–259. doi: 10.1046/j.1471-4159.1996.67010251.x

Longinetti, E., and Fang, F. (2019). Epidemiology of amyotrophic lateral sclerosis: an update of recent literature. *Curr. Opin. Neurol.* 32, 771–776. doi: 10.1097/wco.0000000000000730

Lukaszewicz-Zajac, M., Mroczko, B., and Slowik, A. (2014). Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) in amyotrophic lateral sclerosis (ALS). *J. Neural Transm. (Vienna).* 121, 1387–1397. doi: 10.1007/s00702-014-1205-3

Marin, B., Fontana, A., Arcuti, S., Copetti, M., Boumediene, F., Couratier, P., et al. (2018). Age-specific ALS incidence: a dose-response meta-analysis. *Eur. J. Epidemiol.* 33, 621–634. doi: 10.1007/s10654-018-0392-x

Miyazaki, K., Ohta, Y., Nagai, M., Morimoto, N., Kurata, T., Takehisa, Y., et al. (2011). Disruption of neurovascular unit prior to motor neuron degeneration in amyotrophic lateral sclerosis. *J. Neurosci. Res.* 89, 718–728. doi: 10.1002/jnr.22594

Moeendarbary, E., Weber, I. P., Sheridan, G. K., Koser, D. E., Soleman, S., Haenzi, B., et al. (2017). The soft mechanical signature of glial scars in the central nervous system. *Nat. Commun.* 8:14787.

Mongiat, M., Andreuzzi, E., Tarticchio, G., and Paulitti, A. (2016). Extracellular matrix, a hard player in angiogenesis. *Int. J. Mol. Sci.* 17:1822. doi: 10.3390/ijms17111822

Nguyen, H. P., Van Broeckhoven, C., and van der Zee, J. A. L. S. (2018). Genes in the Genomic Era and their Implications for FTD. *Trends Genet.* 34, 404–423. doi: 10.1016/j.tig.2018.03.001

Niebroj-Dobosz, I., Janik, P., Sokolowska, B., and Kwiecinski, H. (2010). Matrix metalloproteinases and their tissue inhibitors in serum and cerebrospinal fluid of patients with amyotrophic lateral sclerosis. *Eur. J. Neurol.* 17, 226–231. doi: 10.1111/j.1468-1331.2009.02775.x

Ono, S., Imai, T., Shimizu, N., Nakayama, M., Mihori, A., Kaneda, K., et al. (2000). Decreased plasma levels of fibronectin in amyotrophic lateral sclerosis. *Acta Neurol. Scand.* 101, 391–394. doi: 10.1034/j.1600-0404.2000.90289a.x

Oskarsson, B., Gendron, T. F., and Staff, N. P. (2018). Amyotrophic lateral sclerosis: an update for 2018. *Mayo Clin. Proc.* 93, 1617–1628.

Ouali Alami, N., Schurr, C., Olde Heuvel, F., Tang, L., Li, Q., Tasdogan, A., et al. (2018). NF-kappaB activation in astrocytes drives a stage-specific beneficial neuroimmunological response in ALS. *EMBO J.* 37:e98697.

Patel, A. N., and Mathew, D. (2020). A study of gene expression changes in human spinal and oculomotor neurons; identifying potential links to sporadic ALS. *Genes (Basel)* 11:448. doi: 10.3390/genes11040448

Peters, S., Zitzelsperger, E., Kuespert, S., Iberl, S., Heydn, R., Johannesen, S., et al. (2017). The TGF-beta system as a potential pathogenic player in disease modulation of amyotrophic lateral sclerosis. *Front. Neurol.* 8:669.

Picher-Martel, V., Dutta, K., Phaneuf, D., Sobue, G., and Julien, J.-P. (2015). Ubiquilin-2 drives NF-κB activity and cytosolic TDP-43 aggregation in neuronal cells. *Mol. Brain* 8:71.

Previtali, S. C., Malaguti, M. C., Riva, N., Scarlato, M., Dacci, P., Dina, G., et al. (2008). The extracellular matrix affects axonal regeneration in peripheral neuropathies. *Neurology* 71, 322–331. doi: 10.1212/01.wnl.0000319736.43628.04

Rabin, S. J., Kim, J. M. H., Baughn, M., Libby, R. T., Kim, Y. J., Fan, Y., et al. (2010). Sporadic ALS has compartment-specific aberrant exon splicing and altered cell-matrix adhesion biology. *Hum. Mol. Genet.* 19, 313–328. doi: 10.1093/hmg/ddp498

Renton, A. E., Chio, A., and Traynor, B. J. (2014). State of play in amyotrophic lateral sclerosis genetics. *Nat. Neurosci.* 17, 17–23.

Saxena, T., Gilbert, J., Stelzner, D., and Hasenwinkel, J. (2012). Mechanical characterization of the injured spinal cord after lateral spinal hemisection injury in the rat. *J. Neurotrauma* 29, 1747–1757. doi: 10.1089/neu.2011.1818

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., et al. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 13, 2498–2504. doi: 10.1101/gr.1239303

Siggers, T., Chang, A. B., Teixeira, A., Wong, D., Williams, K. J., Ahmed, B., et al. (2011). Principles of dimer-specific gene regulation revealed by a comprehensive characterization of NF-kappaB family DNA binding. *Nat. Immunol.* 13, 95–102. doi: 10.1038/ni.2151

Singh, D., Srivastava, S. K., Chaudhuri, T. K., and Upadhyay, G. (2015). Multifaceted role of matrix metalloproteinases (MMPs). *Front. Mol. Biosci.* 2:19.

Song, C., He, L., Zhang, J., Ma, H., Yuan, X., Hu, G., et al. (2016). Fluorofenidone attenuates pulmonary inflammation and fibrosis via inhibiting the activation of NALP3 inflammasome and IL-1beta/IL-1R1/MyD88/NF-kappaB pathway. *J. Cell Mol. Med.* 20, 2064–2077. doi: 10.1111/jcmm.12898

Spiller, K. J., Khan, T., Dominique, M. A., Restrepo, C. R., Cotton-Samuel, D., Levitan, M., et al. (2019). Reduction of matrix metalloproteinase 9 (MMP-9) protects motor neurons from TDP-43-triggered death in rNLS8 mice. *Neurobiol. Dis.* 124, 133–140. doi: 10.1016/j.nbd.2018.11.013

Stoffels, J. M., de Jonge, J. C., Stancic, M., Nomden, A., van Strien, M. E., Ma, D., et al. (2013). Fibronectin aggregation in multiple sclerosis lesions impairs remyelination. *Brain* 136(Pt 1), 116–131. doi: 10.1093/brain/aws313

Swarup, V., Phaneuf, D., Dupre, N., Petri, S., Strong, M., Kriz, J., et al. (2011). Deregulation of TDP-43 in amyotrophic lateral sclerosis triggers nuclear factor kappaB-mediated pathogenic pathways. *J. Exp. Med.* 208, 2429–2447. doi: 10.1084/jem.20111313

Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., et al. (2019). STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* 47, D607–D613.

Thapa, N., Lee, B. H., and Kim, I. S. (2007). TGFBIp/betaig-h3 protein: a versatile matrix molecule induced by TGF-beta. *Int. J. Biochem. Cell Biol.* 39, 2183–2194. doi: 10.1016/j.biocel.2007.06.004

Tonge, D. A., de Burgh, H. T., Docherty, R., Humphries, M. J., Craig, S. E., and Pizzey, J. (2012). Fibronectin supports neurite outgrowth and axonal regeneration of adult brain neurons in vitro. *Brain Res.* 1453, 8–16. doi: 10.1016/j.brainres.2012.03.024

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.

Wehner, D., Tsarouchas, T. M., Michael, A., Haase, C., Weidinger, G., Reimer, M. M., et al. (2017). Wnt signaling controls pro-regenerative Collagen XII in functional spinal cord regeneration in zebrafish. *Nat. Commun.* 8:126.

Wei, R., Qiu, H., Xu, J., Mo, J., Liu, Y., Gui, Y., et al. (2020). Expression and prognostic potential of GPX1 in human cancers based on data mining. *Ann. Transl. Med.* 8:124. doi: 10.21037/atm.2020.02.36

Werkman, I., Sikkema, A. H., Versluijs, J. B., Qin, J., de Boer, P., and Baron, W. (2020). TLR3 agonists induce fibronectin aggregation by activated astrocytes: a role of pro-inflammatory cytokines and fibronectin splice variants. *Sci. Rep.* 10:532.

Wong, C.-O., and Venkatachalam, K. (2019). Motor neurons from ALS patients with mutations in C9ORF72 and SOD1 exhibit distinct transcriptional landscapes. *Hum. Mol. Genet.* 28, 2799–2810. doi: 10.1093/hmg/ddz104

Yurchenco, P. D. (2011). Basement membranes: cell scaffoldings and signaling platforms. *Cold Spring Harb. Perspect. Biol.* 3:a004911. doi: 10.1101/cshperspect.a004911

Zhong, C., Wang, G., Xu, T., Zhu, Z., Guo, D., Zheng, X., et al. (2019). Tissue inhibitor metalloproteinase-1 and clinical outcomes after acute ischemic stroke. *Neurology* 93, e1675–e1685.

Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A. H., Tanaseichuk, O., et al. (2019). Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat. Commun.* 10:1523.

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.* 88, 540–549.

Zufiria, M., Gil-Bea, F. J., Fernandez-Torron, R., Poza, J. J., Munoz-Blanco, J. L., Rojas-Garcia, R., et al. (2016). ALS: a bucket of genes, environment, metabolism and unknown ingredients. *Prog. Neurobiol.* 142, 104–129. doi: 10.1016/j.pneurobio.2016.05.004

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Searching Far and Genome-Wide: The Relevance of Association Studies in Amyotrophic Lateral Sclerosis

Kelly A. Rich[1], Jennifer Roggenbuck[1] and Stephen J. Kolb[1,2]*

[1] Department of Neurology, The Ohio State University Wexner Medical Center, Columbus, OH, United States, [2] Department of Biological Chemistry and Pharmacology, The Ohio State University Wexner Medical Center, Columbus, OH, United States

Genome-wide association studies (GWAS) and rare variant association studies (RVAS) are applied across many areas of complex disease to analyze variation in whole genomes of thousands of unrelated patients. These approaches are able to identify variants and/or biological pathways which are associated with disease status and, in contrast to traditional linkage studies or candidate gene approaches, do so without requiring multigenerational affected families, prior hypotheses, or known genes of interest. However, the novel associations identified by these methods typically have lower effect sizes than those found in classical family studies. In the motor neuron disease amyotrophic lateral sclerosis (ALS), GWAS, and RVAS have been used to identify multiple disease-associated genes but have not yet resulted in novel therapeutic interventions. There is significant urgency within the ALS community to identify additional genetic markers of disease to uncover novel biological mechanisms, stratify genetic subgroups of disease, and drive drug development. Given the widespread and increasing application of genetic association studies of complex disease, it is important to recognize the strengths and limitations of these approaches. Here, we review ALS gene discovery via GWAS and RVAS.

Keywords: amyotrophic lateral sclerosis, genetic testing, gene therapy, genome, genetic variants, rare variant association study (RVAS), genome wide association studies (GWAS)

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United States

****Correspondence:***
Kelly A. Rich
Kelly.Rich@osumc.edu

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INTRODUCTION

In the timeline of gene discovery for hereditary disease, high penetrance genes are historically identified by linkage analysis in multi-generational family studies and subsequently replicated in high-risk case-control studies of independent disease cohorts. These Mendelian genes, with highly significant (or moderately significant) effect sizes, generally represent the "low-hanging fruit" of gene discovery. Identifying the genetic underpinnings of complex diseases requires an approach to assess variation in many genes simultaneously. Genome-wide association studies (GWAS) were developed using single nucleotide variant (SNV) array technology to identify disease-associated variation in large cohorts of cases and controls and became widely adopted in the late 2000s. GWAS are able to interrogate millions of common genetic variants [minor allele frequency (MAF) > 5%]

in thousands of unrelated individuals to identify associations with disease that potentially explain some percentage of disease heritability within a population (Tam et al., 2019).

Despite the impact of GWAS in identifying disease-associated genetic changes, the majority of genetic contribution to many complex diseases remains unexplained. Rare variant association studies (RVAS) extend the genome-wide approach by using massively parallel sequencing to identify less-common variants (MAF < 0.5 or 0.1%) that would be missed by GWAS (Lee et al., 2014). This has been made possible by increasing sample sizes in disease cohorts as well as advances in sequencing technology, leading to greater genomic resolution. Next generation sequencing approaches such as whole exome sequencing (WES) and whole genome sequencing (WGS), sequence the coding regions and the entirety of the genome, respectively, allowing for inclusion of rare variants into large association studies of complex disease (Kosmicki et al., 2016).

Rare variant studies extend the reach of traditional association studies by identifying rare and potentially more clinically significant variants using powerful sequencing technologies. Variants identified via GWAS only explain a fraction of missing heritability in most diseases, limiting the clinical relevance of GWAS findings (Manolio et al., 2009). Targeted candidate gene studies have revealed that rare coding variants may produce large effect sizes in complex disease, motivating further investigation into rare variant contribution (Kosmicki et al., 2016). Rare variants are known to play important roles in human disease (Rivas et al., 2011; Gudmundsson et al., 2012) and explain phenotypic differences across the disease spectrum (Cohen, 2004; Cohen et al., 2005).

While GWAS can be performed on WGS or WES, it is most commonly conducted using SNV array to maximize sample size. The associations evaluated via GWAS often do not include variants of less than 0.1% allele frequency. High-depth WGS offers the greatest opportunity for assessing low-frequency or rare variants using an RVAS approach. RVAS is able to assess both single-variants or the cumulative effects of multiple variants on a gene or region (Lee et al., 2014). The latter includes approaches such as burden tests, variance-component tests, and exponential-combination tests (Lee et al., 2014). Further, RVAS can also be used to confirm candidate associations identified via GWAS or screen a known disease-associated gene in a separate cohort (Auer and Lettre, 2015).

The typical association study includes four components; (1) accrual of a large group of individuals with the disease of interest as well as a carefully matched control group for comparison; (2) genotyping of hundreds of thousands to millions of variants in disease and control groups, traditionally via SNV arrays in GWAS and sequencing in RVAS; (3) statistical analyses to test for common- or rare-variant association with disease; and (4) prioritizing and replicating significant findings in a non-overlapping, independent cohort or performing functional experiments to examine variant consequences (Pearson, 2008). Data from association studies can be easily visualized via a Manhattan plot in which significant regions or variants appear as "skyscrapers," an example of which is provided in **Figure 1**.

As opposed to candidate gene studies assessing variation in specific genes, neither approach requires prior hypotheses of associations between genetic variants and disease. Given the widespread and increasing application of GWAS and RVAS to uncover genetic associations in complex diseases, it is important to recognize the strengths and limitations of each approach. Here we will consider the contribution of association studies in unraveling the genetic etiologies of the motor neuron disease amyotrophic lateral sclerosis (ALS).

STRENGTHS AND SUCCESSES IN ASSOCIATION STUDIES

Association studies have been used to identify significant risk loci in conditions such as type 2 diabetes (Zhao et al., 2017), schizophrenia (Li et al., 2017), hypocholesterolemia (Cohen, 2004; Cohen et al., 2005) and coronary artery disease (Nikpay et al., 2015). Beyond identifying novel disease associations, GWAS and RVAS may also serve as a first step in uncovering biological mechanisms and/or pathways for therapeutic intervention. In schizophrenia, GWAS identified a significant association signal within the major histocompatibility complex (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014) and sparked investigation into complement factor haplotypes including C4 (Sekar et al., 2016). C4 is a known marker of synaptic pruning which was later found to be overexpressed in the brain tissue of individuals with schizophrenia. This biological context supports the theory of excessive synaptic elimination ("pruning") as a mechanism of disease. Similarly, a GWAS approach to type 2 diabetes identified the risk locus *SLC30A8,* and follow-up investigation discovered that loss-of-function variants at this locus are protective against disease. This discovery led to the development of several drugs which aim to antagonize the product of *SLC30A8*, a zinc transporter in pancreatic islet cells (Flannick and Florez, 2016). RVAS identified rare variants in *PCSK9* as a key component of low-density lipoprotein metabolism and individuals with loss-of-function variants in this gene had consistently low cholesterol levels throughout their lifetimes (Cohen et al., 2005). Since then, three PCSK9 inhibitors have been tested in human trials, two approved in the United States (Shapiro et al., 2018). This association finding is among the most compelling examples of translation from genetic findings to therapeutic intervention.

Association studies may also provide an avenue for disease subgroup stratification, where a subgroup may have a particular clinical course (Ridker et al., 2008; Reiner et al., 2009; Owen et al., 2010; Thanabalasingham et al., 2011) or may be more likely to benefit from a certain intervention (Nelson et al., 2015). These approaches can provide insight into the impact of geoancestry in disease (Choquet et al., 2013; Wen et al., 2014; Liu et al., 2015; Minster et al., 2016; Visscher et al., 2017). GWAS and RVAS may uncover modifier genes and shed light on the contribution of multiple variants (Pigeyre et al., 2016; Whitacre et al., 2017; Tam et al., 2019). Polygenic risk scores (PRS), which predict an individual's risk for disease based on the combination of multiple risk alleles, can be calculated using tens

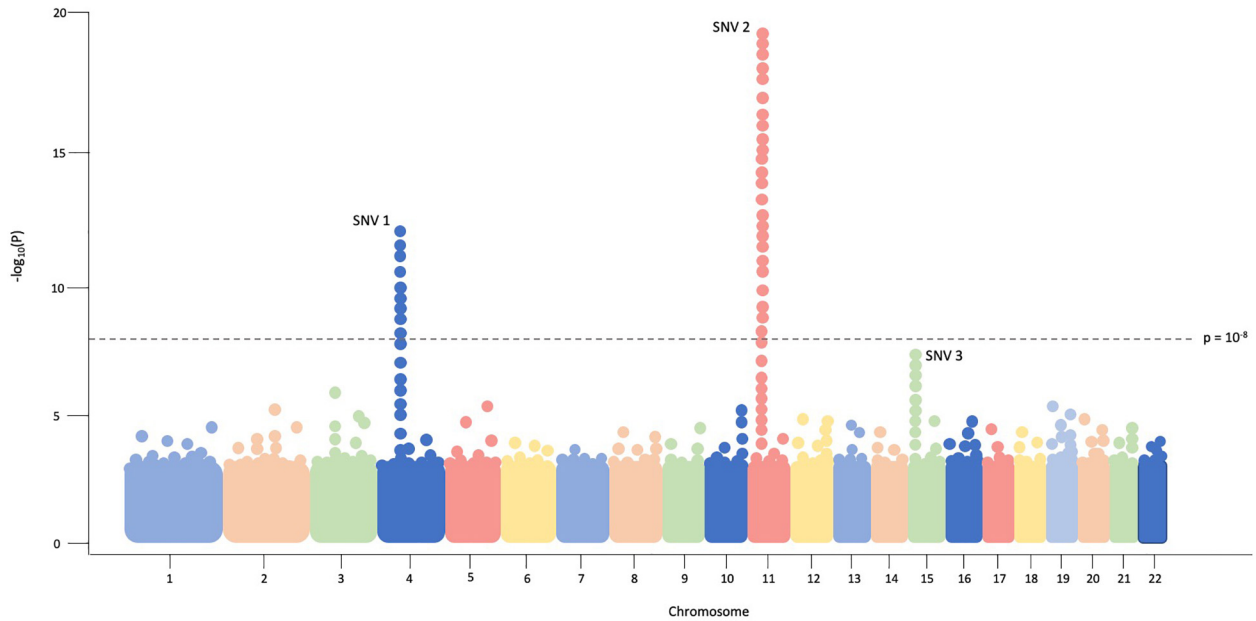

FIGURE 1 | In this example Manhattan plot, each dot represents a single SNV assessed in every individual in the cohort. Genomic coordinates are displayed in ascending fashion on the *X*-axis, beginning with chromosome 1 on the left. The negative logarithm of the association p-value for each SNV is shown on the *Y*-axis. The SNVs with the strongest association will have the most negative p-value, so the negative logarithm will be greatest for these SNVs. In this example plot, SNV1 and SNV2 exceed the threshold of significance ($p = 10^{-8}$) while SNV3 does not.

of thousands of association "hits" together (Wray et al., 2007). Individuals in the top 1–5% of risk profile may face a disease risk that approaches that of individuals who inherit a single monogenic pathogenic mutation (Khera et al., 2018). Informed by association results, PRS have shown modest but reliable prediction capability in a number of disease areas (Barrett et al., 2009; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Hoffmann et al., 2017; Seibert et al., 2018) as well as the ability to modify risk prediction for monogenic variants (Fahed et al., 2020).

LIMITATIONS OF ASSOCIATION STUDIES

Association studies require large datasets and a stringent threshold for significance to avoid false positives. Given the nature of studying rare variants, even larger sample sizes are required with RVAS so that patients with such rare variants will be included. For less-common diseases such as ALS, the sample size required to identify risk variants of low effect size may not be feasible. Further, the overwhelming majority of association studies have been performed in European cohorts (Haga, 2010; Duncan et al., 2019). Since variant frequencies as well as linkage disequilibrium vary between ethnic groups, findings may not be applicable across racial and ethnic groups (Need and Goldstein, 2009; Gravel et al., 2011).

Perhaps most importantly, association studies, by their nature, only measure the association of a risk loci with a disease but cannot determine the impact of a SNV on lifetime risk nor the mechanism by which it confers such risk (Altshuler et al., 2008). Specifically, GWAS findings often highlight non-coding SNVs in linkage disequilibrium with several other genes or regions, making it difficult to specifically identify causal genes. Variants that are associated with disease may in fact act as direct drivers of disease progression, or such a link to disease or phenotype may not be understood, potentially because the true causal variant at that locus has not yet been identified or multiple variants at a locus must work together. Even though association to a particular variant may be statistically significant with cases compared to controls, causality cannot necessarily be assigned by the GWAS approach. Thus, GWAS may reveal synthetic associations (Dickson et al., 2010). Typical GWAS reveal multiple variants associated with disease due to linkage disequilibrium, and functional studies are necessary to determine which are truly meaningful in the context of disease (Pearson, 2008).

Additionally, in such large cohorts the cost of uniform, deep sequencing approaches such as WGS can be a prohibiting factor, so other testing approaches may be considered, each with important caveats. A number of statistical methods have been developed to increase the power of RVAS in the context of sample size limitations.

GENE DISCOVERY IN AMYOTROPHIC LATERAL SCLEROSIS

Amyotrophic lateral sclerosis is a progressive neurodegenerative disorder affecting 1–2 per 100,000, involving selective loss of

upper and lower motor neurons and typically resulting in death in 2–5 years (van Es et al., 2017). The discovery of multiple genes associated with ALS has led to an era of targeted gene therapies and multiple lines of mechanistic inquiry. As such, the story of gene discovery in ALS provides a useful context in which to understand the significance of GWAS- and RVAS-identified variants in a genetically heterogeneous disease population.

The first gene identified to cause familial ALS (fALS, defined as having a history of ALS in a first-, second- or third-degree relative), *SOD1*, was identified via linkage studies in 1993 (Rosen, 1993). Since then, variants in over 50 genes have been identified in individuals with both familial and sporadic ALS (an up-to-date list of these genes can be found at alsod.ac.uk). Many of these genes were identified via linkage analysis in high-penetrance fALS families and confirmed in follow-up case-control studies that utilized either Sanger sequencing, SNV arrays, or exome sequencing (Siddique et al., 1989; Hosler et al., 1998; Nishimura, 2004). Other studies utilized prior biological knowledge to identify candidate genes and then conducted case-control sequencing studies (Kwiatkowski et al., 2009; Fecto, 2011). Currently, a monogenic etiology can be identified in up to two-thirds of fALS and 10% of sporadic ALS (sALS) cases (Chia et al., 2018). As with other genetically complex diseases, traditional linkage or candidate gene approaches were responsible for the discovery of the most highly penetrant ALS genes, including *C9orf72* (DeJesus-Hernandez et al., 2011; Renton et al., 2011), *SOD1* (Rosen, 1993), and *FUS* (Kwiatkowski et al., 2009).

ASSOCIATION STUDIES IN AMYOTROPHIC LATERAL SCLEROSIS: DISCOVERY AND REPRODUCIBILITY

The primary goal of most association studies in ALS is to identify new ALS-associated genes, either common (via GWAS) or rare (via RVAS), and this has been successful in recent years as evidenced by the identification of multiple disease-associated ALS genes (summarized in **Table 1**).

Rare variation appears to play an important role in explaining missing heritability within ALS (van Rheenen et al., 2016). As such, the field has made significant strides in applying RVAS using large, international collaborations (Smith et al., 2014; van Rheenen et al., 2016; Nicolas et al., 2018), the most recent of which identified *KIF5A* as a Mendelian ALS gene (Nicolas et al., 2018). Some GWAS studies (using SNV genotyping data) have performed RVAS (using sequencing data on smaller cohorts) as a follow-on validation step. Other approaches leverage additional sources of genetic data, such as gene expression data, to prioritize GWAS findings (Diekstra et al., 2012).

Association studies may be used to confirm previously identified findings, resulting either from early linkage studies or from other association studies in separate human ALS cohorts (**Table 1**). For example, *SOD1* was originally identified via linkage (Rosen, 1993) and in subsequent GWAS a clear signal was found at the *SOD1* locus (Laaksovirta et al., 2010). Additionally, association studies can help to more completely characterize a linkage finding. A 9p21.2 locus causing dominant ALS was originally discovered via linkage analysis, with multiple reports defining a minimum linkage region of 3.7 Mb including only five known genes (Luty et al., 2008; Le Ber et al., 2009; Boxer et al., 2011). This region was ultimately pinpointed to *C9orf72* in part by association studies which condensed the locus to a few genes (van Es et al., 2009b; Laaksovirta et al., 2010; Shatunov et al., 2010), providing avenues for targeted repeat-mapping in *C9orf72* (DeJesus-Hernandez et al., 2011).

Positive replication studies add support that the original finding was in fact a true association. If the replication cohort differs in geographical origin and/or phenotypic features to the original cohort, the findings may be more applicable in additional disease populations. Cross-ethnic analyses have uncovered such genes (Benyamin et al., 2017). Identifying genes and variants that are robustly replicated over time and across populations is a critical first step in characterizing the biological mechanisms underlying ALS. For example, association studies in ALS have replicated *C9orf72* as a disease-associated gene across ethnic groups (Laaksovirta et al., 2010; Shatunov et al., 2010). Additional examples of gene replication exist in small cohorts (Cronin et al., 2007; Li et al., 2009) and in larger meta-analyses (van Rheenen et al., 2016; Benyamin et al., 2017). In addition, RVAS studies have also lent support to prior GWAS findings (Kenna et al., 2016).

Nevertheless, across the board, many gene-specific replication studies have failed to replicate association findings (Chiò et al., 2009; Cronin et al., 2009; van Es et al., 2009a; Daoud et al., 2010; Fernández-Santiago et al., 2011; Fogh et al., 2011; Chen et al., 2012; Cai et al., 2014). Lack of reproducibility of association findings is common and may reflect several issues common in human genetic studies. As the risk variants often confer very small increases in risk, small sample sizes of less than 10,000 individuals are frequently underpowered to detect these risk variants. Larger study sizes (>50,000), which are most commonly assembled via large, international collaborations, are much more likely to reproduce association findings (Feliciano et al., 2018; Nishino et al., 2018; Zhang et al., 2020). The sample sizes in ALS, GWAS, and RVAS have grown steadily over time but are much smaller than those in other disease areas (Michailidou et al., 2017). Inherent population stratification also influences varying allele frequencies between individuals from different geographical regions and/or different ancestral backgrounds (Tam et al., 2019). Thus, positive associations may not be found in a subsequent study if population differences exist. Selection criteria for each cohort are not always consistent and may be subject to bias based on clinical or demographic standards (McClellan and King, 2010). Phenotypic variation in different cohorts may influence diagnosis and inclusion in genomic studies. Finally, variation in genetic testing technology and analysis as well as genotyping errors may occur between cohorts.

Genetically homogenous ethnic populations are typically selected for GWAS and RVAS because they introduce the least amount of genetic diversity and maximize the chances of identifying variants that are disease-related rather

TABLE 1 | A chronological overview of ALS-related genes initially identified via association studies.

Gene(s) Identified	Study Type	Sequencing Method	Discovery Cohort	Replication Cohort	Reported functional validation	Biological role of gene product	Gene(s) Replicated
FLJ10986 (Dunckley et al., 2007)	GWAS	SNV array	386 sALS patients and 542 controls (Caucasian)	+	Western blot expression of FLJ10986 was detected in spinal cord samples from both sALS cases and controls.	Uncharacterized at time of publication. Subsequently found to be involved in carbohydrate phosphorylation (Singh et al., 2017).	
ITPR2 (van Es et al., 2007)	GWAS	SNV array	461 ALS patients and 450 controls (Netherlands)	+ (Netherlands, Belgium, Sweden)	ITPR2 mRNA expression was greater in the peripheral blood of 126 ALS patients than in that of 126 healthy controls ($p = 0.00016$).	Glutamate-mediated neurotransmission, intracellular calcium concentration and apoptosis specifically in motor neurons (Gambardella et al., 2020).	
DPP6 (van Es et al., 2008)	GWAS	SNV array	1,767 ALS patients and 1,916 controls (Netherlands, United States)	+ (Netherlands, Belgium, Sweden)		Neuronal excitability via binding of voltage-gated potassium channels. Mice with *DPP6* knockout demonstrate neuronal hyperexcitability and behavioral alterations (Lin et al., 2018).	
UNC13A *MOBKL2B* (van Es et al., 2009b)	GWAS	SNV array	2,323 sALS patients and 9,013 controls (Netherlands, United States, Ireland, Sweden Belgium)	+ (Netherlands, United States, United Kingdom, France, Ireland Poland, Germany)		*UNC13A*: Release,of neurotransmitters, such as glutamate, at neuromuscular synapses (Varoqueaux et al., 2005). *MOBKL2B*: Kinase activity (Hergovich, 2011)	
KIFAP3 (Landers et al., 2009)	GWAS	SNV array	1,821 sALS cases and 2,258 controls (United States, Britain, France, Netherlands)	+ (United States, Europe)		*KIFAP3*: Encodes a kinesin-associated protein (Shimizu et al., 1996) Neurite outgrowth and cortical development (Ozeki et al., 2003)	
CYP27A1 (Diekstra et al., 2012)	GWAS with eQTL	SNV array and mRNA expression	Genotyping: 2,261 sALS patients and 8,328 controls (Netherlands, Belgium, France, Ireland, United Kingdom, Sweden, United States) eQTL: 162 sALS patients and 207 controls	+		Cholesterol metabolism. Variants in this gene have been found in individuals with cerebrotendinous xanthomatosis (CTX) (Gallus et al., 2006). The progressive upper motor neuron symptoms involved in CTX overlap with those found in ALS.	*UNC13A*
ZNF512B (Iida et al., 2011)	GWAS	SNV array	92 ALS patients and 233 control (Japan)	+ (Japan)	ZNF512B overexpression increased TGF-β signaling and knockdown decreased TGF-β signaling. ZNF512B expression was increased in the spinal cord anterior horn motor neurons of patients.		
CAMK1G *CABIN1-SUSD2* (Deng et al., 2013)	GWAS	SNV array	506 sALS patients and 1,859 controls (Han Chinese)	+ (Han Chinese)		*CAMK1G*: Calcium kinase signaling (Takemoto-Kimura et al., 2003) *CABIN1*: Inhibition of calcineurin-mediated signal transduction (Sun et al., 1998) *SUSD2*: Cell membrane signaling (Watson et al., 2013)	

(Continued)

TABLE 1 | Continued

Gene(s) Identified	Study Type	Sequencing Method	Discovery Cohort	Replication Cohort	Reported functional validation	Biological role of gene product	Gene(s) Replicated
SARM1 (Fogh et al., 2014)	GWAS	SNV array	6,100 sALS patients and 7,125 controls (Italy Netherlands, Belgium, Sweden, France, Ireland, United States, Britain)	+ (Italy, Netherlands, Germany)		Axonal degeneration (Osterloh et al., 2012)	*C9orf72*
TBK1 *NEK1* (Cirulli et al., 2015)	GWAS	Exome sequencing	2,869 patients with ALS and 6,405 controls (Caucasian)	+ (Caucasian)		*TBK1*: Autophagy (Duan et al., 2019) *NEK1*: Kinase linked to multiple cellular processes including cell cycle control and cilia repair (Thiel et al., 2011)	*SOD1, TARDBP, OPTN, VCP**
TUBA4A (Smith et al., 2014)	RVAS	Exome sequencing	363 unrelated fALS probands and 4,331 (European American)	+ (European American)	Mutant TUB4A constructs transfected in HEK293 and primary motor neurons show altered incorporation into microtubules as well as altered microtubule polymerization and stability.	Cytoskeletal organization and maintenance (Smith et al., 2014)	*MATR3***
C21orf2 *MOBP* *SCFD1* (van Rheenen et al., 2016)	GWAS RVAS	Genome sequencing and SNV array	1,861 ALS patients and matched controls (Australia, Belgium, France, Germany, Ireland, Italy, Netherlands and Turkey)	+ (Australia, Belgium, France, Germany, Ireland, Italy, Netherlands and Turkey)		*C21orf2:* Development and maintenance of cilia, DNA repair mechanisms (Khan et al., 2015; Wang et al., 2016). *MOBP:* Expressed in myelin, neurodegeneration (Yamamoto et al., 1994) *SCFD1:*Intracellular transport (Laufman et al., 2009)	*UNC13A, SARM1, C9orf72, TBK1, C21orf2*
GPX3-TNIP1 (Benyamin et al., 2017)	GWAS	SNV array	13,811 sALS patients and 26,325 controls (European and Chinese)	+ (Australian)	Investigation of differential expression of GPX3 and TNIP1 between ALS patients and controls was not conclusive	*GPX3*: Antioxidant molecule functionally related to SOD1 (Chi et al., 2007) *TNIP1*: Previously associated with inflammation and immune disorders (Gateva et al., 2009; Nair et al., 2009)	*C9orf72, MOPB, SARM1, UNC13A, SCFD1*
KIF5A (Nicolas et al., 2018)	GWAS RVAS	SNV array (GWAS) Exome sequencing (RVAS)	20,806 ALS patients and 59,804 control samples (Caucasian European and United States)	+ (Caucasian European and United States)	Splice site prediction software (ASSEDA) predicted all *KIF5A* variants to result in aberrant splicing leading to skipping of *KIF5A* exon 27	Axonal transport (Kanai et al., 2004)	*C9orf72, TBK1, UNC13A, C21orf2, TNIP1*

sALS, sporadic ALS; fALS, familial ALS; SNV, single nucleotide variant.
**This study did not assess repeat disorders including those found in C9orf72.*
***This study excluded patients with variants in several known ALS genes.*

than geoancestry-related. However, the results from such cohorts are often not replicable in subsequent analyses and/or generalizable in other populations, often due to varying allele prevalence and unequal representation of different populations in case and control groups. For decades, Caucasian individuals have made up the vast majority of people studied in association studies across all diseases, including ALS (Popejoy and Fullerton, 2016). In ALS association studies, there is notable lack of replication between Asian and European ALS cohorts, which may reflect inherent population differences in SNV frequencies and disease phenotypes (Gravel et al., 2011). For example, ALS onset occurs at a younger age in Han Chinese patients and is more likely to present with bulbar-onset, as compared to limb-onset (Deng et al., 2013).

GWAS AND RVAS STUDIES RESULT IN APPRECIATION OF RELEVANT MECHANISTIC PATHWAYS

Experiments to determine the functional consequences of ALS-associated variants in genes identified via GWAS and RVAS have further characterized the pathology underlying disease either via a specific gene product itself or the network or pathway in which it operates. Such ALS genes play roles in

glutamate-mediated neurotransmission and excitability (*ITPR2* and *UNC13A*; Varoqueaux et al., 2005; Gambardella et al., 2020), regulation of neuronal excitability (*DPP6;* Lin et al., 2018), autophagy (*TBK1;* Duan et al., 2019), cytoskeletal organization (*TUBA4A;* Smith et al., 2014), and axonal transport (*KIF5A*; Nicolas et al., 2018).

Some association findings have led to promising results in *in vitro* and *in vivo* models of ALS. For example, conditional knockout of *TBK1* was reported to result in motor and cognitive defects in mice as well as pathological features typical of autophagy dysfunction (Duan et al., 2019). In $SOD1^{G93A}$-transfected cells, *TBK1* overexpression reduced the number and size of SOD1 aggregates. $SOD1^{G93A}$ transgenic mice demonstrating an ALS phenotype show increased survival and decreased protein aggregates after intracerebroventricular injection of AAV vectors encoding *TBK1* (Duan et al., 2019). *TBK1* expression may have the therapeutic potential to promote autophagy even in the absence of *TBK1* variants.

Other disease pathway studies for GWAS- and RVAS-identified genes have lent support to the approaches of current therapeutic options for ALS. For example, UNC13A functions to regulate the release of neurotransmitters, such as glutamate at neuromuscular synapses (Rossner et al., 2004; Engel et al., 2016). In mice, UNC13A acts in synaptic vesicle priming, and mice lacking UNC13A demonstrate altered glutamatergic neurotransmission (Varoqueaux et al., 2005; Gambardella et al., 2020). *UNC13A* variants may therefore promote disease via glutamate-mediated excitotoxicity. Riluzole, one of two FDA-approved treatments for ALS, is a glutamate release inhibitor and can lead to a 2-3 month increase in survival for some patients (Bellingham, 2011; Dharmadasa and Kiernan, 2018). However, in clinical trials, other treatments aimed at decreasing glutamate neurotransmission have demonstrated limited or negative results (Bedlack, 2019).

CURRENT IMPACT OF GWAS AND RVAS ON PRECLINICAL AND CLINICAL THERAPY

Efforts to translate genetic discoveries into therapeutic clinical trials in ALS have thus far been et with limited success, in contrast to other diseases in which GWAS had led to new drugs currently in clinical trials or clinical practice (Visscher et al., 2017). The high degree of clinical and genetic heterogeneity, unknown influence of endogenous and exogenous factors on disease susceptibility, and unknown reasons for selective vulnerability of certain cell types present significant challenges to therapeutic development (Katyal and Govindarajan, 2017).

Gene-targeted clinical trials for ALS patients with variants in three genes (*FUS, C9orf72*, and *SOD1*) are underway. Antisense oligonucleotides targeted at C9orf72 mutant transcripts have shown promising results in ALS models and are currently in development for patients with *C9orf72*-related ALS (Riboldi et al., 2014; Martier et al., 2019). Recently, two approaches to down-regulate SOD1 expression in patients with SOD1-ALS [one utilizing an antisense oligonucleotide (Miller et al., 2020) and the other an adeno-associated viral vector (Mueller et al., 2020)] have been reported. The genes targeted in ALS genomic therapies were each originally identified in high-penetrance families demonstrating Mendelian inheritance, not via GWAS or RVAS. They also represent the most common, known genetic causes of ALS and were discovered prior to the widespread application of association-based technology.

There is potential utility of association studies in identifying subgroups of medication responders. This has been demonstrated in a survival analysis of patients with a particular *UNC13A* genotype who were treated with lithium carbonate (van Eijk et al., 2017). *UNC13A* was originally identified via an association study (van Es et al., 2009b). This finding suggests that GWAS and RVAS may lead to more targeted studies in the future and/or improved interpretation of clinical trial results.

Currently, results from ALS association studies are used broadly in several ways, such as improving understanding of the genetic architecture of ALS, illuminating tissue- or cell-specific pathways involving ALS-associated genes, and informing variable expressivity and penetrance of disease. Moving forward, GWAS and RVAS findings may assist in the design of combinatorial therapies that target multiple gene products and disease pathways, reflecting the proposed oligogenic nature of disease (Nguyen et al., 2018). Finally, larger, more powerful association studies may one day enable the calculation of clinical PRS to identify healthy individuals at highest risk of disease, who may be candidates for neuroprotective interventions.

DISCUSSION

Advances in genetic testing and identification of genetic subtypes of disease have been the cornerstone of ALS research in recent years, marked by widespread genetic testing in larger and more diverse cohorts, bioinformatic and molecular characterization of identified variants, and progress toward clinical trials for genetic subtypes of disease. Gene discovery has been driven by linkage analysis of families with high-penetrance genes, candidate gene approaches and more recently, association studies such as GWAS and RVAS. Association studies represent an attractive option for novel gene discovery because they do not require prior knowledge or hypotheses, compared with hypothesis-confirming sequencing studies.

Currently, there are no effective treatment options to halt progression of ALS and only two FDA-approved medications. Significant urgency exists within the ALS community to identify additional genetic markers of disease in order to uncover novel biological mechanisms, stratify genetic subgroups of disease, and drive drug development. Lower-penetrance genes and risk factors identified via association studies may serve as important components of combinatorial gene-targeted therapies in the future. Gene-targeted clinical trials are currently underway, though to date, no ALS genes initially identified via GWAS or RVAS have been developed for gene therapy approaches. In general, consideration of the potential of a GWAS or RVAS finding must be approached with measured expectations,

particularly when such genes are quickly added to ALS clinical genetic testing panels. Association studies of common and rare genetic variation, when critically evaluated and contextualized properly, are a powerful tool in understanding the genetic basis of complex diseases such as ALS.

AUTHOR CONTRIBUTIONS

KR: background research. All authors: manuscript writing and development.

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REFERENCES

Altshuler, D., Daly, M. J., and Lander, E. S. (2008). Genetic mapping in human disease. *Science (New York, N.Y.)* 322, 881–888. doi: 10.1126/science.1156409

Auer, P. L., and Lettre, G. (2015). Rare variant association studies: considerations, challenges and opportunities. *Genome Med.* 7:16. doi: 10.1186/s13073-015-0138-2

Barrett, J. C., Clayton, D. G., Concannon, P., Akolkar, B., Cooper, J. D., Erlich, H. A., et al. (2009). Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat. Genet.* 41, 703–707. doi: 10.1038/ng.381

Bedlack, R. (2019). ALSUntangled 48: perampanel (Fycompa). *Amyotroph. Lateral Scler. Frontotemporal Degener.* 20, 453–456. doi: 10.1080/21678421.2019.1573850

Bellingham, M. C. (2011). A review of the neural mechanisms of action and clinical efficiency of riluzole in treating amyotrophic lateral sclerosis: what have we learned in the last decade?: neural mechanisms of action and clinical efficiency of riluzole in treating ALS. *CNS Neurosci. Ther.* 17, 4–31. doi: 10.1111/j.1755-5949.2009.00116.x

Benyamin, B., He, J., Zhao, Q., Gratten, J., Garton, F., Leo, P. J., et al. (2017). Cross-ethnic meta-analysis identifies association of the *GPX3-TNIP1* locus with amyotrophic lateral sclerosis. *Nat. Commun.* 8:611. doi: 10.1038/s41467-017-00471-1

Boxer, A. L., Mackenzie, I. R., Boeve, B. F., Baker, M., Seeley, W. W., Crook, R., et al. (2011). Clinical, neuroimaging and neuropathological features of a new chromosome 9p-linked FTD-ALS family. *J. Neurol. Neurosurg. Psychiatry* 82, 196–203. doi: 10.1136/jnnp.2009.204081

Cai, B., Tang, L., Zhang, N., and Fan, D. (2014). The single-nucleotide polymorphism rs6690993 in FGGY is not associated with amyotrophic lateral sclerosisin a large Chinese cohort. *Neurobiol. Aging* 35, 1512.e3–1512.e4. doi: 10.1016/j.neurobiolaging.2013.12.018

Chen, Y., Zeng, Y., Huang, R., Yang, Y., Chen, K., Song, W., et al. (2012). No association of five candidate genetic variants with amyotrophic lateral sclerosis in a Chinese population. *Neurobiol. Aging* 33, 2721.e3–5. doi: 10.1016/j.neurobiolaging.2012.06.004

Chi, L., Ke, Y., Luo, C., Gozal, D., and Liu, R. (2007). Depletion of reduced glutathione enhances motor neuron degeneration *in vitro* and in vivo. *Neuroscience* 144, 991–1003. doi: 10.1016/j.neuroscience.2006.09.064

Chia, R., Chiò, 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

Chiò, A., Schymick, J. C., Restagno, G., Scholz, S. W., Lombardo, F., Lai, S.-L., et al. (2009). A two-stage genome-wide association study of sporadic amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 18, 1524–1532. doi: 10.1093/hmg/ddp059

Choquet, H., Kasberger, J., Hamidovic, A., and Jorgenson, E. (2013). Contribution of common PCSK1 genetic variants to obesity in 8,359 subjects from multi-ethnic American population. *PLoS One* 8:e57857. doi: 10.1371/journal.pone.0057857

Cirulli, E. T., Lasseigne, B. N., Petrovski, S., Sapp, P. C., Dion, P. A., Leblond, C. S., et al. (2015). Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. *Science* 347, 1436–1441. doi: 10.1126/science.aaa3650

Cohen, J., Pertsemlidis, A., Kotowski, I. K., Graham, R., Garcia, C. K., and Hobbs, H. H. (2005). Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. *Nat. Genet.* 37, 161–165. doi: 10.1038/ng1509

Cohen, J. C. (2004). Multiple rare alleles contribute to low plasma levels of HDL Cholesterol. *Science* 305, 869–872. doi: 10.1126/science.1099870

Cronin, S., Berger, S., Ding, J., Schymick, J. C., Washecka, N., Hernandez, D. G., et al. (2007). A genome-wide association study of sporadic ALS in a homogenous Irish population. *Hum. Mol. Genet.* 17, 768–774. doi: 10.1093/hmg/ddm361

Cronin, S., Tomik, B., Bradley, D. G., Slowik, A., and Hardiman, O. (2009). Screening for replication of genome-wide SNP associations in sporadic ALS. *Eur. J. Hum. Genet.* 17, 213–218. doi: 10.1038/ejhg.2008.194

Daoud, H., Valdmanis, P. N., Dion, P. A., and Rouleau, G. A. (2010). Analysis of *DPP6* and *FGGY* as candidate genes for amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* 11, 389–391. doi: 10.3109/17482960903358857

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

Deng, M., Wei, L., Zuo, X., Tian, Y., Xie, F., Hu, P., et al. (2013). Genome-wide association analyses in Han Chinese identify two new susceptibility loci for amyotrophic lateral sclerosis. *Nat. Genet.* 45, 697–700. doi: 10.1038/ng.2627

Dharmadasa, T., and Kiernan, M. C. (2018). Riluzole, disease stage and survival in ALS. *Lancet Neurol.* 17, 385–386. doi: 10.1016/S1474-4422(18)30091-7

Dickson, S. P., Wang, K., Krantz, I., Hakonarson, H., and Goldstein, D. B. (2010). Rare variants create synthetic genome-wide associations. *PLoS Biol.* 8:e1000294. doi: 10.1371/journal.pbio.1000294

Diekstra, F. P., Saris, C. G. J., van Rheenen, W., Franke, L., Jansen, R. C., van Es, M. A., et al. (2012). Mapping of Gene Expression Reveals CYP27A1 as a susceptibility gene for sporadic ALS. *PLoS One* 7:e35333. doi: 10.1371/journal.pone.0035333

Duan, W., Guo, M., Yi, L., Zhang, J., Bi, Y., Liu, Y., et al. (2019). Deletion of Tbk1 disrupts autophagy and reproduces behavioral and locomotor symptoms of FTD-ALS in mice. *Aging (Albany NY)* 11, 2457–2476. doi: 10.18632/aging.101936

Duncan, L., Shen, H., Gelaye, B., Meijsen, J., Ressler, K., Feldman, M., et al. (2019). Analysis of polygenic risk score usage and performance in diverse human populations. *Nat. Commun.* 10:3328. doi: 10.1038/s41467-019-11112-0

Dunckley, T., Huentelman, M. J., Craig, D. W., Pearson, J. V., Szelinger, S., Joshipura, K., et al. (2007). Whole-genome analysis of sporadic amyotrophic lateral sclerosis. *N. Engl. J. Med.* 357, 775–788. doi: 10.1056/NEJMoa070174

Engel, A. G., Selcen, D., Shen, X.-M., Milone, M., and Harper, C. M. (2016). Loss of MUNC13-1 function causes microcephaly, cortical hyperexcitability, and fatal myasthenia. *Neurol. Genet.* 2:e105. doi: 10.1212/NXG.0000000000000105

Fahed, A. C., Wang, M., Homburger, J. R., Patel, A. P., Bick, A. G., Neben, C. L., et al. (2020). Polygenic background modifies penetrance of monogenic variants for tier 1 genomic conditions. *Nat. Commun.* 11:3635. doi: 10.1038/s41467-020-17374-3

Fecto, F. (2011). SQSTM1 mutations in familial and sporadic amyotrophic lateral sclerosis. *Arch. Neurol.* 68:1440. doi: 10.1001/archneurol.2011.250

Feliciano, P., Daniels, A. M., Green Snyder, L., Beaumont, A., Camba, A., Esler, A., et al. (2018). SPARK: a US cohort of 50,000 families to accelerate autism research. *Neuron* 97, 488–493. doi: 10.1016/j.neuron.2018.01.015

Fernández-Santiago, R., Sharma, M., Berg, D., Illig, T., Anneser, J., Meyer, T., et al. (2011). No evidence of association of FLJ10986 and ITPR2 with ALS in a large German cohort. *Neurobiol. Aging* 32, 551.e1–4. doi: 10.1016/j.neurobiolaging.2009.04.018

Flannick, J., and Florez, J. C. (2016). Type 2 diabetes: genetic data sharing to advance complex disease research. *Nat. Rev. Genet.* 17, 535–549. doi: 10.1038/nrg.2016.56

Fogh, I., D'Alfonso, S., Gellera, C., Ratti, A., Cereda, C., Penco, S., et al. (2011). No association of DPP6 with amyotrophic lateral sclerosis in an Italian population. *Neurobiol. Aging* 32, 966–967. doi: 10.1016/j.neurobiolaging.2009.05.014

Fogh, I., Ratti, A., Gellera, C., Lin, K., Tiloca, C., Moskvina, V., et al. (2014). A genome-wide association meta-analysis identifies a novel locus at 17q11.2 associated with sporadic amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 23, 2220–2231. doi: 10.1093/hmg/ddt587

Gallus, G. N., Dotti, M. T., and Federico, A. (2006). Clinical and molecular diagnosis of cerebrotendinous xanthomatosis with a review of the mutations in the CYP27A1 gene. *Neurol. Sci.* 27, 143–149. doi: 10.1007/s10072-006-0618-7

Gambardella, J., Lombardi, A., Morelli, M. B., Ferrara, J., and Santulli, G. (2020). Inositol 1,4,5-Trisphosphate receptors in human disease: a comprehensive update. *J. Clin. Med.* 9:1096. doi: 10.3390/jcm9041096

Gateva, V., Sandling, J. K., Hom, G., Taylor, K. E., Chung, S. A., Sun, X., et al. (2009). A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. *Nat. Genet.* 41, 1228–1233. doi: 10.1038/ng.468

Gravel, S., Henn, B. M., Gutenkunst, R. N., Indap, A. R., Marth, G. T., Clark, A. G., et al. (2011). Demographic history and rare allele sharing among human populations. *Proc. Natl. Acad. Sci. U. S. A.* 108, 11983–11988. doi: 10.1073/pnas.1019276108

Gudmundsson, J., Sulem, P., Gudbjartsson, D. F., Masson, G., Agnarsson, B. A., Benediktsdottir, K. R., et al. (2012). A study based on whole-genome sequencing yields a rare variant at 8q24 associated with prostate cancer. *Nat. Genet.* 44, 1326–1329. doi: 10.1038/ng.2437

Haga, S. B. (2010). Impact of limited population diversity of genome-wide association studies. *Genet. Med.* 12, 81–84. doi: 10.1097/GIM.0b013e3181ca2bbf

Hergovich, A. (2011). MOB control: reviewing a conserved family of kinase regulators. *Cell. Signal.* 23, 1433–1440. doi: 10.1016/j.cellsig.2011.04.007

Hoffmann, T. J., Ehret, G. B., Nandakumar, P., Ranatunga, D., Schaefer, C., Kwok, P.-Y., et al. (2017). Genome-wide association analyses using electronic health records identify new loci influencing blood pressure variation. *Nat. Genet.* 49, 54–64. doi: 10.1038/ng.3715

Hosler, B. A., Sapp, P. C., Berger, R., O'Neill, G., Bejaoui, K., Hamida, M. B., et al. (1998). Refined mapping and characterization of the recessive familial amyotrophic lateral sclerosis locus (ALS2) on chromosome 2q33. *Neurogenetics* 2, 34–42. doi: 10.1007/s100480050049

Iida, A., Takahashi, A., Kubo, M., Saito, S., Hosono, N., Ohnishi, Y., et al. (2011). A functional variant in *ZNF512B* is associated with susceptibility to amyotrophic lateral sclerosis in Japanese. *Hum. Mol. Genet.* 20, 3684–3692. doi: 10.1093/hmg/ddr268

Kanai, Y., Dohmae, N., and Hirokawa, N. (2004). Kinesin transports RNA: isolation and characterization of an RNA-transporting granule. *Neuron* 43, 513–525. doi: 10.1016/j.neuron.2004.07.022

Katyal, N., and Govindarajan, R. (2017). Shortcomings in the current amyotrophic lateral sclerosis trials and potential solutions for improvement. *Front. Neurol.* 8:521. doi: 10.3389/fneur.2017.00521

Kenna, K. P., van Doormaal, P. T. C., Dekker, A. M., Ticozzi, N., Kenna, B. J., Diekstra, F. P., et al. (2016). NEK1 variants confer susceptibility to amyotrophic lateral sclerosis. *Nat. Genet.* 48, 1037–1042. doi: 10.1038/ng.3626

Khan, A. O., Eisenberger, T., Nagel-Wolfrum, K., Wolfrum, U., and Bolz, H. J. (2015). C21orf2 is mutated in recessive early-onset retinal dystrophy with macular staphyloma and encodes a protein that localises to the photoreceptor primary cilium. *Br. J. Ophthalmol.* 99, 1725–1731. doi: 10.1136/bjophthalmol-2015-307277

Khera, A. V., Chaffin, M., Aragam, K. G., Haas, M. E., Roselli, C., Choi, S. H., et al. (2018). Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nat. Genet.* 50, 1219–1224. doi: 10.1038/s41588-018-0183-z

Kosmicki, J. A., Churchhouse, C. L., Rivas, M. A., and Neale, B. M. (2016). Discovery of rare variants for complex phenotypes. *Hum. Genet.* 135, 625–634. doi: 10.1007/s00439-016-1679-1

Kwiatkowski, T. J., 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

Laaksovirta, H., Peuralinna, T., Schymick, J. C., Scholz, S. W., Lai, S.-L., Myllykangas, L., et al. (2010). Chromosome 9p21 in amyotrophic lateral sclerosis in Finland: a genome-wide association study. *Lancet. Neurol.* 9, 978–985. doi: 10.1016/S1474-4422(10)70184-8

Landers, J. E., Melki, J., Meininger, V., Glass, J. D., van den Berg, L. H., van Es, M. A., et al. (2009). Reduced expression of the *Kinesin-Associated Protein 3* (*KIFAP3*) gene increases survival in sporadic amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. U. S. A.* 106, 9004–9009. doi: 10.1073/pnas.0812937106

Laufman, O., Kedan, A., Hong, W., and Lev, S. (2009). Direct interaction between the COG complex and the SM protein, Sly1, is required for Golgi SNARE pairing. *EMBO J.* 28, 2006–2017. doi: 10.1038/emboj.2009.168

Le Ber, I., Camuzat, A., Berger, E., Hannequin, D., Laquerriere, A., Golfier, V., et al. (2009). Chromosome 9p-linked families with frontotemporal dementia associated with motor neuron disease. *Neurology* 72, 1669–1676. doi: 10.1212/WNL.0b013e3181a55f1c

Lee, S., Abecasis, G. R., Boehnke, M., and Lin, X. (2014). Rare-Variant Association analysis: study designs and statistical tests. *Am. J. Hum. Genet.* 95, 5–23. doi: 10.1016/j.ajhg.2014.06.009

Li, X.-G., Zhang, J.-H., Xie, M.-Q., Liu, M.-S., Li, B.-H., Zhao, Y.-H., et al. (2009). Association between DPP6 polymorphism and the risk of sporadic amyotrophic lateral sclerosis in Chinese patients. *Chin. Med. J.* 122, 2989–2992.

Li, Z., Chen, J., Yu, H., He, L., Xu, Y., Zhang, D., et al. (2017). Genome-wide association analysis identifies 30 new susceptibility loci for schizophrenia. *Nat. Genet.* 49, 1576–1583. doi: 10.1038/ng.3973

Lin, L., Murphy, J. G., Karlsson, R.-M., Petralia, R. S., Gutzmann, J. J., Abebe, D., et al. (2018). DPP6 loss impacts hippocampal synaptic development and induces behavioral impairments in recognition, learning and memory. *Front. Cell Neurosci.* 12:84. doi: 10.3389/fncel.2018.00084

Liu, J. Z., van Sommeren, S., Huang, H., Ng, S. C., Alberts, R., Takahashi, A., et al. (2015). Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nat. Genet.* 47, 979–986. doi: 10.1038/ng.3359

Luty, A. A., Kwok, J. B., Thompson, E. M., Blumbergs, P., Brooks, W. S., Loy, C. T., et al. (2008). Pedigree with frontotemporal lobar degeneration – motor neuron disease and Tar DNA binding protein-43 positive neuropathology: genetic linkage to chromosome 9. *BMC Neurol.* 8:32. doi: 10.1186/1471-2377-8-32

Manolio, T. A., Collins, F. S., Cox, N. J., Goldstein, D. B., Hindorff, L. A., Hunter, D. J., et al. (2009). Finding the missing heritability of complex diseases. *Nature* 461, 747–753. doi: 10.1038/nature08494

Martier, R., Liefhebber, J. M., García-Osta, A., Miniarikova, J., Cuadrado-Tejedor, M., Espelosin, M., et al. (2019). Targeting RNA-Mediated Toxicity in C9orf72 ALS and/or FTD by RNAi-based gene therapy. *Mol. Ther. Nucleic Acids* 16, 26–37. doi: 10.1016/j.omtn.2019.02.001

McClellan, J., and King, M.-C. (2010). Genetic heterogeneity in human disease. *Cell* 141, 210–217. doi: 10.1016/j.cell.2010.03.032

Michailidou, K., Lindström, S., Dennis, J., Beesley, J., Hui, S., Kar, S., et al. (2017). Association analysis identifies 65 new breast cancer risk loci. *Nature* 551, 92–94. doi: 10.1038/nature24284

Miller, T., Cudkowicz, M., Shaw, P. J., Andersen, P. M., Atassi, N., Bucelli, R. C., et al. (2020). Phase 1–2 trial of antisense oligonucleotide tofersen for *SOD1* ALS. *N. Engl. J. Med.* 383, 109–119. doi: 10.1056/NEJMoa2003715

Minster, R. L., Hawley, N. L., Su, C.-T., Sun, G., Kershaw, E. E., Cheng, H., et al. (2016). A thrifty variant in CREBRF strongly influences body mass index in Samoans. *Nat. Genet.* 48, 1049–1054. doi: 10.1038/ng.3620

Mueller, C., Berry, J. D., McKenna-Yasek, D. M., Gernoux, G., Owegi, M. A., Pothier, L. M., et al. (2020). *SOD1* suppression with adeno-associated virus and MicroRNA in Familial ALS. *N. Engl. J. Med.* 383, 151–158. doi: 10.1056/NEJMoa2005056

Nair, R. P., Duffin, K. C., Helms, C., Ding, J., Stuart, P. E., Goldgar, D., et al. (2009). Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat. Genet.* 41, 199–204. doi: 10.1038/ng.311

Need, A. C., and Goldstein, D. B. (2009). Next generation disparities in human genomics: concerns and remedies. *Trends Genet. TIG* 25, 489–494. doi: 10.1016/j.tig.2009.09.012

Nelson, M. R., Tipney, H., Painter, J. L., Shen, J., Nicoletti, P., Shen, Y., et al. (2015). The support of human genetic evidence for approved drug indications. *Nat. Genet.* 47, 856–860. doi: 10.1038/ng.3314

Nguyen, H. P., Van Broeckhoven, C., and van der Zee, J. (2018). ALS genes in the genomic era and their implications for FTD. *Trends Genet.* 34, 404–423. doi: 10.1016/j.tig.2018.03.001

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, e1268–e1283. doi: 10.1016/j.neuron.2018.02.027

Nikpay, M., Goel, A., Won, H.-H., Hall, L. M., Willenborg, C., Kanoni, S., et al. (2015). A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat. Genet.* 47, 1121–1130. doi: 10.1038/ng.3396

Nishimura, A. L. (2004). A novel locus for late onset amyotrophic lateral sclerosis/motor neurone disease variant at 20q13. *J. Med. Genet.* 41, 315–320. doi: 10.1136/jmg.2003.013029

Nishino, J., Ochi, H., Kochi, Y., Tsunoda, T., and Matsui, S. (2018). Sample size for successful genome-wide association study of major depressive disorder. *Front. Genet.* 9:227. doi: 10.3389/fgene.2018.00227

Osterloh, J. M., Yang, J., Rooney, T. M., Fox, A. N., Adalbert, R., Powell, E. H., et al. (2012). dSarm/Sarm1 is required for activation of an injury-induced axon death pathway. *Science* 337, 481–484. doi: 10.1126/science.1223899

Owen, K. R., Thanabalasingham, G., James, T. J., Karpe, F., Farmer, A. J., McCarthy, M. I., et al. (2010). Assessment of high-sensitivity C-reactive protein levels as diagnostic discriminator of maturity-onset diabetes of the young due to HNF1A mutations. *Diabetes Care* 33, 1919–1924. doi: 10.2337/dc10-0288

Ozeki, Y., Tomoda, T., Kleiderlein, J., Kamiya, A., Bord, L., Fujii, K., et al. (2003). Disrupted-in-Schizophrenia-1 (DISC-1): mutant truncation prevents binding to NudE-like (NUDEL) and inhibits neurite outgrowth. *Proc. Natl. Acad. Sci. U. S. A.* 100, 289–294. doi: 10.1073/pnas.0136913100

Pearson, T. A. (2008). How to interpret a genome-wide association study. *JAMA* 299:1335. doi: 10.1001/jama.299.11.1335

Pigeyre, M., Yazdi, F. T., Kaur, Y., and Meyre, D. (2016). Recent progress in genetics, epigenetics and metagenomics unveils the pathophysiology of human obesity. *Clin. Sci. (London, England: 1979)* 130, 943–986. doi: 10.1042/CS20160136

Popejoy, A. B., and Fullerton, S. M. (2016). Genomics is failing on diversity. *Nature* 538, 161–164. doi: 10.1038/538161a

Reiner, A. P., Gross, M. D., Carlson, C. S., Bielinski, S. J., Lange, L. A., Fornage, M., et al. (2009). Common coding variants of the HNF1A gene are associated with multiple cardiovascular risk phenotypes in community-based samples of younger and older European-American adults: the Coronary Artery Risk Development in Young Adults Study and The Cardiovascular Health Study. *Circ. Cardiovasc. Genet.* 2, 244–254. doi: 10.1161/CIRCGENETICS.108.839506

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

Riboldi, G., Zanetta, C., Ranieri, M., Nizzardo, M., Simone, C., Magri, F., et al. (2014). Antisense oligonucleotide therapy for the treatment of C9ORF72 ALS/FTD Diseases. *Mol. Neurobiol.* 50, 721–732. doi: 10.1007/s12035-014-8724-7

Ridker, P. M., Pare, G., Parker, A., Zee, R. Y. L., Danik, J. S., Buring, J. E., et al. (2008). Loci related to metabolic-syndrome pathways including LEPR,HNF1A, IL6R, and GCKR associate with plasma C-reactive protein: the Women's Genome Health Study. *Am. J. Hum. Genet.* 82, 1185–1192. doi: 10.1016/j.ajhg.2008.03.015

Rivas, M. A., Beaudoin, M., Gardet, A., Stevens, C., Sharma, Y., Zhang, C. K., et al. (2011). Deep resequencing of GWAS loci identifies independent rare variants associated with inflammatory bowel disease. *Nat. Genet.* 43, 1066–1073. doi: 10.1038/ng.952

Rosen, D. (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 364, 362–362. doi: 10.1038/364362c0

Rossner, S., Fuchsbrunner, K., Lange-Dohna, C., Hartlage-Rübsamen, M., Bigl, V., Betz, A., et al. (2004). Munc13-1-mediated vesicle priming contributes to secretory amyloid precursor protein processing. *J. Biol. Chem.* 279, 27841–27844. doi: 10.1074/jbc.C400122200

Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 511, 421–427. doi: 10.1038/nature13595

Seibert, T. M., Fan, C. C., Wang, Y., Zuber, V., Karunamuni, R., Parsons, J. K., et al. (2018). Polygenic hazard score to guide screening for aggressive prostate cancer: development and validation in large scale cohorts. *BMJ* 360:j5757. doi: 10.1136/bmj.j5757

Sekar, A., Bialas, A. R., de Rivera, H., Davis, A., Hammond, T. R., Kamitaki, N., et al. (2016). Schizophrenia risk from complex variation of complement component 4. *Nature* 530, 177–183. doi: 10.1038/nature16549

Shapiro, M. D., Tavori, H., and Fazio, S. (2018). PCSK9: from basic science discoveries to clinical trials. *Circ. Res.* 122, 1420–1438. doi: 10.1161/CIRCRESAHA.118.311227

Shatunov, A., Mok, K., Newhouse, S., Weale, M. E., Smith, B., Vance, C., et al. (2010). Chromosome 9p21 in sporadic amyotrophic lateral sclerosis in the UK and seven other countries: a genome-wide association study. *Lancet. Neurol.* 9, 986–994. doi: 10.1016/S1474-4422(10)70197-6

Shimizu, K., Kawabe, H., Minami, S., Honda, T., Takaishi, K., Shirataki, H., et al. (1996). SMAP, an Smg GDS-associating protein having arm repeats and phosphorylated by Src tyrosine kinase. *J. Biol. Chem.* 271, 27013–27017. doi: 10.1074/jbc.271.43.27013

Siddique, T., Pericak-Vance, M. A., Brooks, B. R., Roos, R. P., Hung, W. Y., Antel, J. P., et al. (1989). Linkage analysis in familial amyotrophic lateral sclerosis. *Neurology* 39, 919–925. doi: 10.1212/wnl.39.7.919

Singh, C., Glaab, E., and Linster, C. L. (2017). Molecular identification of d-ribulokinase in budding yeast and mammals. *J. Biol. Chem.* 292, 1005–1028. doi: 10.1074/jbc.M116.760744

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

Sun, L., Youn, H. D., Loh, C., Stolow, M., He, W., and Liu, J. O. (1998). Cabin 1, a negative regulator for calcineurin signaling in T lymphocytes. *Immunity* 8, 703–711. doi: 10.1016/s1074-7613(00)80575-0

Takemoto-Kimura, S., Terai, H., Takamoto, M., Ohmae, S., Kikumura, S., Segi, E., et al. (2003). Molecular cloning and characterization of CLICK-III/CaMKIgamma, a novel membrane-anchored neuronal Ca2+/calmodulin-dependent protein kinase (CaMK). *J. Biol. Chem.* 278, 18597–18605. doi: 10.1074/jbc.M300578200

Tam, V., Patel, N., Turcotte, M., Bossé, Y., Paré, G., and Meyre, D. (2019). Benefits and limitations of genome-wide association studies. *Nat. Rev. Genet.* 20, 467–484. doi: 10.1038/s41576-019-0127-1

Thanabalasingham, G., Shah, N., Vaxillaire, M., Hansen, T., Tuomi, T., Gašperíková, D., et al. (2011). A large multi-centre European study validates high-sensitivity C-reactive protein (hsCRP) as a clinical biomarker for the diagnosis of diabetes subtypes. *Diabetologia* 54, 2801–2810. doi: 10.1007/s00125-011-2261-y

Thiel, C., Kessler, K., Giessl, A., Dimmler, A., Shalev, S. A., der Haar, S., et al. (2011). NEK1 mutations cause short-rib polydactyly syndrome type majewski. *Am. J. Hum. Genet.* 88, 106–114. doi: 10.1016/j.ajhg.2010.12.004

van Eijk, R. P. A., Jones, A. R., Sproviero, W., Shatunov, A., Shaw, P. J., Leigh, P. N., et al. (2017). Meta-analysis of pharmacogenetic interactions in amyotrophic lateral sclerosis clinical trials. *Neurology* 89, 1915–1922. doi: 10.1212/WNL.0000000000004606

van Es, M. A., Van Vught, P. W., Blauw, H. M., Franke, L., Saris, C. G., Andersen, P. M., et al. (2007). *ITPR2* as a susceptibility gene in sporadic amyotrophic lateral sclerosis: a genome-wide association study. *Lancet Neurol.* 6, 869–877. doi: 10.1016/S1474-4422(07)70222-3

van Es, M. A., van Vught, P. W., Blauw, H. M., Franke, L., Saris, C. G., Van Den Bosch, L., et al. (2008). Genetic variation in *DPP6* is associated with

susceptibility to amyotrophic lateral sclerosis. *Nat. Genet.* 40, 29–31. doi: 10.1038/ng.2007.52

van Es, M. A., Van Vught, P. W. J., Veldink, J. H., Andersen, P. M., Birve, A., Lemmens, R., et al. (2009a). Analysis of *FGGY* as a risk factor for sporadic amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* 10, 441–447. doi: 10.3109/17482960802673042

van Es, M. A., Veldink, J. H., Saris, C. G. J., Blauw, H. M., van Vught, P. W. J., Birve, A., et al. (2009b). Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. *Nat. Genet.* 41, 1083–1087. doi: 10.1038/ng.442

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 Rheenen, W., Shatunov, A., Dekker, A. M., McLaughlin, R. L., Diekstra, F. P., Pulit, S. L., et al. (2016). Genome-wide association analyses identify new risk variants and the genetic architecture of amyotrophic lateral sclerosis. *Nat. Genet.* 48, 1043–1048. doi: 10.1038/ng.3622

Varoqueaux, F., Sons, M. S., Plomp, J. J., and Brose, N. (2005). Aberrant morphology and residual transmitter release at the Munc13-deficient mouse neuromuscular synapse. *Mol. Cell. Biol.* 25, 5973–5984. doi: 10.1128/MCB.25.14.5973-5984.2005

Visscher, P. M., Wray, N. R., Zhang, Q., Sklar, P., McCarthy, M. I., Brown, M. A., et al. (2017). 10 Years of GWAS discovery: biology, function, and translation. *Am. J. Hum. Genet.* 101, 5–22. doi: 10.1016/j.ajhg.2017.06.005

Wang, Z., Iida, A., Miyake, N., Nishiguchi, K. M., Fujita, K., Nakazawa, T., et al. (2016). Axial spondylometaphyseal dysplasia is caused by C21orf2 Mutations. *PLoS One* 11:e0150555. doi: 10.1371/journal.pone.0150555

Watson, A. P., Evans, R. L., and Egland, K. A. (2013). Multiple functions of sushi domain containing 2 (SUSD2) in breast tumorigenesis. *Mol. Cancer Res.* 11, 74–85. doi: 10.1158/1541-7786.MCR-12-0501-T

Wen, W., Zheng, W., Okada, Y., Takeuchi, F., Tabara, Y., Hwang, J.-Y., et al. (2014). Meta-analysis of genome-wide association studies in East Asian-ancestry populations identifies four new loci for body mass index. *Hum. Mol. Genet.* 23, 5492–5504. doi: 10.1093/hmg/ddu248

Whitacre, L. K., Hoff, J. L., Schnabel, R. D., Albarella, S., Ciotola, F., Peretti, V., et al. (2017). Elucidating the genetic basis of an oligogenic birth defect using whole genome sequence data in a non-model organism. *Bubalus bubalis. Sci. Rep.* 7:39719. doi: 10.1038/srep39719

Wray, N. R., Goddard, M. E., and Visscher, P. M. (2007). Prediction of individual genetic risk to disease from genome-wide association studies. *Genome Res.* 17, 1520–1528. doi: 10.1101/gr.6665407

Yamamoto, Y., Mizuno, R., Nishimura, T., Ogawa, Y., Yoshikawa, H., Fujimura, H., et al. (1994). Cloning and expression of myelin-associated oligodendrocytic basic protein. A novel basic protein constituting the central nervous system myelin. *J. Biol. Chem.* 269, 31725–31730.

Zhang, Y. D., Hurson, A. N., Zhang, H., Choudhury, P. P., Easton, D. F., Milne, R. L., et al. (2020). Assessment of polygenic architecture and risk prediction based on common variants across fourteen cancers. *Nat. Commun.* 11:3353. doi: 10.1038/s41467-020-16483-3

Zhao, W., Rasheed, A., Tikkanen, E., Lee, J.-J., Butterworth, A. S., Howson, J. M. M., et al. (2017). Identification of new susceptibility loci for type 2 diabetes and shared etiological pathways with coronary heart disease. *Nat. Genet.* 49, 1450–1457. doi: 10.1038/ng.3943

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REVIEW
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Moving Toward Patient-Tailored Treatment in ALS and FTD: The Potential of Genomic Assessment as a Tool for Biological Discovery and Trial Recruitment

Iris J. Broce[1,2], Patricia A. Castruita[1] and Jennifer S. Yokoyama[1,3]**

[1] Memory and Aging Center, Department of Neurology, Weill Institute for Neurosciences, University of California, San Francisco, San Francisco, CA, United States, [2] Department of Family Medicine and Public Health, University of California, San Diego, San Diego, CA, United States, [3] Department of Radiology and Biomedical Imaging, University of California, San Francisco, San Francisco, CA, United States

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***Correspondence:**
Iris J. Broce
iris.broce@ucsf.edu
Jennifer S. Yokoyama
jennifer.yokoyama@ucsf.edu

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are two devastating and intertwined neurodegenerative diseases. Historically, ALS and FTD were considered distinct disorders given differences in presenting clinical symptoms, disease duration, and predicted risk of developing each disease. However, research over recent years has highlighted the considerable clinical, pathological, and genetic overlap of ALS and FTD, and these two syndromes are now thought to represent different manifestations of the same neuropathological disease spectrum. In this review, we discuss the need to shift our focus from studying ALS and FTD in isolation to identifying the biological mechanisms that drive these diseases—both common and distinct—to improve treatment discovery and therapeutic development success. We also emphasize the importance of genomic data to facilitate a "precision medicine" approach for treating ALS and FTD.

Keywords: clinical trials, genomics, precision medicine, amyotrophic lateral sclerosis, frontotemporal dementia

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are two devastating and intertwined neurodegenerative diseases. ALS is a progressive and fatal motor neuron disease (MND), leading to muscle atrophy, paralysis, and eventual death from respiratory failure within 3–5 years from symptom onset (Rowland and Shneider, 2001). FTD is characterized by changes in social behavior and/or language abilities at disease onset due to neurodegeneration of the frontal and temporal lobes, leading to death within 3–12 years from symptom onset (Mitchell et al., 2015; Kansal et al., 2016; Ahmed et al., 2020). Historically, ALS and FTD were considered distinct disorders given differences in presenting clinical symptoms, disease duration, and predicted risk of developing each disease. However, research over recent years has highlighted the considerable clinical, imaging, pathological, and genetic overlap of ALS and FTD, and these two syndromes are now thought to represent different manifestations of the same neuropathological disease spectrum.

Amyotrophic lateral sclerosis and FTD represent opposite ends of the same disease continuum, defined by underlying TDP-43 neuropathology (Geser et al., 2010). Indeed, the terms ALS and FTD remain useful for clinical diagnostic and prognostic purposes. Patients clinically diagnosed with pure forms of FTD have a prolonged survival compared to patients diagnosed with pure ALS and patients diagnosed with combined ALS-FTD (Hodges et al., 2003; Govaarts et al., 2016; Kansal et al., 2016; Xu et al., 2017). We speculate that distinct molecular etiologies—driven in part through genetic differences—result in distinct clinical manifestations of disease from a common neuropathological entity. Although there are currently no disease-modifying drugs that halt or reverse the progression of either ALS or FTD, leveraging both the unique and shared genetic contributions to ALS and FTD may facilitate therapeutic discovery. In this review, we discuss the need to shift our focus from studying ALS and FTD in isolation to identifying the biological mechanisms that drive these diseases—both common and distinct—to improve treatment discovery and therapeutic development success. We also emphasize the importance of genomic data to facilitate a "precision medicine" approach for treating ALS and FTD.

CLINICAL PHENOTYPES OF FTD AND ALS

Frontotemporal dementia is an umbrella term and represents one of the most common forms of dementia diagnosed in people younger than 65 years old. The global prevalence and incidence of FTD is uncertain, with estimates among people in the United States aged 45–64 between 15–22 per 100,000 and 2.7–4.3 per 100,000 person-years, respectively (Onyike and Diehl-Schmid, 2013; Knopman and Roberts, 2011; Logroscino et al., 2019; Turcano et al., 2020). Incidence of FTD increases with age (Rosso et al., 2003; Logroscino et al., 2019) and is roughly equal between males compared to females, though studies report greater risk in males (Onyike and Diehl-Schmid, 2013; Turcano et al., 2020). Depending on the signs and symptoms, FTD patients are classified into one of three different clinical syndromes: behavioral variant FTD (bvFTD) or one of two forms of primary progressive aphasias (PPA), including non-fluent variant PPA (nfvPPA) and semantic variant PPA (svPPA). People with FTD may also experience movement symptoms, such as bradykinesia, dystonia, rigidity, and apraxia, with 12.5% of patients with bvFTD meeting clinical criteria for either corticobasal syndrome (CBS) or progressive supranuclear palsy syndrome (PSPS) (Ljubenkov and Miller, 2016). About 15% of people with bvFTD, 11% of patients with nfvPPA, and 19% of patients with svPPA may also eventually develop motor symptoms consistent with ALS (Rascovsky et al., 2011; Vinceti et al., 2019).

Amyotrophic lateral sclerosis is the most common form of adult-onset MND. It is classically characterized by progressive degeneration of *both* upper motor neurons (UMN) of the motor cortex and lower motor neurons (LMN) of the brainstem and spinal cord at disease onset. Although motor neuron damage is predominating in ALS, other neurons in the fronto-executive circuits, temporal and parietal cortical regions, basal ganglia, and dorsal root ganglia are also involved in some patients (Bede et al., 2013; Westeneng et al., 2016; Cykowski et al., 2017; Omer et al., 2017; Nakamura-Shindo et al., 2020; Riancho et al., 2020). ALS is considered a rare "orphan" disease. The global prevalence and incidence of ALS is 4.42 (95% CI 3.92–4.96) per 100,000 and 1.59 (95% CI 1.39–1.81) per 100,000 person-years, respectively (Xu et al., 2020). ALS prevalence and incidence increases with age until about the age of 70–79 and is higher in males compared to females (Xu et al., 2020). Because ALS shows extensive phenotypic heterogeneity in disease presentation, redefining ALS as a group of clinical syndromes is gaining favor (Kim et al., 2009; de Vries et al., 2019; Shefner et al., 2020). For example, according to the current consensus, primary lateral sclerosis (PLS), which results from UMN loss exclusively, and progressive muscular atrophy (PMA), which results from LMN loss exclusively, are distinct clinical phenotypes of MND relying on a continuum with ALS. This poses a delicate diagnostic problem since some patients initially diagnosed with PLS or PMA eventually develop LMN and UMN loss, respectively, and meet clinical criteria for ALS later on (Gordon et al., 2006; Kim et al., 2009). Several factors, including survival, distinguish PLS or PMA from ALS, with shorter survival in ALS patients (Turner et al., 2020). Further, about 50% of ALS patients develop cognitive and behavioral impairment, with 13% meeting diagnostic criteria for bvFTD (Ljubenkov and Miller, 2016). Recently, one study showed that cognitive and behavioral impairment, including FTD, are also common in PLS and PMA (de Vries et al., 2019). Together, these findings provide valuable insight into ALS's clinical heterogeneity and overlapping clinical features between MNDs and FTD.

The FTD and ALS clinical syndromes described above represent the manifestations of underlying neuropathology that results in the dysfunction and death of neurons in specific neuroanatomical regions. That is: individuals with bvFTD manifest with dysexecutive and behavioral symptoms because neurons within specific regions of the brain underlying executive function and social behavior (e.g., anterior cingulate, frontoinsular, striatum, and amygdala regions) are impacted (Perry et al., 2017); individuals with svPPA manifest semantic loss because neurons within anterior temporal lobe related to semantic knowledge are impacted (Gorno-Tempini et al., 2011). Neuroimaging studies additionally support the notion of ALS and FTD as a continuum showing that motor cortex and anterior cingulate as well as their underlying white matter tracts are impacted in ALS patients, while widespread frontal, anterior cingulate, insular, and temporal lobes are impacted in ALS-FTD and bvFTD patients (Lillo et al., 2012; Crespi et al., 2018; Trojsi et al., 2018; Seeley, 2019; Vinceti et al., 2019). Furthermore, while most forms of ALS are due to pathological inclusions of TDP-43 (with some exceptions, noted in the next section), about half of all bvFTD (Neumann et al., 2006; Perry et al., 2017), most svPPA (Grossman, 2010; Josephs et al., 2011; Borghesani et al., 2020), and a portion of nfvPPA cases are due to TDP-43 (Adams-Carr et al., 2020). The other half of bvFTD, a small minority to few svPPA, over half of

nfvPPA, CBS, and PSPS result from underlying tau pathology (Josephs et al., 2011).

SHARED GENETIC RISK HIGHLIGHTS MOLECULAR OVERLAP OF FTD AND ALS

Since the clinical phenotypes of FTD and ALS can be heterogeneous, it may seem that these syndromes have different underlying biological mechanisms. However, clinical, genetic, and neuropathological overlap between these diseases is well-established (Lomen-Hoerth et al., 2002; DeJesus-Hernandez et al., 2011; Renton et al., 2011; Weishaupt et al., 2016; Abramzon et al., 2020). About 30 disease-causing mutations have been repeatedly associated with ALS (for a review see, Abramzon et al., 2020). Fewer genes have been associated with FTD. The most common disease-causing mutations occur in chromosome 9 open reading frame 72 (*C9ORF72*) and progranulin (*GRN*), which both result in TDP-43 neuropathology, and microtubule-associated protein tau (*MAPT*), resulting in tau neuropathology (Olney et al., 2017). The most common causes of either ALS, FTD, or combined ALS and FTD (ALS-FTD), even within the same families, are pathogenic hexanucleotide repeat expansions in *C9ORF72*. Nearly 25–40% of all familial ALS and FTD cases carry this mutation and 5–7% of sporadic cases—cases without an established family history of neurodegenerative disease—also screen positive for *C9ORF72* pathogenic expansions (DeJesus-Hernandez et al., 2011; Renton et al., 2011). These estimates are based on European ancestry; *C9ORF72* mutations are relatively rare in people of Asian ancestry, and other ancestral populations remain understudied (Majounie et al., 2012). Beyond *C9ORF72*, mutations in several other genes have been associated with both ALS and FTD, including *TARDBP, SQSTM1, VCP, FUS, TBK1, CHCHD10,* and *UBQLN2* (Abramzon et al., 2020). The pathological hallmark of ALS and FTD patients harboring these particular mutations, with the exception of *FUS*, is the presence of ubiquitinated protein deposits primarily composed of TDP-43. How mutations in the same genes cause different clinical syndromes despite similar neuropathology remains unknown and may relate to differences in mutation localization on downstream processes or modifying genetic and/or environmental factors. Nevertheless, shared genetic contributions to FTD and ALS suggest that there are, at least in part, common molecular mechanisms driving disease pathology. Novel disease-modifying treatments are underway for ALS and FTD patients that target specific molecular subtypes. Since half of FTD patients show TDP-43 proteinopathy and the other half shows tau proteinopathy, it will be increasingly important to use genetic information to accurately predict the underlying pathology.

Characterizing genotype-phenotype interactions in both ALS and FTD have diagnostic and prognostic value and can help in selecting patients for clinical trials. A known genotype/phenotype association provides information about how certain genes or genetic variants result in specific features of ALS or FTD, including symptoms at onset, severity of motor/cognitive impairment, rate of disease progression, and survival. These genotype-phenotype interactions for ALS and FTD have been recently reviewed in prior reports (Van Mossevelde et al., 2018; Connolly et al., 2020). ALS patients with specific *TARDBP* mutations show differences in survival; patients with G298S mutations have shorter survival (27 months) compared to patients with A315T and M337V mutations (100 months) (Regal et al., 2006). Patients with A4V *SOD1* mutations have shorter survival (less than 12 months) compared to patients with other mutations, such as D90A or G93C (5–10 years) (Cudkowicz et al., 1997; Regal et al., 2006; Corcia et al., 2012). Patients with *TARDBP* and *SOD1* mutations have earlier onset (53.4, 50.1 years, respectively) compared to sporadic cases (61.9 years) (Corcia et al., 2012). For patients with mutations in *FUS*, age of onset depends on the location of the mutation and type of mutations (missense, nonsense, and deletion) (Waibel et al., 2013). Different mutations have been associated with different symptoms at onset – higher proportion of patients with mutations in *SOD1*, *hnRNAP1*, and *TUBA4A* manifest with limb onset (>80%), compared to patients with mutations in other genes: *VCP* (50%), *NEK1* (50%), and *TBK1* (50%), *C9orf72* (33%), *UBQLN2* (40%) (Connolly et al., 2020). Also, the prevalence of cognitive impairment and FTD in ALS varies in cases with different mutations: *C9orf72*, *SQSTM1*, *TBK1, TARDBP* (36, 67, 43, and 12%, respectively) (Connolly et al., 2020). Furthermore, people with mutations in different genes show differing prevalence of FTD subtypes (Van Mossevelde et al., 2018). FTD patients who carry mutations in *C9ORF72, GRN, TBK1,* and *VCP* are all associated with TDP-43 pathology. However, patients with *C9ORF72* repeat expansions with bvFTD often present with psychotic symptoms, whereas patients with FTD who carry a *GRN* mutation often present with apathetic behavior and language impairment. Patients with FTD who carry *TBK1* mutations often present with MND and language and behavioral impairment but no psychotic symptoms, whereas patients with FTD who have a *VCP* mutation may present with or without myopathy or Paget disease of the bone and show apathy, anomia, and/or psychotic symptoms. Knowing that patients with FTD carrying mutations in *C9ORF72* or *VCP* are likely to show psychotic symptoms is valuable because clinicians can anticipate complications of these symptoms, with the potential of managing the condition more effectively. In sum, known genotype-phenotype relationships provide information about how genes may work in causing certain features (e.g., age of onset, survival, and clinical features such as concomitant FTD and psychosis or Paget disease). This information helps clinicians in estimating prognosis, provides insight into molecular mechanisms of motor neuron cell death, and can also provide a framework to test new therapies.

In familial forms of ALS and FTD, the underlying neuropathology is predictable. However, in sporadic disease, there is more variability, particularly in FTD. Given that sporadic ALS and FTD may have distinct molecular etiology of disease and future therapeutics will likely target a specific protein or biological pathway (e.g., inflammation), it will be critical to have tools in place that can effectively distinguish between these *in vivo*. PET ligand-based imaging has begun to try to address

this question through tau imaging in FTD (Dani et al., 2016; Tsai et al., 2019) and translocator protein (TSPO) PET imaging, a neuroinflammation biomarker, in ALS (Van Weehaeghe et al., 2020). However, numerous technical challenges remain to be addressed. In addition to lack of specificity, PET imaging represents an expensive procedure dependent upon specific infrastructure to create the radioligand. Additionally, TSPO PET studies require genotyping of the rs6971 polymorphism to determine TSPO binding affinity since approximately 5–10% of individuals may have a lower specific signal (Owen et al., 2012; Van Weehaeghe et al., 2020). For this reason, molecular-based biomarkers such as genetic profiles represent an appealing means of identifying underlying neurodegenerative disease pathology in a relatively inexpensive, non-invasive way. Indeed, individuals with genetic forms of FTD and their unaffected family members are currently being recruited for longitudinal study, setting the stage for clinical trials in large families with predictable disease trajectories (Tsai and Boxer, 2016; Rosen et al., 2020). Although current clinical trials in ALS only recruit symptomatic cases despite evidence that ALS has a long presymptomatic phase (Eisen et al., 2014), clinical trials aimed at better understanding the clinical and biological changes that occur in individuals with genetic forms of ALS and are asymptomatic are underway[1]. Taken together, there is a critical need for identifying novel molecular mechanisms driving sporadic ALS and FTD pathogenesis and a critical need for developing novel mechanism-based biomarkers that can assist in patient selection for molecular-based clinical trials. Also, studies of familial disease provide an opportunity to test early-stage or even presymptomatic disease given the established underlying disease etiology.

TECHNOLOGICAL ADVANCEMENT DRIVES GENETIC DISCOVERY

To facilitate identification of new treatment approaches and preventative measures, FTD and ALS genetics research seeks to elucidate the underlying mechanisms of disease; these biological discoveries can then be probed as potential targets for disease modification. The human genome contains about 3 billion base pairs of DNA, which reside within the nucleus of every cell of the body. Perhaps unsurprisingly, the genomes of any two unrelated individuals are more similar than different since genomes define our species. At the same time, no two individuals–even identical twins–are genetically identical (Vadgama et al., 2019; Homfray, 2020). Strong efforts from various international groups, including 1000 Genomes Project and the Genome Aggregation Database (gnomAD), have curated comprehensive catalogs of human genetic variation by genotyping and sequencing hundreds of thousands of individuals from diverse populations (Genomes Project et al., 2015; Koch, 2020). These large databases serve as a global reference for human genetic variation. A genetic variant is a term used to refer to differences in a DNA sequence (e.g., C and T) at a specific location between two genomes (e.g., patient genome compared to human reference genome). The genome of any two unrelated individuals shows differences in roughly 0.5% of their genome, which equals approximately 15 million genetic variants (Jackson et al., 2018). In this context, researchers aim to identify genetic variants that are enriched in patients with ALS and FTD versus unaffected controls.

Next-generation sequencing (NGS) technologies and high-throughput genotyping platforms have revolutionized the ability to detect genetic variation in humans. Genotyping-based approaches use microarray technology, which is well-established, highly accurate, and less expensive than NGS. Clinicians are very familiar with these tests, helping maintain their place as a critical diagnostic tool. However, microarray-based approaches are limited in number of genetic variants they can probe and are only able to survey "known" variants by nature of their design. Thus, microarray technology is better suited for profiling well-established single nucleotide polymorphisms (SNPs) or established disease-causing genetic variants but not suitable for discovering novel or extremely rare genetic variants that have not been observed in previously generated datasets.

Next-generation sequencing refers to high-throughput technology that determines the sequence of nucleotides across an entire surveyed region, including genome (whole-genome sequencing; WGS), exons within all known genes (whole-exome sequencing; WES), or pre-selected coding or non-coding regions that target only a portion of the genome (target panel). Unlike genotyping, there is no requirement for *a priori* knowledge of the genetic variants of interest. We can identify known genetic variants and novel genetic variants that may be unique to each patient. NGS technology also enables us to capture multiple types of genetic variants, including SNVs, indels, structural variants, common and rare variants in the population: common variants [minor allele frequency (MAF) $\geq$ 5%], low-frequency variants (0.5% $\leq$ MAF $<$ 5%), rare variants (MAF $<$ 0.5%), and *de novo* mutations. While WGS in particular seems promising in providing an all-in-one solution for detecting multiple types of variants, there are several drawbacks. It is expensive relative to other sequencing and microarray technologies on a per-sample basis, though given its flexibility in detecting multiple variants, the per-patient cost is cheaper (van Nimwegen et al., 2016; Schwarze et al., 2020). WGS also requires robust bioinformatics capabilities for variant annotation, classification, interpretation and enormous amounts of space and server capabilities for processing, storing, and backing up the data in a reasonable timeframe. Finally, variant detection relies on DNA quality, sequencing coverage, fidelity of the reference assembly, and the number and population background of samples being assessed. Given these technical complexities and constraints in infrastructure and resources, WGS has been slower to enter the clinical space. Identifying ways to overcome these challenges will be critical to translating these genomic advances into patient care.

GENOMIC CONTRIBUTIONS TO FAMILIAL AND SPORADIC ALS AND FTD

An important question is, how much does genetics matter for predicting ALS and FTD risk in non-familial forms of disease?

[1]https://www.neals.org/als-trials/1330

The term heritability is a statistical concept used to describe the proportion of phenotypic variation that can be explained by genetic variation. Heritability estimates are important and widely used for determining how well we can predict a trait from genetics. Heritability estimates range from 0 to 1, with a score of 1 indicating that genetics explains all the variance in the trait and a score of zero indicating that it explains none. The heritability estimates of ALS based on pedigree and twin-studies is moderate to high, ranging between 0.40–0.60 and 0.38–0.78, respectively (Al-Chalabi et al., 2010). The heritability estimates of ALS based on genome-wide genotyping SNP data is 0.12–0.21 which is low-to-moderate (Fogh et al., 2014; Keller et al., 2014; McLaughlin et al., 2015). Interestingly, all the genetic signal from the largest sporadic ALS GWAS to date, which is based on 36,052 people (12,577 cases and 23,475 controls), comes from chromosome 9 (van Rheenen et al., 2016; Holland et al., 2021). Therefore, ALS GWAS, based on SNP data, may not capture rare variants or low-frequency variants pertinent to ALS. Additionally, since the current ALS GWAS is underpowered, the extent to which increases in sample size can help identify novel SNPs in other regions remains an important question. Collectively, these findings suggest that genetic factors do play an important role in predicting ALS risk. Increases in sample sizes may help explain additional phenotypic variance from SNP data beyond the signal from *C9ORF72*. The genetic contribution to ALS may be underestimated based on the way heritability is calculated (e.g., using pedigree information, twin data, or case-control data and with genome-wide SNP data, WES, or WGS) and the variant information generated through different genotyping/sequencing technologies (e.g., low-frequency, rare variants, structural variants, or *de novo* variants). Taken together, these studies provide justification for continued efforts toward data collection in additional ALS patients to enhance the field's ability to identify novel genetic variants influencing disease risk. Indeed, WGS data collection efforts in ALS patients would enable capturing the full spectrum of genetic variation—including rare variants—associated with disease risk and may provide novel insights into additional genetic contributions to ALS.

The heritability estimates in FTD based on pedigree data range between 0.26 and 0.31 (Rohrer et al., 2009; Wood et al., 2013; Greaves and Rohrer, 2019). These estimates vary across the different clinical subtypes, with bvFTD showing highest heritability at 0.58, compared to svPPA at 0.22, nfvPPA 0.30, and 0.10–0.40 in FTD-ALS (Goldman et al., 2005; Rohrer et al., 2009). Genetic variants captured by exome array data (predominantly low frequency) explains 53% of the total phenotypic variance of sporadic FTD (excluding FTD-MND patients) (Chen et al., 2015). However, whether common variants explain a significant portion of phenotypic variance in FTD is also difficult to say. The small sample size and wide range of clinical and neuropathological variability pose important challenges in estimating FTD heritability. Despite these limitations, as shown in the original FTD GWAS paper, the per-variant effect size (odds ratio $\approx$ 1.3) found in the major histocompatibility complex (MHC) area on chromosome 6 is quite strong (Ferrari et al., 2014). This indicates the need for complex haplotype analysis, which could be addressed through access to sequencing data. WGS and WES in sporadic disease has only begun to emerge and several mutations and CNVs have been identified in 11% of sporadic cases (Blauwendraat et al., 2018). Taken together, the genetic architecture of FTD is highly complex. Low-frequency, rare variants and CNVs may likely explain more genetic variation in sporadic FTD compared to common variants. Further, better understanding the complex haplotype driving risk associations in the MHC region may improve both prediction and inference of the genetic contribution to FTD risk.

GENETIC INFLUENCES ON DISEASE BEYOND RISK

In addition to overall disease risk, genetic information can inform our understanding of how individual differences in people's genes affects their response to drugs (pharmacogenetics). Pharmacogenetics is important for improving targeted treatment and minimizing adverse drug reactions. For example, recent ALS research has shown that genetic variants can modify the effects of drugs on patient outcomes. Lithium carbonate, which is proposed to boost autophagy and remove misfolded proteins that accumulate in motor neurons during the course of disease, was found not to benefit people with ALS in a past clinical trial. However, a recent *post hoc* meta-analysis across three clinical trials found that the effect of lithium was dependent on patient genotype (van Eijk et al., 2017). Individuals with a particular genotype at a SNP in *UNC13A* (C/C genotype; rs12608932) showed better 12-month survival compared to non-carriers and the control group. Similarly, in separate *post hoc* analysis of two other randomized clinical trials, the same authors evaluated whether the effect of creatine monohydrate and valproic acid also depended on the genotype of patients. They found a dose-dependent pharmacogenetic interaction between creatine and the A allele of a SNP (i.e., rs616147) in *MOBP*, suggesting a qualitative interaction in a recessive model (van Eijk et al., 2020). These studies highlight the importance of incorporating genetic information into clinical trials to identify patient subgroups that are most likely to benefit from a particular treatment, either through direct or indirect effects of genetic variation on drug metabolism, disease biology and/or downstream processes influenced by the intervention. Results from conducting pharmacogenetic *post hoc* analyses may serve as a powerful method for refining the inclusion criteria for subsequent trials and minimizing the sample size required to detect a therapeutic effect.

We may also be able to improve treatment discovery and success rates by leveraging the observation that ALS and FTD—particularly in their familial forms—represent a disease spectrum. Rather than studying diseases in isolation or restricting inclusion criteria to patients with a single clinical syndrome, it may be more useful to modify inclusion criteria for clinical trials and recruit patients based on a common genetic etiology and/or molecular phenotype of disease, regardless of showing different clinical syndromes (e.g., PLS, ALS, and FTD).

Basket design clinical trials, which have been widely used in oncology research, are a newer trial design that offers this type of flexibility. Basket trials allow investigation of therapeutic efficacy of a candidate therapeutic simultaneously in multiple clinical syndromes that result from the same genetic or molecular aberration (**Figure 1**). In this framework, patients can be assessed for treatment response using individualized endpoint measures that are most relevant to their diagnosis. This method was recently attempted in neurodegenerative disease. For example, Tsai et al. (2020) assessed the safety, tolerability, and pharmacodynamics of the microtubule stabilizer TPI-287 in Alzheimer's disease (AD) and in the 4-repeat tauopathies (4RT) PSPS and CBS. They found that patients with AD tolerated TPI-287 less than those with 4RT as a result of the presence of anaphylactoid reactions. Similar to this tau-based study, basket trial approaches could be applied to ALS and FTD by grouping patients with similar genetic forms of disease. If we can use genetic information to identify specific molecular profiles unique to each patient's particular form of disease, we may be able to leverage genetic information to drive a "precision medicine" strategy for disease treatment, even at the clinical trial phase.

Similarly, genetic information may be useful in identifying the most relevant therapeutic target(s) based on each patient's specific etiology or molecular profile of disease. Several new potential drugs are currently being tested in Phase 1 to Phase 3 clinical trials for ALS and FTD: therapeutic approaches to neuroinflammation (e.g., masitinib and *Cannabis sativa*), autophagy and protein quality control (e.g., arimoclomol and NCT03491462), apoptosis (e.g., tauroursodeoxycholic acid; TUDCA), mitochondria and endoplasmic reticulum function (e.g., AMX0035), among others. The various genes associated with ALS and FTD encode proteins associated with different classes of cellular processes (Yousefian-Jazi et al., 2020). For example, *GRN, TREM2,* and *TYROBP* are implicated in immune response and neuroinflammation (Jay et al., 2017; Bright et al., 2019; Rostalski et al., 2019); *SOD1, CHCHD10,* and *C19ORF12* are implicated in mitochondrial and oxidative stress (Deschauer et al., 2012; Tan et al., 2014; Anderson et al., 2019); and *EWSR1, FUS, SETX, TAF15,* and *TARDBP* are implicated in DNA damage and repair (Higelin et al., 2016; Hill et al., 2016). Rather than randomly assigning patients to the placebo and treatment group in a drug-mediated clinical trial, it may prove useful to instead profile patients for deleterious variants in genes associated with one of these common disease-associated biological pathways. Then, enroll patients into basket trials for treatment using a therapeutic candidate most relevant for the given pathway implicated by underlying genetic risk (i.e., target neuroinflammation in individuals with risk profiles consistent with immune dysregulation) (**Figure 1**).

Taken together, collecting genetic information in ALS and FTD patients is important for investigating the pharmacogenetic interactions in clinical trials, improving recruitment accuracy for clinical trials, and may accelerate drug discovery.

LEVERAGING BIOINFORMATICS INNOVATION FOR GENE DISCOVERY IN ALS AND FTD

There are several analytic techniques that exist for gene discovery and that provide insights into the disease mechanisms of ALS and FTD. Many research groups have exploited pleiotropic methods for gene discovery. Genetic pleiotropy refers to the phenomenon of one gene influencing two or more phenotypes. Beyond *C9ORF72*, studies have identified CAG repeat expansion in the ataxin-2 gene (*ATXN2*) as a genetic cause of spinocerebellar ataxia type 2 (SCA2) and ALS (Elden et al., 2010; Lee et al., 2011;

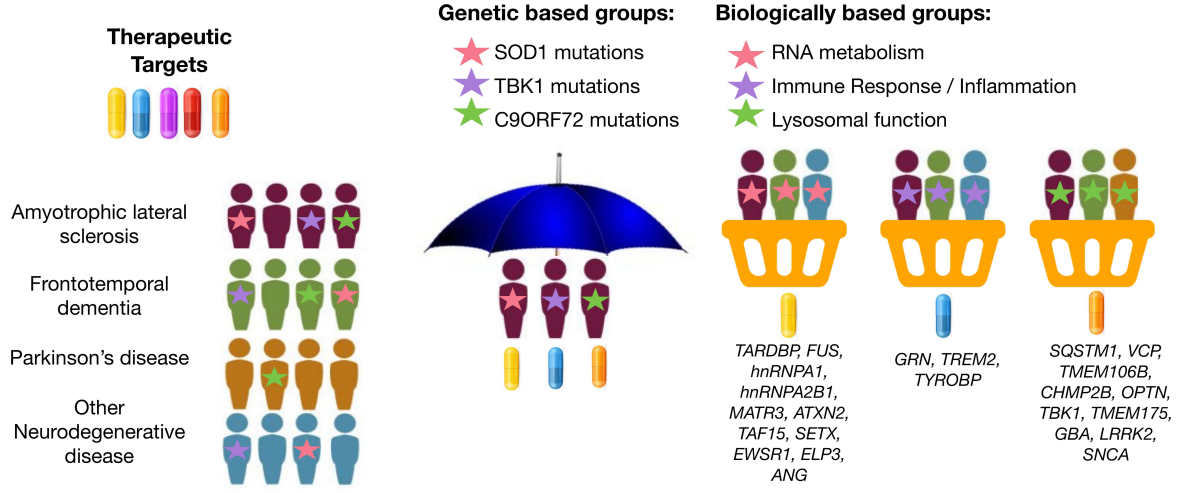

FIGURE 1 | Graphical representation of umbrella and basket trials. Trials have shifted from enrolling patients from a single clinical syndrome and genetic mutation for a selective therapeutic target (umbrella trial) to enrolling patients across different clinical syndromes for treatment using a therapeutic target most relevant for a given molecular or biological pathway implicated by underlying genetic risk (basket trial). Genes were mapped to biologically based groups based on two recent review papers (Wallings et al., 2019; Liscic et al., 2020).

Li et al., 2016; Sproviero et al., 2017). Independent GWAS have identified pleiotropy between FTD and other diseases, such as AD (Ferrari et al., 2017). Using novel pleiotropic methods based on the Bayesian conditional FDR (cFDR) approach (Andreassen et al., 2013; Broce et al., 2019; Frei et al., 2019), we have discovered pleiotropic loci between FTD and ALS beyond *C9ORF72*, as well as pleiotropy between FTD and, CBD and PSP (Yokoyama et al., 2017), and between FTD and other less clearly related traits, such as immune-mediated diseases (Broce et al., 2018). The discovery of pleiotropic genes and biological pathways shared across diseases may be leveraged for repurposing drugs already approved for disorders beyond ALS and FTD and may facilitate basket trials that recruit patients with different diseases that may respond to similar treatments.

In addition to novel analytical methods, machine-learning based approaches are also being leveraged for gene discovery (Grollemund et al., 2019). Recently, one study used phenotypic and biological information from publicly available databases and applied a knowledge graph edge prediction algorithm to predict novel ALS-associated genes (Grollemund et al., 2019; Bean et al., 2020). Across various approaches, they identified over 500 predicted genes linked to ALS. The predicted new candidate genes were linked to a number of biological processes previously associated with ALS, including angiogenesis, lipid metabolism, mitochondria activity, protein kinase activity, superoxide metabolism, vesicle-trafficking, and neurotransmitter regulation. These candidate genes now require validation in large, independent cohorts that include rare and common genetics variants; these data are currently lacking. Thus, as more and more individuals are sequenced, larger patient datasets will be generated that can facilitate validation of these findings and discovery of additional genes, which will both be critical next steps required before this information can be applied to a broader spectrum of patients and used to identify at-risk individuals.

It is important to note that the general consensus is that ALS and FTD have a complex genetic architecture. While a single gene might be associated with increased risk for disease in one patient (monogenic), a few genes might be associated with increased risk in another patient (oligogenic), and many genes may be associated in a third patient (polygenic). Future studies may benefit from implementing polygenic risk scoring approaches incorporating newly identified risk loci. These scores create single, continuous measures composed of the sum of small to moderate contributions from tens to thousands of single genetic variants. In the future, polygenic risk scores could be used to create a personalized genetic diagnostic tool that stratifies people into clear trajectories for disease risk and outcome, and, ultimately, could even inform therapeutic decision-making.

PERSONALIZED GENOMICS TO FACILITATE CLINICAL CARE

Global collaboration between medical centers, academic research institutions, non-profit, and for-profit organizations is necessary to dissect the genetic heterogeneity in ALS and FTD and accelerate discovery of treatments for all patients. No universal database that stores genetic information and other biomarkers for all ALS and FTD patients currently exists. Having a uniform database or registry of both ALS and FTD patients would enable identification of all patients with specific genetic mutations and facilitate analyses that leverage the genetic overlap between diseases. This, in turn, would accelerate recruitment for gene-focused clinical trials and research to identify novel disease-modifying therapies. Currently, different organizations (e.g., AnswerALS[2], ProjectMinE[3], TargetALS[4], ALLFTD[5], dbGAP[6], and GENFI[7]) are leading separate efforts in ALS or FTD. While select groups have plans to "unite" data across studies, open collaboration across geographic regions and granting bodies requires orchestration across a number of challenging fronts. Issues ranging from data normalization and warehousing infrastructure to regulatory agencies and federal guidelines create enormous barriers that will need to be overcome to accelerate global genomic efforts. Also, ALS and FTD are rare diseases, and the percent of people with particular genetic mutations is even rarer. For example, based on three national United States databases (2010–2011), the Center for Disease Control and Prevention reports that about 12,000–15,000 people currently had ALS[8]. Of these, roughly 1,200–1,500 people would have familial form of ALS (10% of all ALS cases) and 10,800–13,500 would have sporadic form of ALS (90% of all ALS cases). Of these, about 120–150 people (10% of all familial ALS cases) would have *SOD1* mutations and about 216–540 people (2–4% of all sporadic ALS cases) would also be expected to harbor *SOD1* mutations. Therefore, approximately 400–700 ALS patients in the United States would have *SOD1* mutations. These approximations are likely underestimated since records on ALS have not been systematically collected throughout the country. In light of this example, it is not surprising that it takes years to recruit the required number of patients for clinical trials. Furthermore, FTD patients are most often diagnosed in memory clinics, while ALS patients are seen in movement disorder clinics; despite the biological overlap and frequent comorbidity of these disorders, both patient groups would benefit from the expertise of these two siloed specialist fields. A unified database would facilitate recruitment for gene-focused clinical trials across both disorders.

The current guidelines for genetic testing in patients with ALS, FTD, or ALS-FTD are conservative. A multi-step process for genetic testing in ALS, FTD, or ALS-FTD patients has been recently proposed (Roggenbuck and Fong, 2020). All patients with ALS or FTD are recommended genetic screening for the *C9ORF72* repeat expansion. However, only patients with a family history of either condition or early onset of

[2]https://www.answerals.org
[3]https://www.projectmine.com
[4]https://www.targetals.org
[5]https://www.allftd.org
[6]https://www.ncbi.nlm.nih.gov/gap/
[7]https://www.genfi.org
[8]https://www.cdc.gov/als/

symptoms are recommended for comprehensive multigene panel testing as a second step. Most ALS/FTD panels include roughly 30 genes. A family history of dementia or motor-neuron disease is not always present for particular mutations (e.g., *MAPT* mutations). Incomplete penetrance of genetic variants that do not always confer disease or have variable age of onset, inaccurate or incomplete family history information (e.g., adoption), misdiagnosis, early death, and other factors also reduce accuracy of records of family history (Roggenbuck and Fong, 2020). Although a family history is useful for genetic counseling, there is no clear biological distinction between familial and sporadic forms of ALS or FTD. Therefore, such stipulations limit a patient's ability to get genetic testing, which in turn limits their access to cutting-edge treatments. Further, while certain mutations (e.g., *C9ORF72*) may be more common in one population (European vs. Asian), ancestry should not limit a patient's ability to access genetic testing. Patients of diverse populations should be able to access information from genetic testing so they, too, can enroll into drug-target clinical trials, which do not exclude patients on the basis of ancestry (e.g., SOD1 AMX035; NCT03127514). Indeed, safety, tolerability, and pharmacodynamics of the therapeutic targets should be assessed in diverse populations during Phase 1 clinical trials. In sum, there are several limitations in the way that the current genetic testing guidelines are designed and recommendations for genetic testing are being made, and these may result in limited access to treatment trials, particularly for individuals from diverse backgrounds.

In this context, it would benefit all patients to have equitable access to WGS and accompanying genetic counseling such that patients can be provided access to relevant genetic information as it becomes clinically actionable. Since no cure for ALS or FTD currently exists, genetic testing for these diseases has been of minimal priority for insurance companies. However, as more genes are discovered that perspective is changing. Some research institutions, such as University of California, San Francisco are now offering genome sequencing paid by research to all of their patients to spearhead their precision medicine health program[9]. Further, we may improve patients' care as well as gene discovery if patients who consent to WGS/WES were permitted to consent to enrolling in a data registry for the purpose of collecting evidence of a genotype–phenotype relationship (Holtzman, 2013). Strong efforts are being made to revise the ethical guidelines of reporting genetic findings to ALS/FTD patients (Leighton et al., 2019; Shoesmith et al., 2020). Close collaboration between physicians, large ALS centers, non-profit and for-profit institutions, ethicists, genetic counselors, and data scientists will be critical for carrying out these tasks that, while complicated, also have the potential to facilitate a tailored approach to clinical care and treatment in ALS and FTD.

[9] https://www.3dhealthstudy.org

CONCLUDING REMARKS

Taken together, ALS and FTD are now thought to represent different manifestations of the same disease spectrum. The field is starting to move toward basket design clinical trials that allow investigation of drug-mediated treatments simultaneously in different clinical syndromes that involve the same genetic or molecular aberration. The genetic architecture of ALS and FTD is complex; different types of genetic variants (e.g., common, rare, low-frequency, and CNV) in different genes cause either ALS, FTD or ALS-FTD in different people. For this reason, WGS is appropriate technology to facilitate identification of disease-causing genetic variants in a single patient and discovery of novel disease-causing genetic variants in large-scale studies. WGS may be particularly valuable for characterizing diverse and understudied populations, where novel variants may be associated with disease and/or where differences in genomic background may modify the clinical presentation of mutations in a given gene. We know that the heritability of ALS and FTD is high, which provides rationale for continuing to expand genomic research in these conditions. Novel approaches for gene discovery, such as machine learning and leveraging pleiotropy, are rapidly growing in ALS and FTD research. In turn, new clinical trials driven by these novel findings will likely be soon underway. In addition to informing therapeutic development, genetic information will be valuable for assessing pharmacogenetic interactions in clinical trials and may ultimately be leveraged to stratify patients to optimize therapeutic efficacy. In this way, collection of genetic data is critical for enhancing our understanding of safety, tolerability, and individual difference in response to therapies. Because ALS and FTD are rare diseases, global collaboration between physicians, large neurodegenerative disease centers, non-profit and for-profit institutions, and other key domain experts will be critical for identifying effective treatments that will stop or slow the progression of disease in clinically and demographically diverse ALS and FTD patients.

AUTHOR CONTRIBUTIONS

IB wrote the initial draft of the manuscript. PC and JY edited the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

Abramzon, Y. A., Fratta, P., Traynor, B. J., and Chia, R. (2020). The overlapping genetics of amyotrophic lateral sclerosis and frontotemporal dementia. *Front. Neurosci.* 14:42. doi: 10.3389/fnins.2020.00042

Adams-Carr, K. L., Bocchetta, M., Neason, M., Holton, J. L., Lashley, T., Warren, J. D., et al. (2020). A case of TDP-43 type C pathology presenting as non-fluent variant primary progressive aphasia. *Neurocase* 26, 1–6. doi: 10.1080/13554794.2019.1690665

Ahmed, R. M., Devenney, E. M., Strikwerda-Brown, C., Hodges, J. R., Piguet, O., Kiernan, M. C. et al. (2020). Phenotypic variability in ALS-FTD and effect on survival. *Neurology* 94, e2005–e2013. doi: 10.1212/WNL.0000000000009398

Al-Chalabi, A., Fang, F., Hanby, M. F., Leigh, P. N., Shaw, C. E., Ye, W., et al. (2010). An estimate of amyotrophic lateral sclerosis heritability using twin data. *J. Neurol. Neurosurg. Psychiatry* 81, 1324–1326. doi: 10.1136/jnnp.2010.207464

Anderson, C. J., Bredvik, K., Burstein, S. R., Davis, C., Meadows, S. M., Dash, J., et al. (2019). ALS/FTD mutant CHCHD10 mice reveal a tissue-specific toxic gain-of-function and mitochondrial stress response. *Acta Neuropathol.* 138, 103–121. doi: 10.1007/s00401-019-01989-y

Andreassen, O. A., Djurovic, S., Thompson, W. K., Schork, A. J., Kendler, K. S., O'Donovan, M. C., et al. (2013). Improved detection of common variants associated with schizophrenia by leveraging pleiotropy with cardiovascular-disease risk factors. *Am. J. Hum. Genet.* 92, 197–209. doi: 10.1016/j.ajhg.2013.01.001

Bean, D. M., Al-Chalabi, A., Dobson, R. J. B., and Iacoangeli, A. (2020). A knowledge-based machine learning approach to gene prioritisation in amyotrophic lateral sclerosis. *Genes* 11:668. doi: 10.3390/genes11060668

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

Blauwendraat, C., Wilke, C., Simon-Sanchez, J., Jansen, I. E., Reifschneider, A., Capell, A., et al. (2018). The wide genetic landscape of clinical frontotemporal dementia: systematic combined sequencing of 121 consecutive subjects. *Genet. Med.* 20, 240–249. doi: 10.1038/gim.2017.102

Borghesani, V., Battistella, G., Mandelli, M. L., Welch, A., Weis, E., Younes, K., et al. (2020). Regional and hemispheric susceptibility of the temporal lobe to FTLD-TDP type C pathology. *Neuroimage Clin.* 28:102369. doi: 10.1016/j.nicl.2020.102369

Bright, F., Werry, E. L., Dobson-Stone, C., Piguet, O., Ittner, L. M., Halliday, G. M., et al. (2019). Neuroinflammation in frontotemporal dementia. *Nat. Rev. Neurol.* 15, 540–555. doi: 10.1038/s41582-019-0231-z

Broce, I., Karch, C. M., Wen, N., Fan, C. C., Wang, Y., Tan, C. H., et al. (2018). Immune-related genetic enrichment in frontotemporal dementia: an analysis of genome-wide association studies. *PLoS Med.* 15:e1002487. doi: 10.1371/journal.pmed.1002487

Broce, I., Tan, C. H., Fan, C. C., Jansen, I., Savage, J. E., Witoelar, A., et al. (2019). Dissecting the genetic relationship between cardiovascular risk factors and Alzheimer's disease. *Acta Neuropathol.* 137, 209–226. doi: 10.1007/s00401-018-1928-6

Chen, J. A., Wang, Q., Davis-Turak, J., Li, Y., Karydas, A. M., Hsu, S. C., et al. (2015). A multiancestral genome-wide exome array study of Alzheimer disease, frontotemporal dementia, and progressive supranuclear palsy. *JAMA Neurol.* 72, 414–422. doi: 10.1001/jamaneurol.2014.4040

Connolly, O., Le Gall, L., McCluskey, G., Donaghy, C. G., Duddy, W. J., Duguez, S. et al. (2020). A systematic review of genotype-phenotype correlation across cohorts having causal mutations of different genes in ALS. *J. Pers. Med.* 10:58. doi: 10.3390/jpm10030058

Corcia, P., Valdmanis, P., Millecamps, S., Lionnet, C., Blasco, H., Mouzat, K., et al. (2012). Phenotype and genotype analysis in amyotrophic lateral sclerosis with TARDBP gene mutations. *Neurology* 78, 1519–1526. doi: 10.1212/WNL.0b013e3182553c88

Crespi, C., Dodich, A., Cappa, S. F., Canessa, N., Iannaccone, S., Corbo, M., et al. (2018). Multimodal MRI quantification of the common neurostructural bases within the FTD-ALS continuum. *Neurobiol. Aging* 62, 95–104. doi: 10.1016/j.neurobiolaging.2017.09.019

Cudkowicz, M. E., McKenna-Yasek, D., Sapp, P. E., Chin, W., Geller, B., Hayden, D. L., et al. (1997). Epidemiology of mutations in superoxide dismutase in amyotrophic lateral sclerosis. *Ann. Neurol.* 41, 210–221. doi: 10.1002/ana.410410212

Cykowski, M. D., Powell, S. Z., Peterson, L. E., Appel, J. W., Rivera, A. L., Takei, H., et al. (2017). Clinical significance of TDP-43 neuropathology in amyotrophic lateral sclerosis. *J. Neuropathol. Exp. Neurol.* 76, 402–413. doi: 10.1093/jnen/nlx025

Dani, M., Brooks, D. J., and Edison, P. (2016). Tau imaging in neurodegenerative diseases. *Eur. J. Nucl. Med. Mol. Imaging* 43, 1139–1150. doi: 10.1007/s00259-015-3231-2

de Vries, B. S., Rustemeijer, L. M. M., Bakker, L. A., Schroder, C. D., Veldink, J. H., van den Berg, L. H., et al. (2019). Cognitive and behavioural changes in PLS and PMA:challenging the concept of restricted phenotypes. *J. Neurol. Neurosurg. Psychiatry* 90, 141–147. doi: 10.1136/jnnp-2018-318788

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 non-coding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 72, 245–256. doi: 10.1016/j.neuron.2011.09.011

Deschauer, M., Gaul, C., Behrmann, C., Prokisch, H., Zierz, S., Haack, T. B. et al. (2012). C19orf12 mutations in neurodegeneration with brain iron accumulation mimicking juvenile amyotrophic lateral sclerosis. *J. Neurol.* 259, 2434–2439. doi: 10.1007/s00415-012-6521-7

Eisen, A., Kiernan, M., Mitsumoto, H., and Swash, M. (2014). Amyotrophic lateral sclerosis: a long preclinical period? *J. Neurol. Neurosurg. Psychiatry* 85, 1232–1238. doi: 10.1136/jnnp-2013-307135

Elden, A. C., Kim, H. J., Hart, M. P., Chen-Plotkin, A. S., Johnson, B. S., Fang, X., et al. (2010). Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. *Nature* 466, 1069–1075. doi: 10.1038/nature09320

Ferrari, R., Hernandez, D. G., Nalls, M. A., Rohrer, J. D., Ramasamy, A., Kwok, J. B., et al. (2014). Frontotemporal dementia and its subtypes: a genome-wide association study. *Lancet Neurol.* 13, 686–699. doi: 10.1016/S1474-4422(14)70065-1

Ferrari, R., Wang, Y., Vandrovcova, J., Guelfi, S., Witeolar, A., Karch, C. M., et al. (2017). Genetic architecture of sporadic frontotemporal dementia and overlap with Alzheimer's and *Parkinson's* diseases. *J. Neurol. Neurosurg. Psychiatry* 88, 152–164. doi: 10.1136/jnnp-2016-314411

Fogh, I., Ratti, A., Gellera, C., Lin, K., Tiloca, C., Moskvina, V., et al. (2014). A genome-wide association meta-analysis identifies a novel locus at 17q11.2 associated with sporadic amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 23, 2220–2231. doi: 10.1093/hmg/ddt587

Frei, O., Holland, D., Smeland, O. B., Shadrin, A. A., Fan, C. C., Maeland, S., et al. (2019). Bivariate causal mixture model quantifies polygenic overlap between complex traits beyond genetic correlation. *Nat. Commun.* 10:2417. doi: 10.1038/s41467-019-10310-0

Genomes Project, C., Auton, A., Brooks, L. D., Durbin, R. M., Garrison, E. P., Kang, H. M., et al. (2015). A global reference for human genetic variation. *Nature* 526, 68–74. doi: 10.1038/nature15393

Geser, F., Lee, V. M., and Trojanowski, J. Q. (2010). Amyotrophic lateral sclerosis and frontotemporal lobar degeneration: a spectrum of TDP-43 proteinopathies. *Neuropathology* 30, 103–112. doi: 10.1111/j.1440-1789.2009.01091.x

Goldman, J. S., Farmer, J. M., Wood, E. M., Johnson, J. K., Boxer, A., Neuhaus, J., et al. (2005). Comparison of family histories in FTLD subtypes and related tauopathies. *Neurology* 65, 1817–1819. doi: 10.1212/01.wnl.0000187068.92184.63

Gordon, P. H., Cheng, B., Katz, I. B., Pinto, M., Hays, A. P., Mitsumoto, H., et al. (2006). The natural history of primary lateral sclerosis. *Neurology* 66, 647–653. doi: 10.1212/01.wnl.0000200962.94777.71

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

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

Greaves, C. V., and Rohrer, J. D. (2019). An update on genetic frontotemporal dementia. *J. Neurol.* 266, 2075–2086. doi: 10.1007/s00415-019-09363-4

Grollemund, V., Pradat, P. F., Querin, G., Delbot, F., Le Chat, G., Pradat-Peyre, J. F., et al. (2019). Machine learning in amyotrophic lateral sclerosis:

achievements, pitfalls, and future directions. *Front. Neurosci.* 13:135. doi: 10.3389/fnins.2019.00135

Grossman, M. (2010). Primary progressive aphasia: clinicopathological correlations. *Nat. Rev. Neurol.* 6, 88–97. doi: 10.1038/nrneurol.2009.216

Higelin, J., Demestre, M., Putz, S., Delling, J. P., Jacob, C., Lutz, A. K., et al. (2016). FUS mislocalization and vulnerability to DNA damage in ALS patients derived hiPSCs and aging motoneurons. *Front. Cell. Neurosci.* 10:290. doi: 10.3389/fncel.2016.00290

Hill, S. J., Mordes, D. A., Cameron, L. A., Neuberg, D. S., Landini, S., Eggan, K., et al. (2016). Two familial ALS proteins function in prevention/repair of transcription-associated DNA damage. *Proc. Natl. Acad. Sci. U S A.* 113, E7701–E7709. doi: 10.1073/pnas.1611673113

Hodges, J. R., Davies, R., Xuereb, J., Kril, J., and Halliday, G. (2003). Survival in frontotemporal dementia. *Neurology* 61, 349–354. doi: 10.1212/01.wnl.0000078928.20107.52

Holland, D., Frei, O., Desikan, R., Fan, C. C., Shadrin, A. A., Smeland, O. B., et al. (2021). *Linkage Disequilibrium and Heterozygosity Modulate the Genetic Architecture of Human Complex Phenotypes. Genet*. Available Online at: https://www.ncbi.nlm.nih.gov/pubmed/32427991

Holtzman, N. A. (2013). ACMG recommendations on incidental findings are flawed scientifically and ethically. *Genet. Med.* 15, 750–751. doi: 10.1038/gim.2013.96

Homfray, T. (2020). *Genetics and epigenetics differences in monozygotic twins: the vision of the geneticist. In Developmental and Fetal Origins of Differences in Monozygotic Twins*. Netherland: Elsevier, 94–110.

Jackson, M., Marks, L., May, G. H. W., and Wilson, J. B. (2018). The genetic basis of disease. *Essays Biochem.* 62, 643–723. doi: 10.1042/EBC20170053

Jay, T. R., von Saucken, V. E., and Landreth, G. E. (2017). TREM2 in neurodegenerative diseases. *Mol. Neurodegener.* 12:56. doi: 10.1186/s13024-017-0197-5

Josephs, K. A., Hodges, J. R., Snowden, J. S., Mackenzie, I. R., Neumann, M., Mann, D. M., et al. (2011). Neuropathological background of phenotypical variability in frontotemporal dementia. *Acta Neuropathol.* 122, 137–153. doi: 10.1007/s00401-011-0839-6

Kansal, K., Mareddy, M., Sloane, K. L., Minc, A. A., Rabins, P. V., McGready, J. B., et al. (2016). Survival in frontotemporal dementia phenotypes: a meta-analysis. *Dement. Geriatr. Cogn. Disord.* 41, 109–122. doi: 10.1159/000443205

Keller, M. F., Ferrucci, L., Singleton, A. B., Tienari, P. J., Laaksovirta, H., Restagno, G., et al. (2014). Genome-wide analysis of the heritability of amyotrophic lateral sclerosis. *JAMA Neurol.* 71, 1123–1134. doi: 10.1001/jamaneurol.2014.1184

Kim, W. K., Liu, X., Sandner, J., Pasmantier, M., Andrews, J., Rowland, L. P., et al. (2009). Study of 962 patients indicates progressive muscular atrophy is a form of ALS. *Neurology* 73, 1686–1692. doi: 10.1212/WNL.0b013e3181c1dea3

Knopman, D. S., and Roberts, R. O. (2011). Estimating the number of persons with frontotemporal lobar degeneration in the US population. *J. Mol. Neurosci.* 45, 330–335. doi: 10.1007/s12031-011-9538-y

Koch, L. (2020). Exploring human genomic diversity with gnomAD. *Nat. Rev. Genet.* 21:448. doi: 10.1038/s41576-020-0255-7

Lee, T., Li, Y. R., Ingre, C., Weber, M., Grehl, T., Gredal, O., et al. (2011). Ataxin-2 intermediate-length polyglutamine expansions in european ALS patients. *Hum. Mol. Genet.* 20, 1697–1700. doi: 10.1093/hmg/ddr045

Leighton, D., de Araujo Martins Moreno, C., Lehrer, H., Pal, S., Chandran, S., Goldstein, D., et al. (2019). *Assessing ACMG Criteria for the Classification of Reported ALS Gene Mutations: Utility, Pitfalls and Recommendations (N2.001)*. Philadelphia, USA: American Academy of Neurologists Annual Meeting.

Li, P. P., Sun, X., Xia, G., Arbez, N., Paul, S., Zhu, S., et al. (2016). ATXN2-AS, a gene antisense to ATXN2, is associated with spinocerebellar ataxia type 2 and amyotrophic lateral sclerosis. *Ann. Neurol.* 80, 600–615. doi: 10.1002/ana.24761

Lillo, P., Mioshi, E., Burrell, J. R., Kiernan, M. C., Hodges, J. R., and Hornberger, M. (2012). Grey and white matter changes across the amyotrophic lateral sclerosis-frontotemporal dementia continuum. *PLoS One* 7:e43993. doi: 10.1371/journal.pone.0043993

Liscic, R. M., Alberici, A., Cairns, N. J., Romano, M., and Buratti, E. (2020). From basic research to the clinic: innovative therapies for ALS and FTD in the pipeline. *Mol. Neurodegener.* 15:31. doi: 10.1186/s13024-020-00373-9

Ljubenkov, P. A., and Miller, B. L. (2016). A clinical guide to frontotemporal dementias. *Focus (Am Psychiatr Publ)* 14, 448–464. doi: 10.1176/appi.focus.20160018

Logroscino, G., Piccininni, M., Binetti, G., Zecca, C., Turrone, R., Capozzo, R., et al. (2019). Incidence of frontotemporal lobar degeneration in italy: the salento-brescia registry study. *Neurology* 92, e2355–e2363. doi: 10.1212/WNL.0000000000007498

Lomen-Hoerth, C., Anderson, T., and Miller, B. (2002). The overlap of amyotrophic lateral sclerosis and frontotemporal dementia. *Neurology* 59, 1077–1079. doi: 10.1212/wnl.59.7.1077

Majounie, E., Renton, A. E., Mok, K., Dopper, E. G., Waite, A., Rollinson, S., et al. (2012). Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study. *Lancet Neurol.* 11, 323–330. doi: 10.1016/S1474-4422(12)70043-1

McLaughlin, R. L., Vajda, A., and Hardiman, O. (2015). Heritability of amyotrophic lateral sclerosis: insights from disparate numbers. *JAMA Neurol.* 72, 857–858. doi: 10.1001/jamaneurol.2014.4049

Mitchell, S., Caso, C., Quimby, M., Kelly, K., Xia, C., Ratti, E., et al. (2015). Survival in primary progressive aphasia: comparison with bvFTD and FTD-ALS (P1.217). *Neurology* 84, 217.

Nakamura-Shindo, K., Sakai, K., Shimizu, A., Ishida, C., and Yamada, M. (2020). Accumulation of phosphorylated TDP-43 in the cytoplasm of schwann cells in a case of sporadic amyotrophic lateral sclerosis. *Neuropathology* 40, 606–610. doi: 10.1111/neup.12673

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

Olney, N. T., Spina, S., and Miller, B. L. (2017). Frontotemporal dementia. *Neurol. Clin.* 35, 339–374. doi: 10.1016/j.ncl.2017.01.008

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

Onyike, C. U., and Diehl-Schmid, J. (2013). The epidemiology of frontotemporal dementia. *Int. Rev. Psychiatry* 25, 130–137. doi: 10.3109/09540261.2013.776523

Owen, D. R., Yeo, A. J., Gunn, R. N., Song, K., Wadsworth, G., Lewis, A., et al. (2012). An 18-kDa translocator protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. *J. Cereb. Blood Flow Metab.* 32, 1–5. doi: 10.1038/jcbfm.2011.147

Perry, D. C., Brown, J. A., Possin, K. L., Datta, S., Trujillo, A., Radke, A., et al. (2017). Clinicopathological correlations in behavioural variant frontotemporal dementia. *Brain* 140, 3329–3345. doi: 10.1093/brain/awx254

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

Regal, L., Vanopdenbosch, L., Tilkin, P., Van den Bosch, L., Thijs, V., Sciot, R., et al. (2006). The G93C mutation in superoxide dismutase 1: clinicopathologic phenotype and prognosis. *Arch. Neurol.* 63, 262–267. doi: 10.1001/archneur.63.2.262

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

Riancho, J., Paz-Fajardo, L., and Lopez de Munain, A. (2020). Clinical and preclinical evidence of somatosensory involvement in amyotrophic lateral sclerosis. *Br. J. Pharmacol.* 2020:15202. doi: 10.1111/bph.15202

Roggenbuck, J., and Fong, J. C. (2020). Genetic testing for amyotrophic lateral sclerosis and frontotemporal dementia: impact on clinical management. *Clin. Lab. Med.* 40, 271–287. doi: 10.1016/j.cll.2020.05.002

Rohrer, J. D., Guerreiro, R., Vandrovcova, J., Uphill, J., Reiman, D., Beck, J., et al. (2009). The heritability and genetics of frontotemporal lobar degeneration. *Neurology* 73, 1451–1456. doi: 10.1212/WNL.0b013e3181bf997a

Rosen, H. J., Boeve, B. F., and Boxer, A. L. (2020). Tracking disease progression in familial and sporadic frontotemporal lobar degeneration: recent findings from ARTFL and LEFFTDS. *Alzheimers Dement* 16, 71–78. doi: 10.1002/alz.12004

Rosso, S. M., Donker Kaat, L., Baks, T., Joosse, M., de Koning, I., Pijnenburg, Y., et al. (2003). Frontotemporal dementia in the netherlands: patient

characteristics and prevalence estimates from a population-based study. *Brain* 126, 2016–2022. doi: 10.1093/brain/awg204

Rostalski, H., Leskela, S., Huber, N., Katisko, K., Cajanus, A., Solje, E., et al. (2019). Astrocytes and microglia as potential contributors to the pathogenesis of C9orf72 repeat expansion-associated FTLD and ALS. *Front. Neurosci.* 13:486. doi: 10.3389/fnins.2019.00486

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

Schwarze, K., Buchanan, J., Fermont, J. M., Dreau, H., Tilley, M. W., Taylor, J. M., et al. (2020). The complete costs of genome sequencing: a microcosting study in cancer and rare diseases from a single center in the united kingdom. *Genet. Med.* 22, 85–94. doi: 10.1038/s41436-019-0618-7

Seeley, W. W. (2019). Behavioral variant frontotemporal dementia. *Continuum* 25, 76–100. doi: 10.1212/CON.0000000000000698

Shefner, J. M., Al-Chalabi, A., Baker, M. R., Cui, L. Y., de Carvalho, M., Eisen, A., et al. (2020). A proposal for new diagnostic criteria for ALS. *Clin. Neurophysiol.* 131, 1975–1978. doi: 10.1016/j.clinph.2020.04.005

Shoesmith, C., Abrahao, A., Benstead, T., Chum, M., Dupre, N., Izenberg, A., et al. (2020). Canadian ALS best practice recommendations (2749). *Neurology* 94:2749.

Sproviero, W., Shatunov, A., Stahl, D., Shoai, M., van Rheenen, W., Jones, A. R., et al. (2017). ATXN2 trinucleotide repeat length correlates with risk of ALS. *Neurobiol. Aging.* 51, 178.e1–178.e9. doi: 10.1016/j.neurobiolaging.2016.11.010

Tan, W., Pasinelli, P., and Trotti, D. (2014). Role of mitochondria in mutant SOD1 linked amyotrophic lateral sclerosis. *Biochim. Biophys. Acta* 1842, 1295–1301. doi: 10.1016/j.bbadis.2014.02.009

Trojsi, F., Sorrentino, P., Sorrentino, G., and Tedeschi, G. (2018). Neurodegeneration of brain networks in the amyotrophic lateral sclerosis-frontotemporal lobar degeneration (ALS-FTLD) continuum: evidence from MRI and MEG studies. *CNS Spectr.* 23, 378–387. doi: 10.1017/S109285291700075X

Tsai, R. M., Bejanin, A., Lesman-Segev, O., LaJoie, R., Visani, A., Bourakova, V., et al. (2019). (18)F-flortaucipir (AV-1451) tau PET in frontotemporal dementia syndromes. *Alzheimers Res. Ther.* 11:13. doi: 10.1186/s13195-019-0470-7

Tsai, R. M., and Boxer, A. L. (2016). Therapy and clinical trials in frontotemporal dementia: past, present, and future. *J. Neurochem.* 138, 211–221. doi: 10.1111/jnc.13640

Tsai, R. M., Miller, Z., Koestler, M., Rojas, J. C., Ljubenkov, P. A., Rosen, H. J., et al. (2020). Reactions to multiple ascending doses of the microtubule stabilizer TPI-287 in patients with alzheimer disease, progressive supranuclear palsy, and corticobasal syndrome: a randomized clinical trial. *JAMA Neurol.* 77, 215–224. doi: 10.1001/jamaneurol.2019.3812

Turcano, P., Stang, C. D., Mielke, M. M., Martin, P. R., Upadhyaya, S. G., Josephs, K. A., et al. (2020). Incidence of frontotemporal disorders in olmsted county: a population-based study. *Alzheimers Dement* 16, 482–490. doi: 10.1016/j.jalz.2019.08.199

Turner, M. R., Barohn, R. J., Corcia, P., Fink, J. K., Harms, M. B., Kiernan, M. C., et al. (2020). Primary lateral sclerosis: consensus diagnostic criteria. *J. Neurol. Neurosurg. Psychiatry* 91, 373–377. doi: 10.1136/jnnp-2019-322541

Vadgama, N., Pittman, A., Simpson, M., Nirmalananthan, N., Murray, R., Yoshikawa, T., et al. (2019). De novo single-nucleotide and copy number variation in discordant monozygotic twins reveals disease-related genes. *Eur. J. Hum. Genet.* 27, 1121–1133. doi: 10.1038/s41431-019-0376-7

van Eijk, R. P. A., Eijkemans, M. J. C., Nikolakopoulos, S., Jansen, M. D., Westeneng, H. J., van Eijk, K. R., et al. (2020). Pharmacogenetic interactions in amyotrophic lateral sclerosis: a step closer to a cure? *Pharmacogenom. J.* 20, 220–226. doi: 10.1038/s41397-019-0111-3

van Eijk, R. P. A., Jones, A. R., Sproviero, W., Shatunov, A., Shaw, P. J., Leigh, P. N., et al. (2017). Meta-analysis of pharmacogenetic interactions in amyotrophic lateral sclerosis clinical trials. *Neurology* 89, 1915–1922. doi: 10.1212/WNL.0000000000004606

Van Mossevelde, S., Engelborghs, S., van der Zee, J., and Van Broeckhoven, C. (2018). Genotype-phenotype links in frontotemporal lobar degeneration. *Nat. Rev. Neurol.* 14, 363–378. doi: 10.1038/s41582-018-0009-8

van Nimwegen, K. J., van Soest, R. A., Veltman, J. A., Nelen, M. R., van der Wilt, G. J., Vissers, L. E., et al. (2016). Is the $1000 genome as near as we think? a cost analysis of next-generation sequencing. *Clin. Chem.* 62, 1458–1464. doi: 10.1373/clinchem.2016.258632

van Rheenen, W., Shatunov, A., Dekker, A. M., McLaughlin, R. L., Diekstra, F. P., Pulit, S. L., et al. (2016). Genome-wide association analyses identify new risk variants and the genetic architecture of amyotrophic lateral sclerosis. *Nat Genet.* 48, 1043–1048. doi: 10.1038/ng.3622

Van Weehaeghe, D., Babu, S., De Vocht, J., Zurcher, N. R., Chew, S., Tseng, C. J., et al. (2020). Moving toward multicenter therapeutic trials in amyotrophic lateral sclerosis: feasibility of data pooling using different translocator protein PET radioligands. *J. Nucl. Med.* 61, 1621–1627. doi: 10.2967/jnumed.119.241059

Vinceti, G., Olney, N., Mandelli, M. L., Spina, S., Hubbard, H. I, Santos-Santos, M. A., et al. (2019). Primary progressive aphasia and the FTD-MND spectrum disorders: clinical, pathological, and neuroimaging correlates. *Amyotroph. Lateral Scler Frontotemporal Degener* 20, 146–158. doi: 10.1080/21678421.2018.1556695

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

Wallings, R. L., Humble, S. W., Ward, M. E., and Wade-Martins, R. (2019). Lysosomal dysfunction at the centre of *Parkinson's* Disease and frontotemporal dementia/amyotrophic lateral sclerosis. *Trends Neurosci.* 42, 899–912. doi: 10.1016/j.tins.2019.10.002

Weishaupt, J. H., Hyman, T., and Dikic, I. (2016). Common molecular pathways in amyotrophic lateral sclerosis and frontotemporal dementia. *Trends Mol. Med.* 22, 769–783. doi: 10.1016/j.molmed.2016.07.005

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. doi: 10.1136/jnnp-2016-313959

Wood, E. M., Falcone, D., Suh, E., Irwin, D. J., Chen-Plotkin, A. S., Lee, E. B., et al. (2013). Development and validation of pedigree classification criteria for frontotemporal lobar degeneration. *JAMA Neurol.* 70, 1411–1417. doi: 10.1001/jamaneurol.2013.3956

Xu, L., Liu, T., Liu, L., Yao, X., Chen, L., Fan, D., et al. (2020). Global variation in prevalence and incidence of amyotrophic lateral sclerosis: a systematic review and meta-analysis. *J. Neurol.* 267, 944–953. doi: 10.1007/s00415-019-09652-y

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

Yokoyama, J. S., Karch, C. M., Fan, C. C., Bonham, L. W., Kouri, N., Ross, O. A., et al. (2017). Shared genetic risk between corticobasal degeneration, progressive supranuclear palsy, and frontotemporal dementia. *Acta Neuropathol.* 133, 825–837. doi: 10.1007/s00401-017-1693-y

Yousefian-Jazi, A., Seol, Y., Kim, J., Ryu, H. L., Lee, J., and Ryu, H. (2020). Pathogenic genome signatures that damage motor neurons in amyotrophic lateral sclerosis. *Cells* 9:2687. doi: 10.3390/cells9122687

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