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Mutation screening of *SLC52A3*, *C19orf12*, and *TARDBP* in Iranian ALS patients

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ABSTRACT

Mutations in the same gene are sometimes the cause of different clinically diagnosed neurologic disorders; this emphasizes interrelationships between various neurologic diseases. In this light, we screened *SLC52A3*, which is the cause of Brown-Vialetto-Van Laere syndrome, and *C19orf12*, which is the cause of neurodegeneration with brain iron accumulation in 60 Iranian amyotrophic lateral sclerosis (ALS) patients without mutations in the 2 most important ALS-causing genes, *SOD1* and *C9orf72*. To the best of our knowledge, neither *SLC52A3* nor *C19orf12* has been mutation-screened previously in ALS cohorts. Justification for screening *SLC52A3* included notable clinical similarities between Brown-Vialetto-Van Laere syndrome and ALS, and justification for screening *C19orf12* was known contribution of mitochondrial dysfunction to ALS etiology. Disease-causing variations in the 2 genes were not found among the ALS patients. *TARDBP* was screened in 107 patients, and a mutation (p.Gly348Cys) was identified in one. Detailed clinical data on the patient are presented. It appears that mutations in *TARDBP* in ALS patients of Iran are rare and occur at similar frequencies to European populations.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal adult onset motor neuron disease that is characterized by dysfunction and degeneration of both upper motor neurons in the cortex and lower motor neurons in the brainstem and spinal cord (Robberecht and Philips, 2013). It is the third most common neurodegenerative disease and the most common motor neuron disease in European populations (Hirtz et al., 2007; Rison and Beydoun, 2010). Within this general framework, clinical features are variable. The earliest presentations are usually in the limbs but sometimes bulbar; time of onset ranges from childhood to 94 years of age, but the mean is 65; progression of disease can be slow or rapid; survival time is usually 2–3 years after onset but can be from less than one to more than 10 years after onset; and cognitive impairment is sometimes present (Alavi et al., 2013; Andersen et al., 1995, 1996; Lattante et al., 2015; Montuschi et al., 2015; Sabatelli et al., 2013; Shirakawa et al., 2009). Ultimately, nearly all motor neurons become affected, and death usually ensues because of respiratory failure. With respect to patterns of inheritance, most cases of ALS are apparently sporadic (SALS), but 5%–10% of patients are classified as familial ALS (FALS) (Therrien et al., 2016; van Rheenen et al., 2016). FALS usually exhibits dominant inheritance (Ittner et al., 2015).

Genetic analysis of FALS families and, more recently, whole-exome sequencing in large patient cohorts have by now led to the identification of over 30 ALS causative genes (http://alsod.iop.kcl.ac.uk//) (Therrien et al., 2016; White and Sreedharan, 2016). Most of these have been identified within the past 10 years. Although most of the genes identified account for disease status in a small fraction of patients, knowledge of the functions of their encoded products has immensely enhanced our understanding of the molecular etiology of ALS. It is now appreciated that nuclear transport, inflammation, protein degradation, mitochondrial functions,

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vesicular trafficking, and RNA processing are among the processes implicated in ALS pathogenesis (Therrien et al., 2016; White and Sreedharan, 2016). Interestingly, the contribution of various genes to disease burden may be variable in different populations. *C9orf72* is the most striking example of this variability. The disease-causing hexanucleotide repeat expansion in this gene was reported as the cause of ALS in approximately 40% and 20%, respectively, of Finish FALS and SALS patients (Renton et al., 2011). Although the mutation was also frequent in various populations of European descent, its contribution to ALS in Asian populations was much lower (0%–2%) (Jang et al., 2013; Ogaki et al., 2012; Tsai et al., 2012). With the exception of a recent important large-scale screening of ALS patients from Turkey, Iranian patients have constituted the only ALS cohort from the Middle East that have been genetically screened (Alavi et al., 2013, 2014; Ozoguz et al., 2015). Screening of *SOD1* and *C9orf72*, which make the largest contribution to disease burden worldwide, revealed that *SOD1* mutations contribute significantly to ALS among Iranians (found in 38.5% of FALS probands and in 4.3% of SALS cases), but *C9orf72* mutations are relatively rare (found in 2.5% of patients). Screenings in various populations have shown that after *SOD1* and *C9orf72*, mutations in *TARDBP* that encodes transactive response DNA-binding protein-43 (TDP-43) are most likely to be found in ALS patients (Alsultan et al., 2016). Here, we report results of mutation screening of *TARDBP* in Iranian patients known not to harbor mutations in *SOD1* and *C9orf72*.

In addition to the recognized ALS-causing genes referred to above, the possible contribution to ALS pathology of genes associated with other neurological disorders, especially those that share some ALS clinical features, can be considered. In fact, mutations in the same gene have sometimes been found in patients diagnosed with different neurological disorders (Lillo et al., 2014). The most notable example is the expansion mutations in *C9orf72* itself, which, in addition to ALS, has also been observed in patients affected with frontotemporal dementia, Alzheimer's disease, corticobasal syndrome, supranuclear palsy, Parkinson's disease, olivopontocerebellar degeneration, and sleeping disorder (Armstrong, 2012; Lesage et al., 2013; Lindquist et al., 2013). In this light, we performed mutation screening of *SLC52A3* (solute carrier family 52, formerly known as *C20orf54*) that encodes human riboflavin transporter 2 and *C19orf12* that encodes a transmembrane mitochondrial protein in Iranian ALS patients without mutations in *SOD1* and *C9orf72*. *SLC52A3* and *C19orf12* are, respectively, recognized causative genes of Brown-Vialetto-Van Laere syndrome (BVVLS; MIM 211530) and neurodegeneration with brain iron accumulation (NBIA) (Green et al., 2010; Hartig et al., 2011). Although the relationship between ALS and BVVLS and also between ALS and NBIA has been referred to in the existing literature, to the best of our knowledge, neither *SLC52A3* nor *C19orf12* has been mutation-screened previously in ALS patient cohorts (Ciccolella et al., 2013; Kim et al., 2016).

The justification for screening of *SLC52A3* in ALS patients included the notable clinical similarities between BVVLS and ALS to the extent that BVVLS was first reported in 1894 as FALS with onset in infancy (Brown, 1894). Even now, differential diagnosis between ALS and BVVLS may be problematic (Gonzalez-Perez et al., 2012). BVVLS is characterized by progressive pontobulbar palsy and bilateral sensorineural hearing loss. Respiratory impairment, upper and lower limb weakness and wasting, cerebellar ataxia, and lower cranial nerve palsies become evident with disease progression (Green et al., 2010). The justification that prompted screening of *C19orf12* was three-fold. First, the gene plays a role in mitochondrial functions, and mitochondrial dysfunction is implicated in ALS etiology because several of ALS-causing genes have mitochondria-associated functions (Smith et al., 2017). Second, mutations in this gene have been reported in 3 unrelated ALS-like affected or initially ALS-diagnosed individuals (Deschauer et al., 2012; Kim et al., 2016). Finally, in contrary to NBIA patients of some other populations, *C19orf12* mutations were earlier shown to be relatively common in Iranian NBIA patients (4 of 11 patients screened) (Dezfouli et al., 2013; Panteghini et al., 2012). NBIA includes a clinically and genetically heterogeneous group of neurodegenerative disorders characterized by iron deposition in the basal ganglia (Gregory and Hayflick, 2011).

2. Materials and methods

The research was performed in accordance with the Declaration of Helsinki and with approval of the ethics board of the University of Tehran. All participants or their responsible guardians consented to participate after being informed of the nature of the research. Unrelated Iranian patients diagnosed with definite ALS based on El Escorial criteria were recruited from the Neurology Section of Hazrat Rassoul Hospital and the neuromuscular clinic of Shariati Hospital that are associated, respectively, with Iran University of Medical Sciences and Tehran University of Medical Sciences. One hundred seven patients without mutations in *SOD1* and without the hexanucleotide repeat mutation in *C9orf72* were included in the present study (Alavi et al., 2013, 2014). Nineteen of the probands (17.8%) were classified as FALS cases because they had affected family members. All exons and flanking intronic sequences of *SLC52A3* and *C19orf12* and the 5 coding exons and flanking intronic sequences of *TARDBP* in the DNA of peripheral blood cells of 60 of the ALS patients were amplified by polymerase chain reaction and sequenced using the dideoxynucleotide termination protocol. The exons of *TARDBP* were later amplified and sequenced in 47 additional SALS patients. The Sequencher 5.4.6 software was used for sequence analysis (Gene Codes Corporation, Ann Arbor, MI). NNsplice (http://www.fruitfly.org) and Human Splicing Finder (http://www.umd.be/HSF3/HSF.shtml) were used to predict potential effects on splicing. Reference sequences used were NC_000001.11, NM_007375.3, and NP_031401.1 for *TARDBP*; NC_000020.10, NM_033409.3, and NP_212134.3 for *SLC52A3*; and NC_000019.9, NM_001031726.3, and NP_001026896.2 for *C19orf12*. Sequences of primers used are available upon request.

Thorough electrodiagnostic examinations that included nerve conduction studies and needle electromyography were carried out on the only patient who carried a disease-causing mutation according to standard procedures (Dantec Keypoint G4, Natus, CA). Whole spine and brain magnetic resonance imaging (MRI) studies were performed using a 1.5-T system (MAGNETOM Avanto 1.5 Tesla, Siemens, Germany).

3. Results

Screening of *SLC52A3* in 60 ALS patients identified 20 previously reported sequence variations, none of which were reasonable candidates as disease-causing mutations (Table 1). The 9 exonic variations among the observed variations were common polymorphisms or did not cause amino acid alterations. Two previously reported variations in *C19orf12* were found among the 60 patients. These were also not disease-causing variations (Table 1).

Four known sequence variations were observed in *TARDBP*, 3 of which are expected not to cause ALS. The intronic variations observed were c.715-126delG and c.715–31C>T. A relatively common synonymous coding variation (c.198 T>C) that causes p.Ala66Ala and that is considered to be benign was observed in 3 patients (Guerreiro et al., 2008; Luquin et al., 2009). A heterozygous variation in exon 6 (c.1042 G>T) which causes p.Gly348Cys was found in 1 FALS patient (ALS163-III9) and is predicted to be the cause of ALS in this individual (Fig. 1A). This variation has earlier

Table 1
SLC52A3 and *C19orf12* sequence variations observed among 60 Iranian ALS patients without mutations in *SOD1* and *C9orf72*

Sequence variation	Exon/intron	Effect on protein	MAF	Reference SNP no.
SLC52A3				
c.-85A>G	Exon (5′UTR)	___	A = 0.4511	rs1884637
c.-52+30T>C	Intron	___	C = 0.1388	rs1884636
c.-52+118C>T	Intron	___	T = 0.0863	rs7270519
c.-14_-6delGGGCAGATA	Exon (5′UTR)	___	0.2364	rs57727892
c.456 C>T	Exon	p.Pro152Pro	T = 0.0980	rs3746807
c.417 C>T	Exon	p.Thr139Thr	___	rs968335720
c.222 C>G	Exon	p.Ile74Met	G = 0.0308	rs35655964
c.568-56G>A	Intron	___	A = 0.0010	rs74462543
c.645 C>T	Exon	p.Pro215Pro	T = 0.1382	rs6054605
c.765 C>T	Exon	p.Leu255Leu	T = 0.3087	rs3746805
c.800 C>T	Exon	p.Pro267Leu	T = 0.1823	rs3746804
c.833 C>T	Exon	p.Thr278Met	T = 0.0905	rs3746803
c.907 A>G	Exon	p.Ile303Val	G = 0.0931	rs3746802
c.1073+92T>C	Intron	___	C = 0.1468	rs8122335
c.1074−163T>C	Intron	___	T = 0.4541	rs873970
c.1197+106A>G	Intron	___	A = 0.0960	rs6054589
c.1197+108C>T	Intron	___	C = 0.4700	rs3746801
c.1197+128G>C	Intron	___	C = 0.2013	rs3746800
c.1233 T>C	Exon	p.Ser411Ser	T = 0.3446	rs910857
c.*16delC	Exon (3′UTR)	___	0.2186	rs3215628
C19orf12				
c.193+107C>T	Intron	___	T = 0.2368/1186	rs1864141
c.324 C>T	Exon	p.Thr119Thr	C = 0.3862/1934	rs10424582

ALS, amyotrophic lateral sclerosis; MAF, minor allele frequency in dbSNP (https://www.ncbi.nlm.nih.gov/snp).

been reported in several European patients (Daoud et al., 2009; Del Bo et al., 2009; Kabashi et al., 2008; Kuhnlein et al., 2008; Ticozzi et al., 2011). The Iranian patient with the p.Gly348Cys mutation was a member of a nonconsanguineous pedigree with at least 8 additional members with ALS-related presentations distributed in 3 generations (Fig. 1B). The proband's mother and brother, both of whom had been diagnosed with ALS, had died at the age of 57 and 40 years, respectively. Four additional available members who are presently at least 7 years older than the proband at the age of diagnosis also underwent genetic analysis. The only ALS-diagnosed individual among these (ALS163-IV1) carries the mutation, and 2 asymptomatic individuals (ALS163-III3 and ALS163-III4) who are 21 and 12 years older than the proband at the age of diagnosis are homozygous for the normal allele. ALS163-III8 who is 7 years older than the proband at the age of diagnosis and who is also presently asymptomatic harbors a mutated allele. As the mutation has been reported to be highly penetrant, she is expected to eventually become symptomatic.

The proband of the pedigree was diagnosed with ALS at the age of 44 years. She had spinal onset ALS as her weakness began from left lower extremity. The disease course continued sequentially as her right lower extremity, right upper extremity, and, at last, left upper extremity became involved; the pattern of myotomal involvement remained asymmetric during the entire disease course. Neuromuscular examination at the age of 44 years showed that muscle force was more prominently weak and atrophic in the right upper and left lower extremities. Deep tendon reflexes were absent and plantar reflex was downward. Sensory examination was normal. No evidence of cranial motor neuron involvement was detected, and cognitive examination results were normal. Two years later, she was using a walker for ambulation and complained about dysphagia of fluid and amnesia. At that time, tongue atrophy was not seen and mini-mental status examination score was 28/30. Although she did no complaint of respiratory difficulty, spirometry showed that forced vital capacity was reduced to 75%. Electromyography showed significant chronic neurogenic changes in cranial region, trunk, and upper and lower extremities, which are suggestive of myotomal involvement at cranial, cervical, thoracic, and lumbosacral levels. Abnormal spontaneous activity manifested in the cranial region as fasciculation potentials and in lower extremities as fibrillation and positive sharp waves. The motor nerve conduction study showed only decreased amplitude in atrophic regions of lower extremity without significant velocity change. The sensory conduction study was completely normal. Whole spine MRI was unremarkable. Brain MRI showed 2 tiny nonenhancing unidentified bright objects (UBOs) in the left frontal region. Laboratory studies were otherwise unremarkable. After identification of the single ALS-causing mutation in *TARDBP*, this gene was also screened in the 47 remaining ALS patients without mutations in *SOD1* and *C9orf72*. The intronic c.715-126delG variation was observed in several patients and the exonic variation that causes p.Ala66Ala was observed in 3 patient. The variant allele (delG) at the c.715-126 position appears to be the more common variant among Iranians as its frequency was 0.93. A candidate disease-causing variation in *TARDBP* was not found in the 47-patient cohort.

4. Discussion

With respect to *SLC52A3* and *C19orf12*, the findings of this study suggest that mutations in these genes do not make a significant contribution to ALS pathology in the Iranian population. Because of similarities in clinical presentations of BVVL and ALS, the need to screen for *SLC52A3* in ALS cohorts was suggested upon discovery of its role in BVVLS pathology (Green et al., 2010). Interestingly, despite the clinical similarities, to date, mutations in *SLC52A3* have not been reported in ALS patients. Although it is yet possible that causative mutations will be found in screenings of larger ALS cohorts, particularly cohorts of juvenile-onset cases, it appears that *SLC52A3* contributes minimally if at all to ALS. With respect to *C19orf12,* as already stated, mutations in this gene have already rarely been found in ALS patients. It would be of interest to have *C19orf12* mutation data on Polish ALS patients, as mutations in this gene are very common in Polish NBIA patients (Hartig et al., 2011).

There exists ample pathologic and genetic evidence that implicate an important role for *TARDBP* in the etiology of various neurodegenerative diseases, including ALS (Alsultan et al., 2016; Chou et al., 2018; Jovicic and Gitler, 2014; Smethurst et al., 2015). The gene's encoded protein, TDP-43, is a major component of the

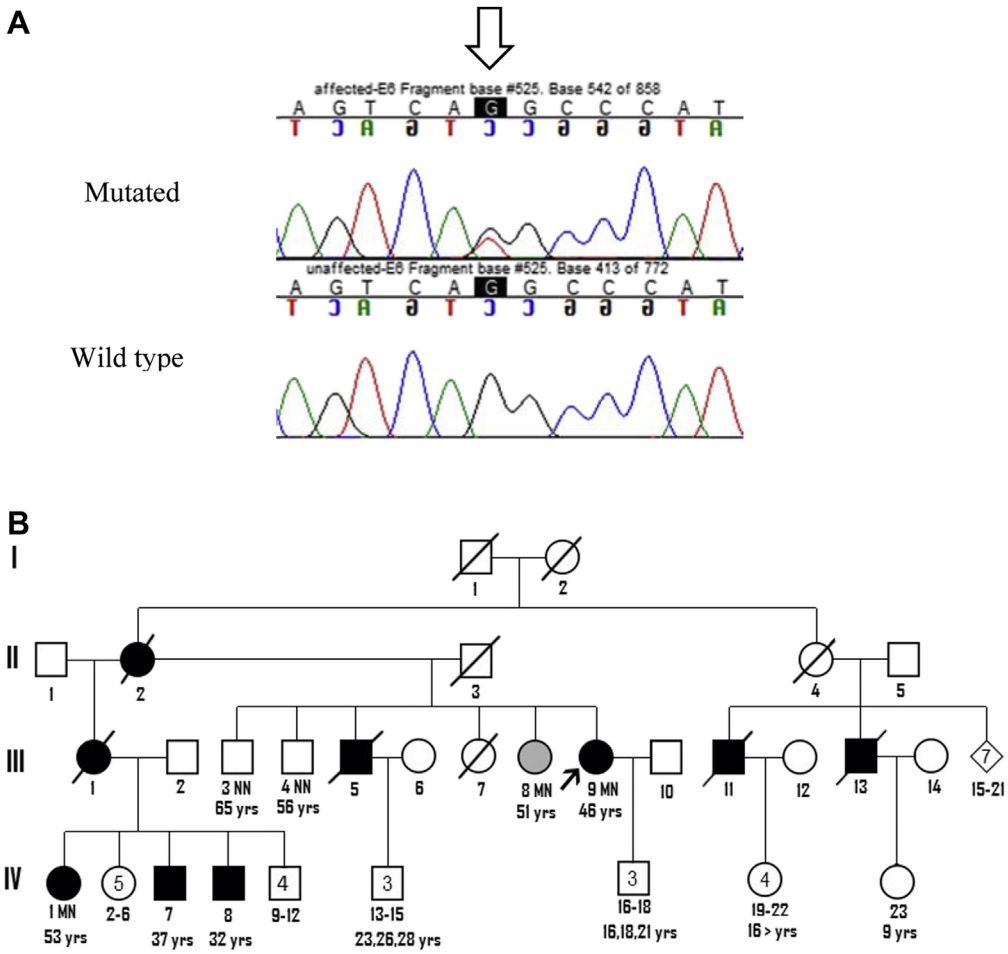

Fig. 1. Iranian FALS pedigree (ALS163) with p.Gly348Cys mutation in *TARDBP*. (A) DNA sequence chromatograms showing the heterozygous c.1042 G>T mutation in *TARDBP* and the wild type sequence. (B) FALS pedigree with the p.Gly348Cys mutation. *TARDBP* genotypes of individuals assessed by sequencing are shown. Present age of individuals is provided when known. Filled circles and squares: ALS affected; unfilled circles and squares: asymptomatic at the time of examination; filled gray circle: individual with affected genotypes who is presently asymptomatic; M: mutated *TARDBP* allele; N: wild-type *TARDBP* allele. Abbreviation: FALS, familial amyotrophic lateral sclerosis.

inclusions found in degenerating neurons of most ALS patients (Neumann et al., 2006). Mutations in the gene are considered the cause of ALS in 4%–5% of FALS patients and nearly 1% of SALS patients from various European populations (Alsultan et al., 2016). The possible cellular and molecular functions affected by mutations in the gene include transcription regulation, splicing (Polymenidou et al., 2011), prion-like toxic gain of functions (Johnson et al., 2009), axonal transport (Alami et al., 2014), microRNA processing (Gregory et al., 2004), apoptosis (Sreedharan et al., 2008), cell division (Ayala et al., 2008), neurite outgrowth (Fallini et al., 2012), and embryo development (Sephton et al., 2010). It has been suggested that the p.Gly348Cys mutation found in the Iranian ALS163 pedigree may affect protein function by promoting formation of intermolecular disulfide bridges that may interfere with protein-protein interactions or increase the aggregation tendency of TDP-43 (Kuhnlein et al., 2008). In terms of contribution to ALS disease burden in the Iranian population, our results suggest that *TARDBP* makes a small contribution as is the case with other populations

Table 2
Features of various ALS patients with the p.Gly348Cys mutation in *TARDBP*[a]

Reference	This study	(Del Bo et al., 2009)	(Daoud et al., 2009)	(Kabashi et al., 2008)	(Kuhnlein et al., 2008)
No. of patients	9 (all in one pedigree)	9 (all in one pedigree)	3 SALS	1 SALS	2 (all in one pedigree)
Nationality	Iranian	Belgian	French	French	German
Sex	4 F, 5 M	6 M, 3 F	M	F	2 F
Initial manifestation	All spinal (lower extremity)	All spinal	2 Bulbar, 1 Spinal	Spinal	All spinal (upper extremity)
Age at onset (y) (range/mean)	30-52/40.7	36-67/52.9	46-55/49.7	-/30	31-55/43
Disease duration (y) (range/mean)	Living patients: >1.7 deceased patients: 5-7/5.8	Living patients: >3 deceased patients: 3-5/3.8	-/-	-/7	3-13/8

-, Information not available.
F, female; M, male; SALS, sporadic amyotrophic lateral sclerosis.
[a] Reference (Ticozzi et al., 2011) not included because no data on the patients with the p.Gly348Cys mutation was provided.

screened (Daoud et al., 2009; Del Bo et al., 2009; Kabashi et al., 2008; Kamada et al., 2009; Kuhnlein et al., 2008; Kwon et al., 2012; Mentula et al., 2012; Nakamura et al., 2016; Ticozzi et al., 2011; Van Deerlin et al., 2008). Extrapolations after considering frequencies of mutations in *SOD1* and *C9orf72* among Iranian ALS patients suggest that approximately 3% of Iranian FALS patients may harbor mutations in *TARDBP*. Of course, a more definitive assessment requires screening in a larger patient cohort. It has been reported that in Turkey, the only other Middle Eastern country for which genetic data are available, 3.7% of ALS patients have mutations in *TARDBP*.

The clinical data on 15 ALS patients with the p.Gly348Cys mutation in *TARDBP* from 4 earlier publications and the data on the 9 patients of this study allow evaluation of consistency of genotype-phenotype correlation among patients with this mutation (Table 2) (Daoud et al., 2009; Del Bo et al., 2009; Kabashi et al., 2008; Kuhnlein et al., 2008; Ticozzi et al., 2011). There was no obvious sex bias. There were variations with respect to age and site of onset of symptoms and with respect to survival time. Although the mean age of onset in most studies was within the 4th decade of life, it ranged from 30 to 67 years among the patients. Onset was most often spinal, but bulbar onset was reported in 2 sporadic cases (Daoud et al., 2009). The length of disease duration among 15 deceased patients ranged from 3 to 13 years (average 5 years).

Disclosure

All authors declare the absence of conflicts of interest and have nothing to disclose including not receiving any payment or services from a third party at any time; no relevant financial activities outside the submitted work; no patents, whether planned, pending, or issued, broadly relevant to the work; and no other relationships, conditions, or circumstances that present a potential conflict of interest.

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References

Alami, N.H., Smith, R.B., Carrasco, M.A., Williams, L.A., Winborn, C.S., Han, S.S., Kiskinis, E., Winborn, B., Freibaum, B.D., Kanagaraj, A., Clare, A.J., Badders, N.M., Bilican, B., Chaum, E., Chandran, S., Shaw, C.E., Eggan, K.C., Maniatis, T., Taylor, J.P., 2014. Axonal transport of TDP-43 mRNA granules is impaired by ALS-causing mutations. Neuron 81, 536–543.

Alavi, A., Nafissi, S., Rohani, M., Shahidi, G., Zamani, B., Shamshiri, H., Safari, I., Elahi, E., 2014. Repeat expansion in C9ORF72 is not a major cause of amyotrophic lateral sclerosis among Iranian patients. Neurobiol. Aging 35, 267.e1–267.e7.

Alavi, A., Nafissi, S., Rohani, M., Zamani, B., Sedighi, B., Shamshiri, H., Fan, J.B., Ronaghi, M., Elahi, E., 2013. Genetic analysis and SOD1 mutation screening in Iranian amyotrophic lateral sclerosis patients. Neurobiol. Aging 34, 1516.e1–1516.e8.

Alsultan, A.A., Rachel, W., Heath, P.R., Kirby, J., 2016. The genetics of amyotrophic lateral sclerosis:current insights. Degenerative Neurol. Neuromuscul. Dis. 6, 49–64.

Andersen, P.M., Forsgren, L., Binzer, M., Nilsson, P., Ala-Hurula, V., Keranen, M.L., Bergmark, L., Saarinen, A., Haltia, T., Tarvainen, I., Kinnunen, E., Udd, B., Marklund, S.L., 1996. Autosomal recessive adult-onset amyotrophic lateral sclerosis associated with homozygosity for Asp90Ala CuZn-superoxide dismutase mutation. A clinical and genealogical study of 36 patients. Brain 119 (Pt 4), 1153–1172.

Andersen, P.M., Nilsson, P., Ala-Hurula, V., Keranen, M.L., Tarvainen, I., Haltia, T., Nilsson, L., Binzer, M., Forsgren, L., Marklund, S.L., 1995. Amyotrophic lateral sclerosis associated with homozygosity for an Asp90Ala mutation in CuZn-superoxide dismutase. Nat. Genet. 10, 61–66.

Armstrong, R.A., 2012. On the 'classification' of neurodegenerative disorders: discrete entities, overlap or continuum? Folia Neuropathol. 50, 201–208.

Ayala, Y.M., Misteli, T., 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.

Brown, C.H., 1894. Infantile amyotrophic lateral sclerosis of the family type. J. Nerv. Ment. Dis. 21, 707–716.

Chou, C.C., Zhang, Y., Umoh, M.E., Vaughan, S.W., Lorenzini, I., Liu, F., Sayegh, M., Donlin-Asp, P.G., Chen, Y.H., Duong, D.M., Seyfried, N.T., Powers, M.A., Kukar, T., Hales, C.M., Gearing, M., Cairns, N.J., Boylan, K.B., Dickson, D.W., Rademakers, R., Zhang, Y.J., Petrucelli, L., Sattler, R., Zarnescu, D.C., Glass, J.D., Rossoll, W., 2018. TDP-43 pathology disrupts nuclear pore complexes and nucleocytoplasmic transport in ALS/FTD. Nat. Neurosci. 21, 228–239.

Ciccolella, M., Corti, S., Catteruccia, M., Petrini, S., Tozzi, G., Rizza, T., Carrozzo, R., Nizzardo, M., Bordoni, A., Ronchi, D., D'Amico, A., Rizzo, C., Comi, G.P., Bertini, E., 2013. Riboflavin transporter 3 involvement in infantile Brown-Vialetto-Van Laere disease: two novel mutations. J. Med. Genet. 50, 104–107.

Daoud, H., Valdmanis, P.N., Kabashi, E., Dion, P., Dupre, N., Camu, W., Meininger, V., Rouleau, G.A., 2009. Contribution of TARDBP mutations to sporadic amyotrophic lateral sclerosis. J. Med. Genet. 46, 112–114.

Del Bo, R., Ghezzi, S., Corti, S., Pandolfo, M., Ranieri, M., Santoro, D., Ghione, I., Prelle, A., Orsetti, V., Mancuso, M., Soraru, G., Briani, C., Angelini, C., Siciliano, G., Bresolin, N., Comi, G.P., 2009. TARDBP (TDP-43) sequence analysis in patients with familial and sporadic ALS: identification of two novel mutations. Eur. J. Neurol. 16, 727–732.

Deschauer, M., Gaul, C., Behrmann, C., Prokisch, H., Zierz, S., Haack, T.B., 2012. C19orf12 mutations in neurodegeneration with brain iron accumulation mimicking juvenile amyotrophic lateral sclerosis. J. Neurol. 259, 2434–2439.

Dezfouli, M.A., Alavi, A., Rohani, M., Rezvani, M., Nekuie, T., Klotzle, B., Tonekaboni, S.H., Shahidi, G.A., Elahi, E., 2013. PANK2 and C19orf12 mutations are common causes of neurodegeneration with brain iron accumulation. Mov. Disord. 28, 228–232.

Fallini, C., Bassell, G.J., Rossoll, W., 2012. The ALS disease protein TDP-43 is actively transported in motor neuron axons and regulates axon outgrowth. Hum. Mol. Genet. 21, 3703–3718.

Gonzalez-Perez, P., Lu, Y., Chian, R.J., Sapp, P.C., Tanzi, R.E., Bertram, L., McKenna-Yasek, D., Gao, F.B., Brown Jr., R.H., 2012. Association of UBQLN1 mutation with Brown-Vialetto-Van Laere syndrome but not typical ALS. Neurobiol. Dis. 48, 391–398.

Green, P., Wiseman, M., Crow, Y.J., Houlden, H., Riphagen, S., Lin, J.P., Raymond, F.L., Childs, A.M., Sheridan, E., Edwards, S., Josifova, D.J., 2010. Brown-Vialetto-Van Laere syndrome, a ponto-bulbar palsy with deafness, is caused by mutations in c20orf54. Am. J. Hum. Genet. 86, 485–489.

Gregory, A., Hayflick, S.J., 2011. Genetics of neurodegeneration with brain iron accumulation. Curr. Neurol. Neurosci. Rep. 11, 254–261.

Gregory, R.I., Yan, K.P., Amuthan, G., Chendrimada, T., Doratotaj, B., Cooch, N., Shiekhattar, R., 2004. The Microprocessor complex mediates the genesis of microRNAs. Nature 432, 235–240.

Guerreiro, R.J., Schymick, J.C., Crews, C., Singleton, A., Hardy, J., Traynor, B.J., 2008. TDP-43 is not a common cause of sporadic amyotrophic lateral sclerosis. PLoS One 3, e2450.

Hartig, M.B., Iuso, A., Haack, T., Kmiec, T., Jurkiewicz, E., Heim, K., Roeber, S., Tarabin, V., Dusi, S., Krajewska-Walasek, M., Jozwiak, S., Hempel, M., Winkelmann, J., Elstner, M., Oexle, K., Klopstock, T., Mueller-Felber, W., Gasser, T., Trenkwalder, C., Tiranti, V., Kretzschmar, H., Schmitz, G., Strom, T.M., Meitinger, T., Prokisch, H., 2011. Absence of an orphan mitochondrial protein, c19orf12, causes a distinct clinical subtype of neurodegeneration with brain iron accumulation. Am. J. Hum. Genet. 89, 543–550.

Hirtz, D., Thurman, D.J., Gwinn-Hardy, K., Mohamed, M., Chaudhuri, A.R., Zalutsky, R., 2007. How common are the "common" neurologic disorders? Neurology 68, 326–337.

Ittner, L.M., Halliday, G.M., Kril, J.J., Gotz, J., Hodges, J.R., Kiernan, M.C., 2015. FTD and ALS–translating mouse studies into clinical trials. Nat. Rev. Neurol. 11, 360–366.

Jang, J.H., Kwon, M.J., Choi, W.J., Oh, K.W., Koh, S.H., Ki, C.S., Kim, S.H., 2013. Analysis of the C9orf72 hexanucleotide repeat expansion in Korean patients with familial and sporadic amyotrophic lateral sclerosis. Neurobiol. Aging 34, 1311.e7–1311.e9.

Johnson, B.S., Snead, D., Lee, J.J., McCaffery, J.M., Shorter, J., 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.

Jovicic, A., Gitler, A.D., 2014. TDP-43 in ALS: stay on target.almost there. Neuron 81, 463–465.

Kabashi, E., Valdmanis, P.N., Dion, P., Spiegelman, D., McConkey, B.J., Vande Velde, C., Bouchard, J.P., Lacomblez, L., Pochigaeva, K., Salachas, F., Pradat, P.F., Camu, W., Meininger, V., Dupre, N., Rouleau, G.A., 2008. TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. Nat. Genet. 40, 572–574.

Kamada, M., Maruyama, H., Tanaka, E., Morino, H., Wate, R., Ito, H., Kusaka, H., Kawano, Y., Miki, T., Nodera, H., Izumi, Y., Kaji, R., Kawakami, H., 2009. Screening for TARDBP mutations in Japanese familial amyotrophic lateral sclerosis. J. Neurol. Sci. 284, 69–71.

Kim, J., Liao, Y.H., Ionita, C., Bale, A.E., Darras, B., Acsadi, G., 2016. Mitochondrial membrane protein-associated neurodegeneration mimicking juvenile amyotrophic lateral sclerosis. Pediatr. Neurol. 64, 83–86.

Kuhnlein, P., Sperfeld, A.D., Vanmassenhove, B., Van Deerlin, V., Lee, V.M., Trojanowski, J.Q., Kretzschmar, H.A., Ludolph, A.C., Neumann, M., 2008. Two

German kindreds with familial amyotrophic lateral sclerosis due to TARDBP mutations. Arch. Neurol. 65, 1185–1189.
Kwon, M.J., Baek, W., Ki, C.S., Kim, H.Y., Koh, S.H., Kim, J.W., Kim, S.H., 2012. Screening of the SOD1, FUS, TARDBP, ANG, and OPTN mutations in Korean patients with familial and sporadic ALS. Neurobiol. Aging 33, 1017.e17–1017.e23.
Lattante, S., Ciura, S., Rouleau, G.A., Kabashi, E., 2015. Defining the genetic connection linking amyotrophic lateral sclerosis (ALS) with frontotemporal dementia (FTD). Trends Genet. 31, 263–273.
Lesage, S., Le Ber, I., Condroyer, C., Broussolle, E., Gabelle, A., Thobois, S., Pasquier, F., Mondon, K., Dion, P.A., Rochefort, D., Rouleau, G.A., Durr, A., Brice, A., French Parkinson's Disease Genetics Study, G., 2013. C9orf72 repeat expansions are a rare genetic cause of parkinsonism. Brain 136 (Pt 2), 385–391.
Lillo, P., Matamala, J.M., Valenzuela, D., Verdugo, R., Castillo, J.L., Ibanez, A., Slachevsky, A., 2014. [Overlapping features of frontotemporal dementia and amyotrophic lateral sclerosis]. Rev. Med. Chil 142, 867–879.
Lindquist, S.G., Duno, M., Batbayli, M., Puschmann, A., Braendgaard, H., Mardosiene, S., Svenstrup, K., Pinborg, L.H., Vestergaard, K., Hjermind, L.E., Stokholm, J., Andersen, B.B., Johannsen, P., Nielsen, J.E., 2013. Corticobasal and ataxia syndromes widen the spectrum of C9ORF72 hexanucleotide expansion disease. Clin. Genet. 83, 279–283.
Luquin, N., Yu, B., Saunderson, R.B., Trent, R.J., Pamphlett, R., 2009. Genetic variants in the promoter of TARDBP in sporadic amyotrophic lateral sclerosis. Neuromuscul. Disord. 19, 696–700.
Mentula, H.K., Tuovinen, L., Penttila, S., Suominen, T., Udd, B., Palmio, J., 2012. TARDBP mutations are not a frequent cause of ALS in Finnish patients. Acta Myol. 31, 134–138.
Montuschi, A., Iazzolino, B., Calvo, A., Moglia, C., Lopiano, L., Restagno, G., Brunetti, M., Ossola, I., Lo Presti, A., Cammarosano, S., Canosa, A., Chio, A., 2015. Cognitive correlates in amyotrophic lateral sclerosis: a population-based study in Italy. J. Neurol. Neurosurg. Psychiatry 86, 168–173.
Nakamura, R., Sone, J., Atsuta, N., Tohnai, G., Watanabe, H., Yokoi, D., Nakatochi, M., Ito, M., Senda, J., Katsuno, M., Tanaka, F., Li, Y., Izumi, Y., Morita, M., Taniguchi, A., Kano, O., Oda, M., Kuwabara, S., Abe, K., Aiba, I., Okamoto, K., Mizoguchi, K., Hasegawa, K., Aoki, M., Hattori, N., Tsuji, S., Nakashima, K., Kaji, R., Sobue, G., 2016. Next-generation sequencing of 28 ALS-related genes in a Japanese ALS cohort. Neurobiol. Aging 39, 219.e1–219.e8.
Neumann, M., Sampathu, D.M., Kwong, L.K., Truax, A.C., Micsenyi, M.C., Chou, T.T., Bruce, J., Schuck, T., Grossman, M., Clark, C.M., McCluskey, L.F., Miller, B.L., Masliah, E., Mackenzie, I.R., Feldman, H., Feiden, W., Kretzschmar, H.A., Trojanowski, J.Q., Lee, V.M., 2006. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314, 130–133.
Ogaki, K., Li, Y., Atsuta, N., Tomiyama, H., Funayama, M., Watanabe, H., Nakamura, R., Yoshino, H., Yato, S., Tamura, A., Naito, Y., Taniguchi, A., Fujita, K., Izumi, Y., Kaji, R., Hattori, N., Sobue, G., 2012. Analysis of C9orf72 repeat expansion in 563 Japanese patients with amyotrophic lateral sclerosis. Neurobiol. Aging 33, 2527.e11–2527.e16.
Ozoguz, A., Uyan, O., Birdal, G., Iskender, C., Kartal, E., Lahut, S., Omur, O., Agim, Z.S., Eken, A.G., Sen, N.E., Kavak, P., Saygi, C., Sapp, P.C., Keagle, P., Parman, Y., Tan, E., Koc, F., Deymeer, F., Oflazer, P., Hanagasi, H., Gurvit, H., Bilgic, B., Durmus, H., Ertas, M., Kotan, D., Akalin, M.A., Gulluoglu, H., Zarifoglu, M., Aysal, F., Dosoglu, N., Bilguvar, K., Gunel, M., Keskin, O., Akgun, T., Ozcelik, H., Landers, J.E., Brown, R.H., Basak, A.N., 2015. The distinct genetic pattern of ALS in Turkey and novel mutations. Neurobiol. Aging 36, 1764.e9-1764.e18.
Panteghini, C., Zorzi, G., Venco, P., Dusi, S., Reale, C., Brunetti, D., Chiapparini, L., Zibordi, F., Siegel, B., Garavaglia, B., Simonati, A., Bertini, E., Nardocci, N., Tiranti, V., 2012. C19orf12 and FA2H mutations are rare in Italian patients with neurodegeneration with brain iron accumulation. Semin. Pediatr. Neurol. 19, 75–81.
Polymenidou, M., Lagier-Tourenne, C., Hutt, K.R., Huelga, S.C., Moran, J., Liang, T.Y., Ling, S.C., Sun, E., Wancewicz, E., Mazur, C., Kordasiewicz, H., Sedaghat, Y., Donohue, J.P., Shiue, L., Bennett, C.F., Yeo, G.W., Cleveland, D.W., 2011. Long pre-mRNA depletion and RNA missplicing contribute to neuronal vulnerability from loss of TDP-43. Nat. Neurosci. 14, 459–468.
Renton, A.E., Majounie, E., Waite, A., Simon-Sanchez, J., Rollinson, S., Gibbs, J.R., Schymick, J.C., Laaksovirta, H., van Swieten, J.C., Myllykangas, L., Kalimo, H., Paetau, A., Abramzon, Y., Remes, A.M., Kaganovich, A., Scholz, S.W., Duckworth, J., Ding, J., Harmer, D.W., Hernandez, D.G., Johnson, J.O., Mok, K., Ryten, M., Trabzuni, D., Guerreiro, R.J., Orrell, R.W., Neal, J., Murray, A., Pearson, J., Jansen, I.E., Sondervan, D., Seelaar, H., Blake, D., Young, K., Halliwell, N., Callister, J.B., Toulson, G., Richardson, A., Gerhard, A., Snowden, J., Mann, D., Neary, D., Nalls, M.A., Peuralinna, T., Jansson, L., Isoviita, V.M., Kaivorinne, A.L., Holtta-Vuori, M., Ikonen, E., Sulkava, R., Benatar, M., Wuu, J., Chio, A., Restagno, G., Borghero, G., Sabatelli, M., Heckerman, D., Rogaeva, E., Zinman, L., Rothstein, J.D., Sendtner, M., Drepper, C., Eichler, E.E., Alkan, C., Abdullaev, Z., Pack, S.D., Dutra, A., Pak, E., Hardy, J., Singleton, A., Williams, N.M., Heutink, P., Pickering-Brown, S., Morris, H.R., Tienari, P.J., Traynor, B.J., 2011. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 72, 257–268.
Rison, R.A., Beydoun, S.R., 2010. Amyotrophic lateral sclerosis-motor neuron disease, monoclonal gammopathy, hyperparathyroidism, and B12 deficiency: case report and review of the literature. J. Med. Case Rep. 4, 298.
Robberecht, W., Philips, T., 2013. The changing scene of amyotrophic lateral sclerosis. Nat. Rev. Neurosci. 14, 248–264.
Sabatelli, M., Conte, A., Zollino, M., 2013. Clinical and genetic heterogeneity of amyotrophic lateral sclerosis. Clin. Genet. 83, 408–416.
Sephton, C.F., Good, S.K., Atkin, S., Dewey, C.M., Mayer 3rd, P., Herz, J., Yu, G., 2010. TDP-43 is a developmentally regulated protein essential for early embryonic development. J. Biol. Chem. 285, 6826–6834.
Shirakawa, K., Suzuki, H., Ito, M., Kono, S., Uchiyama, T., Ohashi, T., Miyajima, H., 2009. Novel compound heterozygous ALS2 mutations cause juvenile amyotrophic lateral sclerosis in Japan. Neurology 73, 2124–2126.
Smethurst, P., Sidle, K.C., Hardy, J., 2015. Review: prion-like mechanisms of transactive response DNA binding protein of 43 kDa (TDP-43) in amyotrophic lateral sclerosis (ALS). Neuropathol. Appl. Neurobiol. 41, 578–597.
Smith, E.F., Shaw, P.J., De Vos, K.J., 2017. The role of mitochondria in amyotrophic lateral sclerosis. Neurosci. Lett. [Epub ahead of print].
Sreedharan, J., Blair, I.P., Tripathi, V.B., Hu, X., Vance, C., Rogelj, B., Ackerley, S., Durnall, J.C., Williams, K.L., Buratti, E., Baralle, F., de Belleroche, J., Mitchell, J.D., Leigh, P.N., Al-Chalabi, A., Miller, C.C., Nicholson, G., Shaw, C.E., 2008. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 319, 1668–1672.
Therrien, M., Dion, P.A., Rouleau, G.A., 2016. ALS: recent developments from genetics studies. Curr. Neurol. Neurosci. Rep. 16, 59.
Ticozzi, N., LeClerc, A.L., van Blitterswijk, M., Keagle, P., McKenna-Yasek, D.M., Sapp, P.C., Silani, V., Wills, A.M., Brown Jr., R.H., Landers, J.E., 2011. Mutational analysis of TARDBP in neurodegenerative diseases. Neurobiol. Aging 32, 2096–2099.
Tsai, C.P., Soong, B.W., Tu, P.H., Lin, K.P., Fuh, J.L., Tsai, P.C., Lu, Y.C., Lee, I.H., Lee, Y.C., 2012. A hexanucleotide repeat expansion in C9ORF72 causes familial and sporadic ALS in Taiwan. Neurobiol. Aging 33, 2232.e11–2232.e18.
Van Deerlin, V.M., Leverenz, J.B., Bekris, L.M., Bird, T.D., Yuan, W., Elman, L.B., Clay, D., Wood, E.M., Chen-Plotkin, A.S., Martinez-Lage, M., Steinbart, E., McCluskey, L., Grossman, M., Neumann, M., Wu, I.L., Yang, W.S., Kalb, R., Galasko, D.R., Montine, T.J., Trojanowski, J.Q., Lee, V.M., Schellenberg, G.D., Yu, C.E., 2008. TARDBP mutations in amyotrophic lateral sclerosis with TDP-43 neuropathology: a genetic and histopathological analysis. Lancet Neurol. 7, 409–416.
van Rheenen, W., Shatunov, A., Dekker, A.M., McLaughlin, R.L., Diekstra, F.P., Pulit, S.L., van der Spek, R.A., Vosa, U., de Jong, S., Robinson, M.R., Yang, J., Fogh, I., van Doormaal, P.T., Tazelaar, G.H., Koppers, M., Blokhuis, A.M., Sproviero, W., Jones, A.R., Kenna, K.P., van Eijk, K.R., Harschnitz, O., Schellevis, R.D., Brands, W.J., Medic, J., Menelaou, A., Vajda, A., Ticozzi, N., Lin, K., Rogelj, B., Vrabec, K., Ravnik-Glavac, M., Koritnik, B., Zidar, J., Leonardis, L., Groselj, L.D., Millecamps, S., Salachas, F., Meininger, V., de Carvalho, M., Pinto, S., Mora, J.S., Rojas-Garcia, R., Polak, M., Chandran, S., Colville, S., Swingler, R., Morrison, K.E., Shaw, P.J., Hardy, J., Orrell, R.W., Pittman, A., Sidle, K., Fratta, P., Malaspina, A., Topp, S., Petri, S., Abdulla, S., Drepper, C., Sendtner, M., Meyer, T., Ophoff, R.A., Staats, K.A., Wiedau-Pazos, M., Lomen-Hoerth, C., Van Deerlin, V.M., Trojanowski, J.Q., Elman, L., McCluskey, L., Basak, A.N., Tunca, C., Hamzeiy, H., Parman, Y., Meitinger, T., Lichtner, P., Radivojkov-Blagojevic, M., Andres, C.R., Maurel, C., Bensimon, G., Landwehrmeyer, B., Brice, A., Payan, C.A., Saker-Delye, S., Durr, A., Wood, N.W., Tittmann, L., Lieb, W., Franke, A., Rietschel, M., Cichon, S., Nothen, M.M., Amouyel, P., Tzourio, C., Dartigues, J.F., Uitterlinden, A.G., Rivadeneira, F., Estrada, K., Hofman, A., Curtis, C., Blauw, H.M., van der Kooi, A.J., de Visser, M., Goris, A., Weber, M., Shaw, C.E., Smith, B.N., Pansarasa, O., Cereda, C., Del Bo, R., Comi, G.P., D'Alfonso, S., Bertolin, C., Soraru, G., Mazzini, L., Pensato, V., Gellera, C., Tiloca, C., Ratti, A., Calvo, A., Moglia, C., Brunetti, M., Arcuti, S., Capozzo, R., Zecca, C., Lunetta, C., Penco, S., Riva, N., Padovani, A., Filosto, M., Muller, B., Stuit, R.J., Blair, I., Zhang, K., McCann, E.P., Fifita, J.A., Nicholson, G.A., Rowe, D.B., Pamphlett, R., Kiernan, M.C., Grosskreutz, J., Witte, O.W., Ringer, T., Prell, T., Stubendorff, B., Kurth, I., Hubner, C.A., Leigh, P.N., Casale, F., Chio, A., Beghi, E., Pupillo, E., Tortelli, R., Logroscino, G., Powell, J., Ludolph, A.C., Weishaupt, J.H., Robberecht, W., Van Damme, P., Franke, L., Pers, T.H., Brown, R.H., Glass, J.D., Landers, J.E., Hardiman, O., Andersen, P.M., Corcia, P., Vourc'h, P., Silani, V., Wray, N.R., Visscher, P.M., de Bakker, P.I., van Es, M.A., Pasterkamp, R.J., Lewis, C.M., Breen, G., Al-Chalabi, A., van den Berg, L.H., Veldink, J.H., 2016. Genome-wide association analyses identify new risk variants and the genetic architecture of amyotrophic lateral sclerosis. Nat. Genet. 48, 1043–1048.
White, M.A., Sreedharan, J., 2016. Amyotrophic lateral sclerosis: recent genetic highlights. Curr. Opin. Neurol. 29, 557–564.