Genetic Epidemiology of Amyotrophic Lateral Sclerosis in Norway: A 2-Year Population-Based Study

Cathrine Goberg Olsen,a,b Øyvind Løvold Buska Tori Navestad Aanjesenc
Karl Bjørnar Alstadhaugd Ingrid Kristine Bjørnåe Geir Julius Braathena Kristin Lif Breivikf
Natasha Demicg Heidi Øyen Flemmenh Erika Hallerstigi Ineke HogenEschi
Øystein Lunde Hollaa Anne Berit Jøntvedth Margitta T. Kampmank Grethe Klevelandl
Helene Ballo Kvernom,m,n Unn Ljøstad,h,p Angelina Maniaolq Åse Hagen Morsundt
Ola Nakkencc Camilla Novyb,ab Tiina Rekandtk Katrin Schlüterui Stephan Schüleru
Kristian Tvetena Ole-Bjørn Tysnesı Trygve Holmøyb,cc Helle Høyerad

aDepartment of Medical Genetics, Telemark Hospital Trust, Skien, Norway; bInstitute of Clinical Medicine, University of Oslo, Nordbyhagen, Norway; cDepartment of Neurology, Akershus University Hospital, Lørenskog, Norway; dDepartment of Neurology, Nordland Hospital Trust, Bodø, Norway; eDepartment of Neurology, Vestre Viken Hospital Trust, Drammen, Norway; fDepartment of Neurology, Førde Hospital Trust, Førde, Norway; gDepartment of Neurology, Vestfold Hospital Trust, Tønsberg, Norway; hDepartment of Neurology, Telemark Hospital Trust, Skien, Norway; iDepartment of Neurology, Østfold Hospital Trust, Grålum, Norway; jDepartment of Neurology, Fonna Hospital Trust, Haugesund, Norway; kDepartment of Neurology, University Hospital of North Norway, Tromsø, Norway; lDepartment of Neurology, Innlandet Hospital Trust, Lillehammer, Norway; mDepartment of Neurology and Clinical Neurophysiology, St.Olav’s Hospital, Trondheim University Hospital, Trondheim, Norway; nDepartment of Neuromedicine and Movement Science, Norwegian University of Science and Technology, Trondheim, Norway; oDepartment of Neurology, Sørlandet Hospital Trust, Kristiansand, Norway; pDepartment of Clinical Medicine, University of Bergen, Bergen, Norway; qDepartment of Neurology, Oslo University Hospital, Oslo, Norway; rDepartment of Neurology, Møre og Romsdal Hospital Trust, Molde, Norway; sDepartment of Neurology, Haukeland University Hospital, Bergen, Norway; tDepartment of Neurology, Stavanger University Hospital, Stavanger, Norway; uDepartment of Neurology, Nord-Trøndelag Hospital Trust, Namsos, Norway

Keywords
Amyotrophic lateral sclerosis · Epidemiology · Genetics · Norway · Population-based study

Abstract
Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects motor neurons. In Europe, disease-causing genetic variants have been identified in 40–70% of familial ALS patients and approximately 5% of sporadic ALS patients. In Norway, the contribution of genetic variants to ALS has not yet been studied. In light of the potential development of personalized medicine, knowledge of the genetic causes of ALS in a population is becoming increasingly important. The present study provides clinical and genetic data on familial and sporadic ALS patients in a Norwegian population-based cohort. Methods: Blood samples and clinical information from ALS patients were obtained at all 17 neurological departments throughout Norway during a 2-year period. Genetic analysis of the samples involved expansion analysis of C9orf72 and exome sequencing targeting 30 known ALS-linked genes. The variants were...
classified using genotype-phenotype correlations and bioinformatics tools. **Results:** A total of 279 ALS patients were included in the study. Of these, 11.5% had one or several family members affected by ALS, whereas 88.5% had no known family history of ALS. A genetic cause of ALS was identified in 31 individuals (11.1%), among which 18 (58.1%) were familial and 13 (41.9%) were sporadic. The most common genetic cause was the C9orf72 expansion (6.8%), which was identified in 8 familial and 11 sporadic ALS patients. Pathogenic or likely pathogenic variants of SOD1 and Tbk1 were identified in 10 familial and 2 sporadic cases. C9orf72 expansions dominated in patients from the Northern and Central regions, whereas SOD1 variants dominated in patients from the South-Eastern region. **Conclusion:** In the present study, we identified several pathogenic gene variants in both familial and sporadic ALS patients. Restricting genetic analysis to only familial cases would miss more than 40 percent of those with a disease-causing genetic variant, indicating the need for genetic analysis in sporadic cases as well.

© 2022 The Author(s). Published by S. Karger AG, Basel

### Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that severely impairs patients’ quality of life and life expectancy. The disease is characterized by the degeneration of both the upper and lower motor neurons [1, 2]. Most affected individuals become symptomatic between 50 and 80 years of age, but onset may occur earlier [3]. Initial disease presentation varies; some patients present with spinal onset characterized by limb weakness, whereas others present with dysarthria and dysphagia, which is also known as bulbar-onset [1]. Patients’ life expectancy is on average 2–5 years after the diagnosis [2, 4]. Approximately 10% of the patients are categorized as having familial ALS (fALS). In these cases, one or more relatives have been diagnosed with ALS. The remaining 90% are categorized as sporadic ALS (sALS) [4].

The cause of ALS is multifactorial with both genetic and environmental factors [1, 5]. To date, more than 30 genes have been implicated in monogenic ALS. Inheritance is usually autosomal dominant [1, 2, 6]. A genetic cause is identified in 40–70% of fALS. A meta-analysis in Europe showed that mutations in C9orf72, SOD1, TARDBP, and FUS account for, on average, 34%, 15%, 4%, and 3% of the familial cases, respectively. The frequencies of genes varied from region to region, possibly related to the patients’ ancestral background. In sALS, a genetic cause is detected in approximately 5% of the cases [7]. The lack of a positive family history in these cases is likely related to reduced penetrance, unknown family history, and variable clinical manifestations, which are common in familial ALS, suggesting the presence of genetic and environmental modifiers [1, 2, 4].

In Norway, the incidence of ALS has increased steadily over the previous decades and has been reported to be 3 per 100,000, with a stable prevalence of 6.9/100,000–7.6/100,000 in the period of 2009–2015 [3]. A similar incidence range has been reported in other European countries [1, 3, 8]. To date, no systematic study on the distribution of fALS versus sALS or the genetic causes of ALS in the Norwegian population has been carried out.

The current European Federation of the Neurological Societies guidelines (2012) recommend that only patients with a familial history of ALS and those with early onset should be offered genetic testing on a routine clinical basis [9]. A previous retrospective study showed that clinical practice in Norway is in accordance with these guidelines [10]. The development of gene-specific therapy targeting ALS-related variants raises the question of whether genetic screening should be offered to all ALS patients [11, 12]. To answer this and to establish the proportion of patients who might benefit from gene-specific therapy, genetic knowledge about fALS and sALS patients in a given population is essential. In the present population-based study, we provide clinical and genetic data on patients with fALS and sALS in a Norwegian ALS cohort.

### Materials and Methods

#### Study Population

Patients from all 17 neurological departments in Norway were included in the study. Three university hospitals and 2 local hospitals recruited patients from August 2019 to August 2021. An additional 12 hospitals started recruitment during the first 6 months of 2020 and recruited patients until August 2021. Blood samples and clinical information were obtained from all participants, along with a questionnaire. The questionnaire consisted of 2 parts; the first part concerning known family history of ALS was answered by the patient. The second part concerning clinical information was answered by the recruiting neurologists. Patients who reported a family history of ALS among first-degree, second-degree, or distant relatives were categorized as having familial ALS. A formal cognitive evaluation was not performed. At the end of inclusion, 2 university hospitals and 2 local hospitals were asked to validate their participants’ diagnoses by reviewing their medical records. These 4 hospitals were chosen due to their large contribution to the study population (124/279) and because they represent both major teaching and non-teaching hospitals. At the end of the study period, all recruiting hospitals were asked to report the number of available ALS patients during the recruitment period, the number of patients who were offered participation but declined, and the number of patients who were not offered participation.

Olsen et al.
Table 1. Overview of analyzed ALS genes

| Gene    | OMIM    | ALS phenotype | Inheritance | Protein                           | Additional neurological phenotype (OMIM)                                      |
|---------|---------|---------------|-------------|-----------------------------------|--------------------------------------------------------------------------------|
| ALS2    | 606352  | ALS 2, juvenile | AR          | Alsin                             | PLS, HSP                                                                      |
| ANG     | 105850  | ALS 9         | AD          | Angiogenin                        |                                                                                |
| ANXA11  | 602572  | ALS 23        | AD          | Annexin A11                       | Inclusion body myopathy and brain white matter abnormalities                   |
| C9ORF72 | 614260  | FTD/ALS 1     | AD          | Guanine nucleotide exchange C9orf72|                                                                                |
| CCNF    | 600227  | FTD/ALS 5     | AD/AR       | Cyclin-F                          |                                                                                |
| CHCHD10 | 615903  | FTD/ALS 2     | AD          | Coiled-coil-helix-coiled-coil-helix domain containing protein 10, mitochondrial| SMA, Myopathy                                                                |
| CHMP2B  | 609512  | FTD/ALS 7     | AD          | Charged multivesicular body protein 2b|                                                                                |
| DAO     | 124050  | NA            | AD          | D-amino-acid oxidase              |                                                                                |
| ERBB4   | 600543  | ALS 19        | AD          | Receptor tyrosine-protein kinase erbB-4|                                                                                |
| FIG4    | 609390  | ALS 11        | AD          | Polyphosphoinositide phosphatase  | CMT, Yunis-Varon syndrome, polymicrogyria                                      |
| FUS     | 137070  | ALS 6 +/- FTD | AD/AR       | RNA-binding protein FUS            | Essential tremor                                                             |
| GLE1    | 603371  | NA            | AR          | mRNA export factor GLE1           | Arthrogryposis                                                               |
| GLTBD1  | 618399  | NA            | AD          | Glycosyltransferase 8 domain-containing protein 1|                                                                                |
| HNRNPA1 | 164017  | ALS 20        | AD          | Heterogeneous nuclear ribonucleoprotein A1 | ?Inclusion body myopathy with Paget disease                                  |
| KIF5A   | 602821  | ALS 25?       | AD          | Kinesin heavy chain isoform 5A    | HSP, Myoclonus                                                               |
| MATR3   | 164015  | ALS 21        | AD          | Matrin-3                         |                                                                                |
| OPTN    | 602432  | ALS 12 +/- FTD| AD/AR       | Optineurin                        |                                                                                |
| PFN1    | 176610  | ALS 18        | AD          | Profilin-1                       |                                                                                |
| SETX    | 608465  | ALS 4, juvenile| AD          | Probable helicase senataxin       | SCA                                                                           |
| SIGMAR1 | 601978  | ALS 16, juvenile| AR          | Sigma non-opioid intracellular receptor 1 | SMA distal                                                                  |
| SOD1    | 14750   | ALS 1         | AD/AR       | Superoxide dismutase [Cu-Zn]     | Spastic tetraplegia and axial hypotonia                                     |
| SPG11   | 610844  | ALS 5, juvenile| AR          | Spatacin                         | CMT, HSP                                                                     |
| SPTLC1  | 605712  | NA            | AD          | Serine palmitoyltransferase 1    | HSAN                                                                          |
| SQSTM1  | 601530  | FTD/ALS 3     | AD          | Sequestosome-1                    | Myopathy, neurodegeneration, Paget disease                                     |
| SS18L1  | 606472  | NA            | AD          | Calcium-responsive transactivator |                                                                                |
| TARDBP  | 605078  | ALS 10 +/- FTD| AD          | TAR DNA-binding protein 43       | FTD                                                                           |
| TBK1    | 604834  | FTD/ALS 4     | AD          | Serine/threonine-protein kinase TBK1|                                                                                |
| TUBA4A  | 191110  | ALS 22 +/- FTD| AD          | Tubulin alpha-4A chain           |                                                                                |
| UBQLN2  | 300264  | ALS 15 +/- FTD| XLD         | Ubiquilin-2                       |                                                                                |
| VAPB    | 605704  | ALS 8         | AD          | Vesicle-associated membrane protein-associated protein B/C | SMA                                                                         |
| VCP     | NA      | FTD/ALS 6     | AD          | Translational endoplasmic reticulum ATPase |                                                                                |

Table is based on information from OMIM [18], UniProtKB [48] and recent published literature [1, 6, 19–21]. AD, autosomal dominant; ALS, amyotrophic lateral sclerosis; AR, autosomal recessive; FTD, frontotemporal dementia; HSAN, hereditary sensory and autonomic neuropathy; HSP, hereditary spastic paraplegia; NA, not available; PLS, primary lateral sclerosis; SCA, spinocerebellar ataxia; SMA, spinal muscular atrophy; XLD, X-linked dominant.
To get a comprehensive view of the patient’s genetic variants in known ALS genes, \(C9orf72\) expansion analysis and exome sequencing were performed on all samples. \(C9orf72\) expansion analysis was performed using the Amplide PCR/CE \(C9orf72\) kit (Asuragen, Inc., Austin, TX, USA) on an ABI3130XL Genetic Analyzer (Life Technologies Ltd., Paisley, UK). The data were analyzed using GeneMarker V2 and V3 (SoftGenetics LLC, West Hartford, CT, USA). Next-Generation Sequencing (NGS) sample preparation and enrichment was performed using the Human Core Exome EF Multiplex kit (Twist Bioscience, San Francisco, CA, USA) according to the manufacturer’s instructions. The samples were sequenced using NextSeq 500 (Illumina Inc., San Diego, CA, USA). The reads were mapped to the reference sequence (GRCh37/hg19) using BWA [13]. The Genome Analysis Toolkit was used for variant calling and filtering [14–16]. The variants were annotated using vc-fanno [17]. During bioinformatic filtering, 30 genes relevant to ALS were included and analyzed (Table 1). The selected genes were chosen based on information from OMIM [18] and recently published literature [1, 6, 19–21]. The identified variants were filtered based on dominant and recessive inheritance models, using gnomAD (https://gnomad.broadinstitute.org/) minor allele frequencies of 0.1% and 2.0%, respectively. Pathogenicity predictions were carried out using genetic frequency databases, the Alamut Visual Interface (SOPHiA GENETICS, Inc. Boston, MA, USA), the Human Gene Mutation Database and literature [22, 23]. The variants were classified according to the guidelines of the American College of Medical Genetics and the Association for Clinical Genomic Science [24, 25]. Principal component analysis based on exome data was used to predict ethnicity [26]. The coefficient of relationship was calculated using somalier (github.com/brentp/somalier) as a quality parameter and to determine whether any of the participants were closely related. This program detects relationships equal to or closer than third-degree relatives.

Table 2. Clinical characteristics of ALS patients

|                      | fALS, \(n = 32\) (11.5%) | sALS, \(n = 247\) (88.5%) | Missing | Total, \(n = 279\) |
|----------------------|---------------------------|---------------------------|---------|-------------------|
| Sex, \(n (%)\)       |                           |                           |         |                   |
| Male                 | 16 (50.0)                 | 150 (60.7)                | –       | 166 (59.5)        |
| Female               | 16 (50.0)                 | 97 (39.3)                 | –       | 113 (40.5)        |
| Clinical characteristics |                           |                           |         |                   |
| Age at onset, \(n = 31\) | n = 245                  | 3                         | n = 276 |
| Mean (95% CI)        | 58 (53–63)                | 62 (61–64)                | –       | 62 (61–63)        |
| Site of onset, \(n = 31\) | n = 245                  | 3                         | n = 276 |
| Bulbar               | 6 (19.4)                  | 59 (24.1)                 | –       | 65 (23.6)         |
| Spinal               | 23 (74.2)                 | 150 (61.2)                | –       | 173 (62.7)        |
| Both                 | 2 (6.5)                   | 36 (14.7)                 | –       | 38 (13.8)         |
| Age at diagnosis, \(n = 31\) | n = 244                  | 4                         | n = 275 |
| Mean (95% CI)        | 61 (56–66)                | 64 (63–65)                | –       | 64 (62–65)        |
| Cognitive affection, \(n = 31\) | n = 244                  | 4                         | n = 275 |
| Yes                  | 2 (6.5)                   | 24 (9.8)                  | –       | 26 (9.5)          |
| No                   | 28 (90.3)                 | 212 (86.9)                | –       | 240 (87.3)        |
| Uncertain            | 1 (3.2)                   | 8 (3.3)                   | –       | 9 (3.3)           |
| Sensory findings, \(n = 31\) | n = 245                  | 3                         | n = 276 |
| Yes                  | 3 (9.7)                   | 17 (6.9)                  | –       | 20 (7.2)          |
| No                   | 27 (87.1)                 | 225 (91.8)                | –       | 252 (91.3)        |
| Uncertain            | 1 (3.2)                   | 3 (1.2)                   | –       | 4 (1.4)           |
| Neurophysiology compatible with ALS, \(n = 31\) | n = 244                  | 4                         | n = 275 |
| Yes                  | 24 (77.4)                 | 211 (86.5)                | –       | 235 (85.5)        |
| No                   | 5 (16.1)                  | 26 (10.7)                 | –       | 31 (11.3)         |
| Uncertain            | 2 (6.5)                   | 7 (2.9)                   | –       | 9 (3.3)           |
| El Escorial fulfilled, \(n = 30\) | n = 245                  | 4                         | n = 275 |
| Yes                  | 21 (70.0)                 | 180 (73.5)                | –       | 201 (73.1)        |
| No                   | 2 (6.7)                   | 41 (16.7)                 | –       | 43 (15.6)         |
| Uncertain            | 7 (23.3)                  | 24 (9.8)                  | –       | 31 (11.3)         |
| Motor neuron signs at diagnosis, \(n = 31\) | n = 239                  | 9                         | n = 270 |
| Upper                | 0 (0.0)                   | 11 (4.6)                  | –       | 11 (4.1)          |
| Lower                | 4 (12.9)                  | 21 (8.8)                  | –       | 25 (9.3)          |
| Both                 | 27 (87.1)                 | 207 (87.1)                | –       | 234 (86.7)        |

–, missing; CI, confidence interval; fALS, familial ALS; sALS, sporadic ALS.
Results

Patient Cohort

A total of 280 ALS patients living in Norway were included in the study. One patient was withdrawn due to an unconfirmed diagnosis, leaving 279 patients for further analysis. The patients’ clinical characteristics are described in Table 2. Thirty-two patients (11.5%) had at least one affected relative (22 first-degree, 5 second-degree, and 5 other relatives) and were classified as fALS. Two hundred and forty-seven patients (88.5%) were classified as sALS. The mean age of onset was 58 (95% CI: 53–63) years for fALS and 62 (95% CI: 61–64) years for sALS. The distribution of fALS was equal between men and women, whereas among sALS patients, a higher frequency in males (60.7%) was observed. Spinal onset was more frequent than bulbar onset in both fALS and sALS patients. At the time of diagnosis, the combinations of upper and lower motor neuron signs were dominant. The neurologists reported that 9.3% of the included patients had cognitive affection. The relatedness analysis showed that 7 patients were related in 3 different families, with 3, 2 and 2 family members, respectively. The principal component analysis revealed that the included patients were of European ethnicity, with the exception of 12 cases (4.3%). Results of the relatedness analysis and principal component analysis are shown in online supplementary File 1 (for all online suppl. material, see www.karger.com doi/10.1159/000525091).

Geographic Distribution and Inclusion Rates

The patients were grouped based on their residence in 4 Norwegian health regions (Fig. 1). In total, 63.7% of the available patients were included in this analysis, varying from 48.3% to 85.4% among the 4 health regions (Table 3). Only 6.7% of the invited patients declined participation. The frequency of fALS varied from 3.5% in the Western region to 16.3% in the South-Eastern region.

---

Table 3. Included, declined, and excluded patients in the 4 health regions

| Norwegian regional health authority | South-Eastern (8 centers) | Western (4 centers) | Central (3 centers) | Northern (2 centers) | Total (17 centers) |
|-------------------------------------|---------------------------|--------------------|---------------------|----------------------|-------------------|
| Population size (Q2 2021)           | 3,060,389                 | 1,123,283          | 736,305             | 482,194              | 5,402,171         |
| Estimated prevalent ALS patients¹  | 233                       | 85                 | 56                  | 37                   | 409               |
| Reported available ALS patients     | 253                       | 79²                | 58                  | 48                   | 438               |
| Participants included, n (%)        | 153 (60.5)                | 57 (72.2)          | 28 (48.3)           | 41 (85.4)            | 279 (63.7)        |
| Familial ALS                        | 25 (16.3)                 | 2 (3.5)            | 2 (7.1)             | 3 (7.3)              | 32 (11.5)         |
| Sporadic ALS                        | 128 (83.7)                | 55 (96.5)          | 26 (92.9)           | 38 (92.7)            | 247 (88.5)        |
| Declined, n (%)                     | 14 (8.4)                  | 2 (3.4)            | 3 (9.7)             | 1 (2.1)              | 20 (6.7)          |
| Patients not attending, n (%)       | 100 (39.5)                | 22 (27.8)          | 30 (51.7)           | 7 (14.6)             | 159 (36.3)        |
| Cognitive impairment¹               | 5 (2.0)                   | 3 (3.8)            | 7 (12.1)            | 1 (2.1)              | 16 (3.7)          |
| Non-invited⁵                        | 81 (32.0)                 | 17 (21.5)          | 20 (34.5)           | 5 (10.4)             | 123 (28.1)        |

¹ Based on the prevalence of 7.6/100,000 [3]. ² Based on the information reported from 2 out of 4 neurological departments (Haukeland University Hospital and Stavanger University Hospital). ³ Based on the total number of invited patients. ⁴ Regarded as unable to consent due to cognitive impairment. ⁵ Not invited for participation due to missed appointments, rapid disease progression, or other medical or social issues.
Disease-Causing Variants
Pathogenic or likely pathogenic variants were identified in 31/279 (11.1%) patients, including 18/32 (56.3%) fALS patients (15 first-degree, 1 second-degree and 1 more distant) and 13/247 (5.3%) sALS patients (Table 4 and Fig. 2). Among the 14 fALS patients without a genetic finding, 7 reported a first degree, 4 a second degree, and 3 a more distant relative with ALS.

The most frequent pathogenic variant was the C9orf72 expansion, detected in 6.8% of our ALS population, including 8/32 (25.0%) fALS patients and 11/247 (4.5%) sALS patients (Table 4 and Fig. 2).
sALS patients. The relatedness analysis (online suppl. File 1) showed that 2 of the C9orf72 patients were related. Based on self-reported family history, the first was registered as sporadic, whereas the second reported a cousin with ALS.

Variants in SOD1 were the second most frequent genetic cause of ALS in our population. They were identified in 10 patients (3.6%), including 9/32 fALS patients (28.1%) and 1/247 sALS patients (0.4%). The c.140A>G p.(His47Arg) variant was identified in 7 fALS patients. The relatedness analysis identified 5 closely related patients in 2 separate families. Moreover, 1 fALS patient was homozygous for the c.272A>C p.(Asp91Ala) variant, 1 fALS patient was heterozygous for the c.301 G>A p.(Glu101Lys) variant, and 1 sALS patient was heterozygous for the c.450T>G p.(Ile150Met) variant. Two individuals with TBK1 variants were identified: 1 was a splice variant, c.701+1 G>A p.(?), and 1 had an in-frame deletion.

| Gene     | cDNA change         | Protein change | Zygosity | European gnomAD MAF | Citations | Patients, n | Family history | Age of onset | Site of onset |
|----------|---------------------|----------------|----------|---------------------|-----------|-------------|----------------|--------------|--------------|
| C9orf72  | GGGGCC expansion    | –              | Het      | –                   | [37, 38, 49] | 19          | Familial²,³  | 40–50        | Bulbar       |
|          |                     |                |          |                     |           |             | Familial¹    | 50–60        | Spinal       |
|          |                     |                |          |                     |           |             | Familial¹    | 50–60        | Spinal       |
| SOD1     | NM_000454.4:c.140A>G | p.(His47Arg)   | Het      | –                   | [40, 50]  | 7           | Familial¹    | 20–30        | Spinal       |
|          |                     |                |          |                     |           |             | Familial¹    | 30–40        | Spinal       |
|          |                     |                |          |                     |           |             | Familial¹    | 30–40        | Spinal       |
|          |                     |                |          |                     |           |             | Familial¹    | 40–50        | Spinal       |
|          |                     |                |          |                     |           |             | Familial¹    | 50–60        | Spinal       |
|          |                     |                |          |                     |           |             | Familial¹    | 60–70        | Spinal       |
| SOD1     | NM_000454.4:c.272A>C | p.(Asp91Ala)   | Hom      | 0.0014              | [41, 51]  | 1           | Familial¹    | 50–60        | Spinal       |
| TBK1     | NM_013254.3:c.701+1G > A | p. (?) | Het | – | [43] | 1 | Familial³ | 60–70 | Spinal |
| SOD1     | NM_000454.4:c.301G>A | p.(Glu101Lys) | Het | – | [52, 53] | 1 | Familial¹ | 30–40 | Spinal |
| SOD1     | NM_000454.4:c.450T>G | p.(Ile150Met) | Het | – | [54] | 1 | Sporadic | 50–60 | Spinal |
| TBK1     | NM_013254.3:c.1928_1930del | p.(Glu643del) | Het | 0.0024 | [45, 55] | 1 | Sporadic | 70–80 | Spinal |

Het, heterozygous; Hom, homozygous; NA, not available. ¹ First-degree relatives (parents, full siblings, or children). ² Second-degree relatives (grandparents, grandparents, aunts, uncles, nephew, nieces or half-siblings). ³ Other relatives.
| Gene  | cDNA change            | Protein change              | Zygosity | European gnomAD MAF | Citations | Patients, n | Family history | Age of onset | Site of onset | Other pathogenic finding |
|-------|------------------------|----------------------------|----------|---------------------|-----------|-------------|----------------|--------------|--------------|-------------------------|
| ANXA11 | NM_001278408.1:c.744+3G>A | p.?                        | Het      | 0.0042              | –         | 1           | Sporadic       | 70–80        | Bulbar       |                         |
| ANXA11 | NM_001278407.1:c.494_496del | p.(Gln165del)              | Het      | 0.0012              | –         | 2           | Sporadic       | 60–70        | Spinal       |                         |
| ANXA11 | NM_001278407.1:c.1481G>A  | p.(Arg494Gln)              | Het      | –                   | –         | 1           | Sporadic       | 50–60        | Spinal       |                         |
| C9orf72 | GGGGGGCG intermediate Repeat expansion, 24-30 repeats | 26 repeats 27 repeats | Het | – | [56] | 2 | Familial2 | 40–50 | Spinal | C9orf72 |
| CCNF   | NM_001761.2:c.419G>A    | p.(Arg140Gln)              | Het      | –                   | –         | 1           | Sporadic       | 60–70        | Bulbar       |                         |
| CCNF   | NM_001761.2:c.1918G>A   | p.(Val640Met)              | Het      | 0.0081              | –         | 1           | Sporadic       | 50–60        | Spinal       |                         |
| DAO    | NM_001917.4:c.34G>A     | p.(Gly12Arg)               | Het      | 0.00077             | –         | 1           | Sporadic       | 70–80        | Bulbar       |                         |
| ERBB4  | NM_005235.2:c.337A>G    | p.(Lys113Glu)              | Het      | –                   | –         | 2           | Sporadic       | 50–60        | Spinal       | C9orf72 |
| FIG4   | NM_014845.5:c.421C>T    | p.(Arg141Trp)              | Het      | 0.0053              | –         | 1           | Sporadic       | 30–40        | Spinal       |                         |
| FIG4   | NM_014845.5:c.337A>G    | p.(Arg141Gln)              | Het      | 0.0044              | –         | 1           | Sporadic       | 60–70        | Spinal       |                         |
| GLT8D1 | NM_001010983.2:c.1108A>G | p.(Ile370Val)              | Het      | –                   | –         | 1           | Sporadic       | 70–80        | Bulbar       |                         |
| KIF5A  | NM_004984.2:c.2146C>T   | p.(Arg716Trp)              | Het      | 0.0047              | [27]      | 1           | Sporadic       | 70–80        | Both         |                         |
| MATR3  | NM_199189.2:c.626A>G    | p.(Gln209Arg)              | Het      | –                   | –         | 1           | Sporadic       | 70–80        | Both         |                         |
| MATR3  | NM_199189.2:c.2321C>T   | p.(Thr774Ile)              | Het      | –                   | –         | 1           | Sporadic       | 50–60        | Spinal       |                         |
| OPTN   | NM_00103722.1:c.1465G>A | p.(Glu489Lys)              | Het      | 0.00090             | –         | 1           | Sporadic       | 50–60        | Spinal       | C9orf72 |
| PFN1   | NM_005023.2:c.86C>T     | p.(Pro29Leu)               | Het      | 0.0036              | –         | 1           | Sporadic       | 50–60        | Spinal       |                         |
| SPTLC1 | NM_001281303.1:c.859C>T | p.(Arg287*?)               | Het      | –                   | –         | 1           | Familial1      | 50–60        | Bulbar       | C9orf72 |
| TUBA4A | NM_006000.2:c.1214T>G    | p.(Val405Gly)              | Het      | 0.00014             | [41, 42]  | 1           | Sporadic       | 60–70        | Spinal       | C9orf72 |

Het, heterozygous; MAF, minor allele frequency; NA, not available. 1 First-degree relatives (parents, full siblings, or children). 2 Second-degree relatives (grandparents, grandsons, aunts, uncles, nephew, nieces or half-siblings). Other relatives.
c.1928_1930del p.(Glu643del). All identified SOD1 and TBK1 variants had previously been reported in the literature (Table 4). No pathogenic or likely pathogenic variants were detected in FUS or TARDPB.

Geographic Distribution of Genetic Variants
Figure 3 shows the distribution of the different genetic findings among the 4 Norwegian health regions. The C9orf72 expansion was found to be frequent in the North and Central regions, whereas SOD1, especially the p.(His47Arg) variant, was frequent in the South-Eastern region. In the Western region, only 1 out of 57 patients had a pathogenic genetic finding.

Variants of Uncertain Significance
Variants of uncertain significance were identified in 30 individuals (Table 5), including 3 familial and 27 sporadic cases. Two intermediate C9orf72 expansions were identified; 1 in a familial patient with full C9orf72 expansion, and 1 in a sporadic patient without other genetic findings. One sporadic patient carrying the C9orf72 expansion was also heterozygous for the SOD1 variant c.272A>C p.(Asp91Ala). Interestingly, 2 patients had identical low-frequency variants in ANXAI1 and ERBB4, and 1 patient had a KIF5A variant previously reported in an individual with spastic paraplegia [27]. In total, 5 variants of uncertain significance were identified among the individuals with the C9orf72 expansion.

Discussion/Conclusion
This study is the first comprehensive genetic screening of patients with ALS in Norway. Both familial and sporadic ALS patients from every neurological department in Norway were included in the 2-year analysis, providing a population-based sample from all parts of Norway.

Our finding of 11.5% fALS versus 88.5% sALS patients was comparable to the findings of previous studies carried out worldwide indicating a 10:90 distribution of fALS versus sALS [7, 28]. It has to be taken into consideration that 5 of the 14 fALS patients (21%) in our study, who had no known disease-causing genetic variant, reported ALS among more distant relatives. This may reflect that not all genes causing ALS have been identified, that ALS occurred by chance in these families, or that it was triggered by a familiar burden of multi-genetic factors.

In our study, the median age of disease onset was lower among fALS (58 [95% CI: 53–63]) than sALS (62 [95% CI: 61–64]). This is a known tendency, as causative genetic variants, more often seen among fALS patients, are known to cause a lower age of onset [29, 30]. Our male-to-female ratio of 1.5 was in accordance with observations in other parts of Europe [31], but slightly higher than that observed in our neighboring country, Sweden [32]. The sex ratio equals in familial cases, indicating that genetic variants are not affected by gender. Spinal onset dominates in both sALS and fALS cases in Norway; the same distribution is generally seen in other populations [33].

In total, 63.7% of the available ALS patients in Norway during the recruitment period were included in the study, which was considered an acceptable inclusion rate. Some of the non-invited patients were not included due to rapid disease progression. Thus, it is possible that ALS genes associated with rapid disease progression, such as certain SOD1 and FUS variants, were missed in our study [34, 35]. Our findings revealed that only 9.5% of the included patients were suspected to have cognitive impairment. In general, clinical studies showed that cognitive impairment occurs in 30–50% of patients with ALS and that 6–14% of patients meets the diagnostic criteria for frontotemporal dementia (FTD) [36]. It is possible that a substantial number of cognitively affected patients were excluded from the study. This could possibly skew our results since several ALS genes, including C9orf72, FUS, and TARDPB, are known to cause both ALS and FTD (Table 1). However, reports on study participation revealed that only 3.7% of the patients were excluded because of cognitive impairment (Table 3). The Norwegian health care system facilitates easy access to specialized health care and most patients were included early in their disease phase. This could indicate that most patients were included before developing a cognitive affection. Another possible bias could be language difficulties, possibly excluding patients with other ethnicities.

A genetic cause of ALS was identified in 11.1% of our enrolled participants, that is, 56.3% of fALS and 5.3% of sALS patients, which is in accordance with the results obtained in other parts of Europe [7]. We reported 19 cases carrying C9orf72 expansion, of which 57.9% had no known familial ALS, emphasizing the need to consider C9orf72 testing among sporadic patients. The C9orf72 expansion is known to cause both ALS and FTD and is characterized by age-dependent reduced penetrance. By the age of 80, almost 100% of the C9orf72 carriers have developed symptoms. The lack of a positive ALS family history is likely caused by the death of relatives before disease
onset or a clinical presentation of FTD [37–39]. It could also be speculated that an unknown genetic mechanism reducing the penetrance of the C9orf72 expansion could be present in families with apparent sALS.

Another major finding of the present study was the identification of SOD1 variants in 11 individuals. Seven individuals carried the p.(His47Arg) variant, which has been previously identified in a large Norwegian family [40]. Surprisingly, we only identified 2 carriers of the p. Asp91Ala variant: 1 homozygous and 1 heterozygous. This variant is an assumed Finno-Scandinavian founder, with a high allele frequency in Finland and Northern Sweden [41]. It is known to be pathogenic when homozygous but is more likely to be a risk factor when heterozygous [41, 42].

Intriguingly, we did not identify any variants in TARDBP or FUS, which are reported to be the third and fourth most common genetic causes of ALS in Europe [7]. However, we identified 2 TBK1 variants. TBK1 was recognized as an ALS gene in 2015 and has not been widely investigated [43], but is the second most common cause of ALS in Belgium [44]. Further, TBK1 variants have been associated with reduced penetrance [44, 45]. This correlates well with our findings, where one of the TBK1 carriers was registered as sALS, whereas the other had a distant family member with ALS and was registered as fALS.

Our geographic investigation revealed that the frequency of the C9orf72 expansion varied, from 17% in the North to only 2% in the West. The C9orf72 expansion has a higher frequency in Finland than in other European countries [46]. Migration from Finland to Northern Norway, which is geographically close to Finland, could possibly contribute to the clustering of cases in these regions of Norway. The principal component analysis used in our study could not separate the non-Finnish Europeans from the Finnish population. Furthermore, SOD1 p.(His47Arg) was frequently seen among familial cases in the South-Eastern region, partly explaining the high number of familial cases in this region.

Our study identified several variants of unknown significance. Particularly interesting variants included the ANXA11 and the ERBB4 variants identified in two ALS patients, and the KIF5A variant, previously reported in an individual with spastic paraplegia [27]. These variants could be good candidates for follow-up studies. Moreover, 5 of the C9orf72 expansion carriers also carried a variant of unknown significance. As commented by other researchers, it could be speculated whether some of these variants might modulate the C9orf72 age of onset [47].

We did not see such a tendency, but our study is too small to make such an assumption.

The largest weakness of this study is that we lack information concerning other specific neurological disorders such as FTD, Parkinson, and schizophrenia among family members. In addition, a substantial proportion of the non-invited patients had rapid disease progression, meaning that pathogenic ALS variants associated with rapid disease might be missed.

In conclusion, this population-based study identified pathogenic ALS variants in both familial and sporadic individuals and reported large regional differences. Interestingly, more than 1/3 of our genetic findings were in patients without a known family history of ALS, supporting the idea that fALS and sALS cannot be sharply distinguished. Restricting genetic testing according to family history would possibly exclude a large proportion of patients from participation in gene-targeted medical trials.

**Acknowledgments**

We thank the participating patients and their families for their cooperation, as well as the nurses and technical personnel for assisting with the inclusion of patients and genetic analysis.

**Statement of Ethics**

The study was approved by the Regional Committees for Medical and Health Research Ethics (REK) #2018/1916, the Norwegian Centre for Research Data, #426990, and the data protection officers at the different hospitals involved in the study. All participants provided written informed consent for their involvement in the study. All patients were offered genetic counseling before agreeing to participate. All procedures were conducted in accordance with the principles of the Declaration of Helsinki.

**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

**Funding Sources**

This study was supported by the Norwegian ALS Patient Organization (ALS Norge). CGO received funds from the Telemark Hospital Trust. HH received funds from the South-Eastern Norway Regional Health Authority (HSØ): research grants #2016133 and #2021097.
Author Contributions

H.H., T.H., and O.B.T.: conception and design of the study; C.G.O., G.J.B., H.O.F., K.B.A., M.T.K., O.L.B., and O.I.H.: contribution to the study design; T.N.A., K.B.A., I.K.B., K.L.B., H.B.K., B.B., N.D., H.O.F., I.H., A.B.J., M.T.K., G.K., U.L., A.M., Å.H.M., O.N., T.R., K.S., S.S., O.B.T., and T.H.: acquisition of data; C.G.O., C.N., O.L.B., and O.I.H.: analysis of data; C.G.O., H.H., O.F., and KT: interpretation of data; C.G.O., H.H., T.H., O.B.T., and O.L.B.: drafting text and figures. All the authors have read and approved the final manuscript.

Data Availability Statement

Data supporting the findings of this study are available from the corresponding author upon request. Variants classified as pathogenic, likely pathogenic, and of uncertain significance have been submitted to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/), accession number SCV002103152-SCV002103184.

References

1. Hardiman O, Al-Chalabi A, Chio A, Corr EM, Logroscino G, Robberecht W, et al. Amyotrophic lateral sclerosis. Nat Rev Dis Primers. 2017 Oct 5;3:17071.
2. Gutman SA, Chen KS, Paez-Colasante X, Feldman EL. Emerging understanding of the genotype-phenotype relationship in amyotrophic lateral sclerosis. Handb Clin Neurol. 2018;148:603–23.
3. Nøkken O, Lindstrom JC, Tynnes OB, Holmøy T. Assessing amyotrophic lateral sclerosis prevalence in Norway from 2009 to 2015 from compulsory nationwide health registers. Amyotroph Lateral Scler Frontotemporal Degener. 2017;19(3–4):303–10.
4. Renton AE, Chiò A, Traynor BJ. State of play in amyotrophic lateral sclerosis genetics. Nat Neurosci. 2014 Jan;17(1):17–23.
5. Zufiria M, Gil-Bea FJ, Fernandez-Torrón R, Poza J, Munoz-Blanco JL, Rojas-García R, et al. ALS: a bucket of genes, environment, metabolism and unknown ingredients. Prog Neurobiol. 2016 Jul;142:104–29.
6. Mathis S, Goizet C, Soulages A, Vallat JM, Masson GL. Genetics of amyotrophic lateral sclerosis: a review. J Neurol Sci. 2019 Apr 15; 399: 217–26.
7. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 2010 Mar 1;26(5):589–95.
8. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010 Sep;20(9):1297–303.
9. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet. 2011 May;43(5):491–8.
10. Van der Auwera GA, Carneiro MO, Hardt C, Poplin R, Del Angel G, Levy-Moonshine A, et al. From FastQ data to high confidence variant calls: the genome analysis toolkit best practices pipeline. Curr Protoc Bioinform. 2013;43(110):11.10.1–33.
11. Pedersen BS, Layer RM, Quinlan AR. Vcfanno: fast, flexible annotation of genetic variants. Genome Biol. 2016;17(1):118–8.
12. OMIM. Online Mendelian Inheritance in Man, OMIM (TM). McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD).
13. Brenner D, Weishaupt JH. Update on amyotrophic lateral sclerosis genetics. Curr Opin Neurol. 2019 Oct;32(5):735–9.
14. Williams KL, Topp S, Yang S, Smith B, Fifita KV, Maguire JR, Hartl C, et al. ACGS best practice guidelines for variant classification in rare disease 2020. The Association for Clinical Genomic Science. 2020. https://www.acgs.uk.com/2020.
15. Pedersen BS, Quinlan AR. Who’s who? Detecting and resolving sample anomalies in human DNA sequencing studies with peddy. Am J Hum Genet. 2017 Mar 2;100(3):406–13.
16. Iqbal Z, Rydning SL, Wedding IM, Koth J, Pihlstrom L, Ringmark AH, et al. Targeted high throughput sequencing in hereditary ataxia and spastic paraplegia. PLoS One. 2017;12(3): e0174667.
17. Nøkken O, Ratnasekera RT, Van Damme P. Amyotrophic lateral sclerosis: a clinical review. Eur J Neurol. 2020;27(10):1918–29.
18. Camu W, Khoris J, Moulard B, Salachas F, Briolotti V, Rouleau GA, et al. Genetics of familial ALS and consequences for diagnosis. French ALS Research Group. J Neurol Sci. 1999 Jun;165(Suppl 1):S21–6.
19. Mehta PR, Jones AR, Opie-Martin S, Shatunov A, Iacoangeli A, Al Khelifat A, et al. Younger age of onset in familial amyotrophic lateral sclerosis is a result of pathogenic gene variants, rather than ascertainment bias. J Neurol Neurosurg Psychiatry. 2019 Mar;90(3):268–71.
20. Fontana A, Marin B, Luna J, Beghi E, Logroscino G, Boumédiene F, et al. Time-trend evolution and determinants of sex ratio in amyotrophic lateral sclerosis: a dose-response meta-analysis. J Neurol. 2021 Aug;268(8):2973–84.
21. Ly CV, Miller TM. Emerging antisense oligonucleotide and viral therapies for amyotrophic lateral sclerosis. Curr Opin Neurol. 2018;31(5):648–54.
22. Stenson PD, Mort M, Ball EV, Chapman M, Evans K, Azevedo L, et al. The human gene mutation database (HGMD®): optimizing its use in a clinical diagnostic or research setting. Hum Genet. 2020 Oct;139(10):1197–207.
23. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. 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. 2015 May;17(5):405–24.
24. Ellard S, Baple EL, Callaway A, Berry I, Forrester N, Turnbull C, et al. ACGS best practice guidelines for variant classification in rare disease 2020. The Association for Clinical Genomic Science. 2020. https://www.acgs.uk.com/2020.
25. Pedersen BS, Quinlan AR. Who’s who? Detecting and resolving sample anomalies in human DNA sequencing studies with peddy. Am J Hum Genet. 2017 Mar 2;100(3):406–13.
26. Iqbal Z, Rydning SL, Wedding IM, Koth J, Pihlstrom L, Ringmark AH, et al. Targeted high throughput sequencing in hereditary ataxia and spastic paraplegia. PLoS One. 2017;12(3):e0174667.
27. Masrori P, Van Damme P. Amyotrophic lateral sclerosis: a clinical review. Eur J Neurol. 2020;27(10):1918–29.
28. Camu W, Khoris J, Moulard B, Salachas F, Briolotti V, Rouleau GA, et al. Genetics of familial ALS and consequences for diagnosis. French ALS Research Group. J Neurol Sci. 1999 Jun;165(Suppl 1):S21–6.
29. Mehta PR, Jones AR, Opie-Martin S, Shatunov A, Iacoangeli A, Al Khelifat A, et al. Younger age of onset in familial amyotrophic lateral sclerosis is a result of pathogenic gene variants, rather than ascertainment bias. J Neurol Neurosurg Psychiatry. 2019 Mar;90(3):268–71.
32 Longinetti E, Regodón Wallin A, Samuelsson K, Press R, Zachau A, Ronnevi I-O, et al. The Swedish motor neuron disease quality registry. Amyotrophic Lateral Sclerosis Frontotemporal Degener. 2018;19(7–8):528–37.
33 Masrori P, Van Damme P. Amyotrophic lateral sclerosis: a clinical review. Eur J Neurol. 2020;27(10):1918–29.
34 Cudkowicz ME, McKenna-Yasek D, Sapp PE, Chua W, Geller B, Hayden DL, et al. Epidemiology of mutations in superoxide dismutase 1 in amyotrophic lateral sclerosis. Ann Neurol. 1997 Feb;41(2):210–21.
35 Yan J, Deng HX, Siddique N, Fecto F, Chen W, Yang Y, et al. Frameshift and novel mutations in FUS in familial amyotrophic lateral sclerosis and ALS/dementia. Neurology. 2010 Aug 31;75(9):807–14.
36 Benbrika S, Desgranges B, Eustache F, Viader F. Cognitive, emotional and psychological manifestations in amyotrophic lateral sclerosis at baseline and overtime: a review. Front Neurol. 2019;13:951–1.
37 DeJesus-Hernandez M, Nilsson P, Ala-Hurula V, Keränen M, Tarvainen I, Halitza T, et al. Amyotrophic lateral sclerosis associated with homozygosity for an Asp90Ala mutation in Cuzn-superoxide dismutase. Nat Genet. 1995 May;10(1):61–6.
38 Renton Alan E, Majounie E, Waite A, Simón-Emparán JL, Waite AP, Rollinson SP, et al. Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2021;92(5):510–8.
39 Murphy NA, Arthur KC, Tienari PJ, Houlden H, Chiò A, Traynor BJ. Age-related penetrance of the C9orf72 repeat expansion. Sci Rep. 2017;7(1):2116–7.
40 Ostern R, Fagerheim T, Orstavik K, Holmoy T, Heiberg A, Lund-Petersen I, et al. Hereditary motor neuron disease in a large Norwegian family with a “H46R” substitution in the superoxide dismutase 1 gene. Neuromuscul Disord. 2012 Jun;22(6):511–21.
41 Andersen PM, Nilsson P, Ala-Hurula V, Keränen M, Tarvainen I, Halitza T, et al. Amyotrophic lateral sclerosis associated with homozygosity for an Asp90Ala mutation in CuZn-superoxide dismutase. Nat Genet. 1995 May;10(1):61–6.
42 Robberecht W, Aguirre T, Van Den Bosch L, Tilkin P, Cassiman JJ, Matthijs G. D90A heterozygosity in the SOD1 gene is associated with familial and apparently sporadic amyotrophic lateral sclerosis. Neurology. 1996;47(5):1336–9.
43 Cirulli ET, Lassagne BN, Petrovski S, Sapp PC, Dion PA, Leblond CS, et al. Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. Science. 2015 Mar 27;347(6229):1436–41.
44 Gjesdal I, Van Mossevelde S, van der Zee J, Sieben A, Philtjens S, Heeman B, et al. Loss of TBK1 is a frequent cause of frontotemporal dementia in a Belgian cohort. Neurology. 2015 Dec 15;85(24):2116–25.
45 van der Zee J, Gijselinck I, Van Mossevelde S, Perrone F, Dillen L, Heeman B, et al. TBK1 Mutation spectrum in an extended European patient cohort with frontotemporal dementia and amyotrophic lateral sclerosis. Hum Mutat. 2017 Mar;38(3):297–309.
46 Majounie EP, Renton AEP, Mok KM, Dopper EGP, Waite AP, Rollinson SP, et al. Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study. Lancet Neurol. 2012;11(4):323–30.
47 Shepheard SR, Parker MD, Cooper-Knock J, Verber NS, Tuddenham L, Heath P, et al. Value of systematic genetic screening of patients with amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2021;92(5):510–8.
48 Consortium TU. UniProt: the universal protein knowledgebase in 2021. Nucleic Acids Res. 2020;49(D1):D480–D89.
49 Ross JP, Leblond CS, Laurent SB, Spiegelman D, Dionne-Laporte A, Camu W, et al. Oligogenicity, C9orf72 expansion, and variant severity in ALS. Neurogenetics. 2020 Jul;21(3):227–42.
50 Ogasawara M, Matsubara Y, Narisawa K, Aoki M, Nakamura S, Itoyama Y, et al. Mild ALS in Japan associated with novel SOD1 mutation. Nat Genet. 1993;5(4):323–4.
51 Luisa Conforti F, Sprovieri T, Mazzei R, Patitucci A, Ungaro C, Zoccolella S, et al. Further evidence that D90A-SOD1 mutation is recessively inherited in ALS patients in Italy. Amyotrophic Lateral Scler. 2009 Feb;10(1):58–60.
52 Siddique T, Deng HX. Genetics of amyotrophic lateral sclerosis. Hum Mol Genet. 1996;5 Spec No:A1465–70.
53 Salehi M, Nikkhah M, Ghasemi A, Arab SS. Mitochondrial membrane disruption by aggre- gation products of ALS-causing superoxide dismutase-1 mutants. Int J Biol Macromol. 2015 Apr;75:290–7.
54 Couthouis J, Raphael AR, Daneshjou R, Gitler AD. Targeted exon capture and sequencing in sporadic amyotrophic lateral sclerosis. PLoS Genet. 2014 Oct;10(10):e1004704.
55 Freischmidt A, Wieland T, Richter B, Ruf W, Schaeffer V, Müller K, et al. Haploinsufficiency of TBK1 causes familial ALS and frontotemporal dementia. Nat Neurosci. 2015 May;18(5):631–6.
56 Iacoangeli A, Al Khleifat A, Jones AR, Sproviero W, Shaturopov A, Opie-Martin S, et al. C9orf72 intermediate expansions of 24–30 repeats are associated with ALS. Acta Neuro- pathol Commun. 2019;7(1):115–5.