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

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Keywords
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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

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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.

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.

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
GLT8D1	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	147450	ALS 1	AD/AR	Superoxide dismutase [Cu-Zn]	Spastic tetraplegia and axial hypotonia
SPG11	610844	ALS 5, juvenile	AR	Spatacsin	CMT, HSP
SPTLC1	605712	NA		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	Transitional 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.

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* (%)	*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* (%)	*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* (%)	*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* (%)	*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* (%)	*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* (%)	*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.

Genetic Analyses

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 vcfanno [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 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 patients[1]	233	85	56	37	409
Reported available ALS patients	253	79[2]	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[3], *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 impairment[4]	5 (2.0)	3 (3.8)	7 (12.1)	1 (2.1)	16 (3.7)
Non-invited[5]	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.

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.

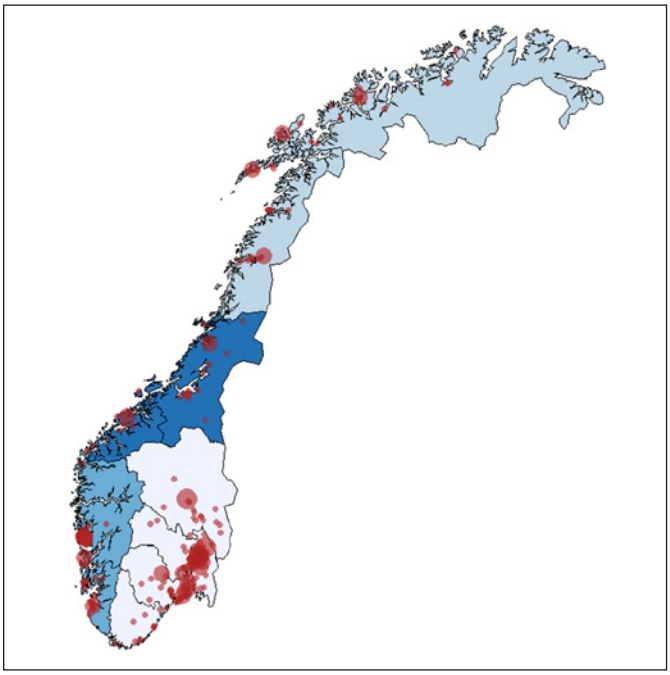

Fig. 1. Geographic distribution of participating Norwegian amyotrophic lateral sclerosis (ALS) patients in the 4 Norwegian health districts; South-Eastern, Western, Central, and Northern. The dots represent patients' postal numbers, and their sizes represent the number of patients at each postal region.

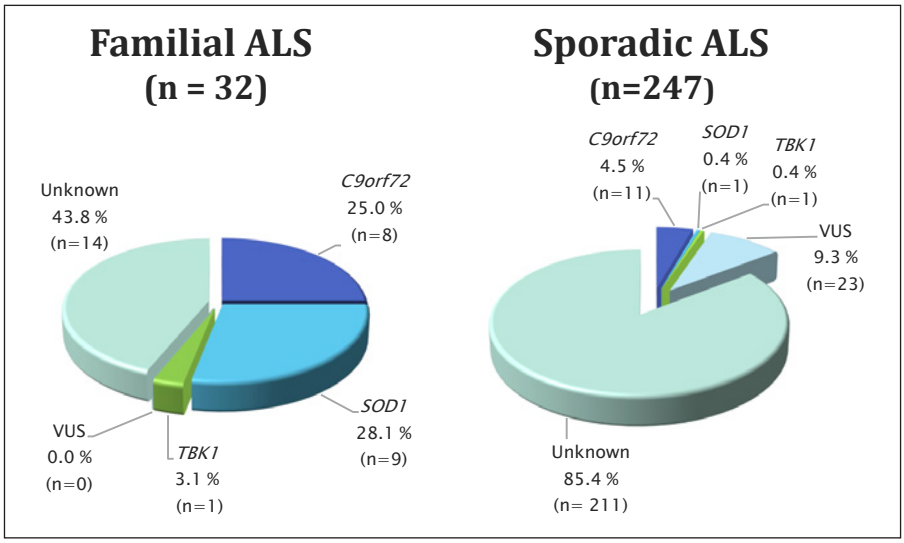

Fig. 2. Summary of genetic findings among familial and sporadic ALS patients. VUS, variant of unknown significance.

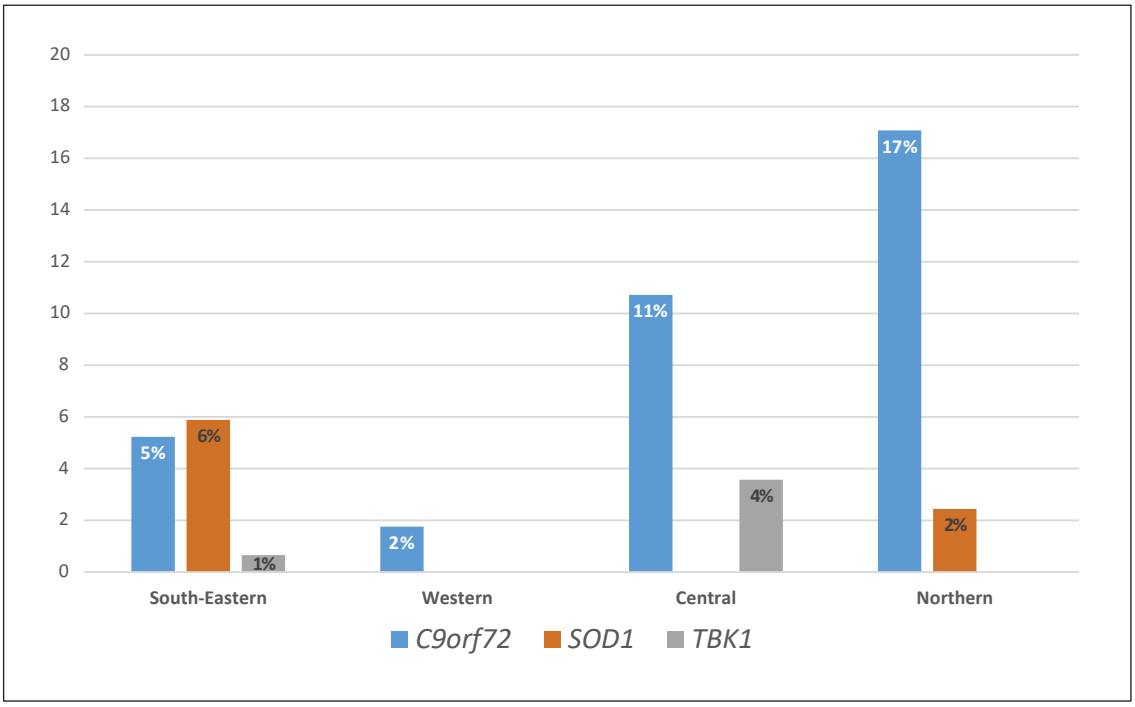

Fig. 3. Genetic findings in *C9orf72*, *SOD1*, and *TBK1* among ALS patients in the 4 Norwegian health districts. Number of patients with a positive genetic finding is shown as the percentage of the total number of included ALS patients in each region.

Genetic Analysis

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%)

Table 4. Pathogenic and likely pathogenic mutations

Gene	cDNA change	Protein change	Zygosity	European gnomAD MAF	Citations	Patients, *n*	Family history	Age of onset	Site of onset
Class 5 – pathogenic									
C9orf72	GGGGCC expansion	–	Het	–	[37, 38, 49]	19	Familial[1]	40–50	Bulbar
							Familial[1]	50–60	Spinal
							Familial[1]	50–60	Bulbar
							Familial[1]	50–60	Spinal
							Familial[1]	50–60	Spinal
							Familial[1,2]	60–70	Spinal
							Familial[2]	60–70	Spinal
							Familial[3]	NA	NA
							Sporadic	50–60	Spinal
							Sporadic	50–60	Both
							Sporadic	50–60	Both
							Sporadic	60–70	Bulbar
							Sporadic	60–70	Spinal
							Sporadic	60–70	Bulbar
							Sporadic	60–70	Bulbar
							Sporadic	60–70	Both
							Sporadic	60–70	Spinal
							Sporadic	70–80	Bulbar
							Sporadic	NA	NA
SOD1	NM_000454.4:c.140A>G	p.(His47Arg)	Het	–	[40, 50]	7	Familial[1]	20–30	Spinal
							Familial[1]	30–40	Spinal
							Familial[1,2,3]	30–40	Spinal
							Familial[1]	40–50	Spinal
							Familial[1]	40–50	Spinal
							Familial[1]	50–60	Spinal
							Familial[1]	60–70	Spinal
SOD1	NM_000454.4:c.272A>C	p.(Asp91Ala)	Hom	0,0014	[41, 51]	1	Familial[1]	50–60	Spinal
TBK1	NM_013254.3:c.701+1G > A	p.(?)	Het	–	[43]	1	Familial[3]	60–70	Spinal
Class 4 – likely pathogenic									
SOD1	NM_000454.4:c.301G>A	p.(Glu101Lys)	Het	–	[52, 53]	1	Familial[1]	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, grandsons, aunts, uncles, nephew, nieces or half-siblings). [3] Other relatives.

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,

Neuroepidemiology
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Table 5. Variants of uncertain significance, Class 3

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 Sporadic	60–70 70–80	Spinal Spinal	
ANXA11	NM_001278407.1:c.1481G>A	p.(Arg494Gln)	Het	–	–	1	Sporadic	50–60	Spinal	
C9orf72	GGGGCC intermediate Repeat expansion, 24–30 repeats	26 repeats 27 repeats	Het	–	[56]	2	Familial[2] Sporadic	40–50 60–70	Spinal 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.34 G>A	p.(Gly12Arg)	Het	0.00077	–	1	Sporadic	70–80	Bulbar	
ERBB4	NM_005235.2:c.3337A>G	p.(Lys1113Glu)	Het	–	–	2	Sporadic Familial[3]	50–60 NA	Spinal NA	*C9orf72*
FIG4	NM_014845.5:c.421C>T	p.(Arg141Trp)	Het	0.0053	–	1	Sporadic	30–40	Spinal	
FIG4	NM_014845.5:c.422G>A	p.(Arg141Gln)	Het	0.0044	–	1	Sporadic	60–70	Spinal	
GLE1	NM_001003722.1:c.1465G>A	p.(Glu489Lys)	Het	0.00090	–	1	Sporadic	50–60	Spinal	*C9orf72*
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_001008211.1:c.447G>T	p.(Arg149Ser)	Het	–	–	1	Sporadic	70–80	Both	
OPTN	NM_001008211.1:c.1533-3C>T	p.?	Het	0.0077	–	1	Sporadic	30–40	Spinal	
PFN1	NM_005022.3:c.86C>T	p.(Pro29Leu)	Het	0.0036	–	1	Sporadic	50–60	Spinal	
SETX	NM_001351528.1:c.1398T>G	p.(Ile466Met)	Het	-	–	1	Sporadic	50–60	Spinal	
SETX	NM_015046.5:c.1934T>A	p.(Phe645Tyr)	Het	–	–	1	Sporadic	70–80	Spinal	
SETX	NM_001351528.1:c.3265G>A	p.(Val1089Met)	Het	–	–	1	Sporadic	70–80	Both	
SETX	NM_001351528.1:c.7199+8A>G	p.?	Het	–	–	1	Sporadic	50–60	Spinal	
SETX	NM_001351528.1:c.7235T>C	p.(Ile2412Thr)	Het	–	–	1	Familial[1]	50–60	Bulbar	*C9orf72*
SOD1	NM_000454.4:c.272A>C	p.(Asp91Ala)	Het	0,0014	[41, 42]	1	Sporadic	60–70	Bulbar	*C9orf72*
SOD1	NM_000454.4:c.-87G>A	p.?	Het	0.013	–	1	Sporadic	80–90	Bulbar	
SPTLC1	NM_001281303.1:c.859C>T	p.(Arg287*)	Het	0.00090	–	1	Sporadic	70–80	Spinal	
TUBA4A	NM_006000.2:c.1214T>G	p.(Val405Gly)	Het	0.0062	–	1	Sporadic	70–80	Spinal	

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). [3] 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,* and especially the p.(His47Arg), variants were 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 *ANXA11* 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 3 of the 14 fALS patients (21%) in our study, who had no known disease-casing 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 equalizes 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.

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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.

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Author Contributions

H.H., T.H., and O.B.T.: conception and design of the study; C.G.O., G.J.B., H.Ø.F., K.B.A., M.T.K., Ø.L.B., and Ø.L.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.Ø.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., Ø.L.B., and Ø.L.H.: analysis of data; CGO, HH, H.Ø.F., and KT: interpretation of data; C.G.O., H.H., T.H., O.B.T., and Ø.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.

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