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RESEARCH ARTICLE

Genetic overlap between ALS and other neurodegenerative or neuromuscular disorders

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Abstract

Objective: In Norway, 89% of patients with Amyotrophic lateral sclerosis (ALS) lacks a genetic diagnose. ALS genes and genes that cause other neuromuscular or neurodegenerative disorders extensively overlap. This population-based study examined whether patients with ALS have a family history of neurological disorders and explored the occurrence of rare genetic variants associated with other neurodegenerative or neuromuscular disorders. *Methods:* During a two-year period, blood samples and clinical data from patients with ALS were collected from all 17 neurological departments in Norway. Our genetic analysis involved exome sequencing and bioinformatics filtering of 510 genes associated with neurodegenerative and neuromuscular disorders. The variants were interpreted using genotype-phenotype correlations and bioinformatics tools. *Results:* A total of 279 patients from a Norwegian population-based ALS cohort participated in this study. Thirty-one percent of the patients had first- or second-degree relatives with other neurodegenerative disorders, most commonly dementia and Parkinson's disease. The genetic analysis identified 20 possible pathogenic variants, in *ATL3, AFG3L2, ATP7A, BICD2, HARS1, KIF1A, LRRK2, MSTO1, NEK1, NEFH,* and *SORL1,* in 25 patients. *NEK1* risk variants were present in 2.5% of this ALS cohort. Only four of the 25 patients reported relatives with other neurodegenerative or neuromuscular disorders. *Conclusion:* Gene variants known to cause other neurodegenerative or neuromuscular disorders, most frequently in *NEK1*, were identified in 9% of the patients with ALS. Most of these patients had no family history of other neurodegenerative or neuromuscular disorders. Our findings indicated that *AFG3L2, ATP7A, BICD2, KIF1A*, and *MSTO1* should be further explored as potential ALS-causing genes.

Keywords: *Amyotrophic lateral sclerosis, amyotrophic lateral sclerosis susceptibility, genetic analysis, pleiotropy*

Introduction

Amyotrophic lateral sclerosis (ALS) affects both the upper and lower motor neurons, leading to muscular weakening and paralysis. Approximately 10% of the diagnosed patients have a family history of ALS (fALS), while the remaining 90% are simplex cases (sALS) (1). ALS is a heterogenic disease and more than 30 genes have been firmly linked to ALS (2, 3). *C9orf72*, *SOD1*, *TARDBP*, and *FUS* are the most frequently mutated genes (3, 4). In Europe, including Norway, a monogenic cause has been identified in approximately 50% of the fALS and 5–10% of the sALS cases (5–8). The low diagnostic yield of sALS is consistent with recent studies demonstrating that ALS is multifaceted; a mix of genetics, environment, and age likely contributes to its development (9). ALS heritability has been estimated at 50–60% (10, 11).

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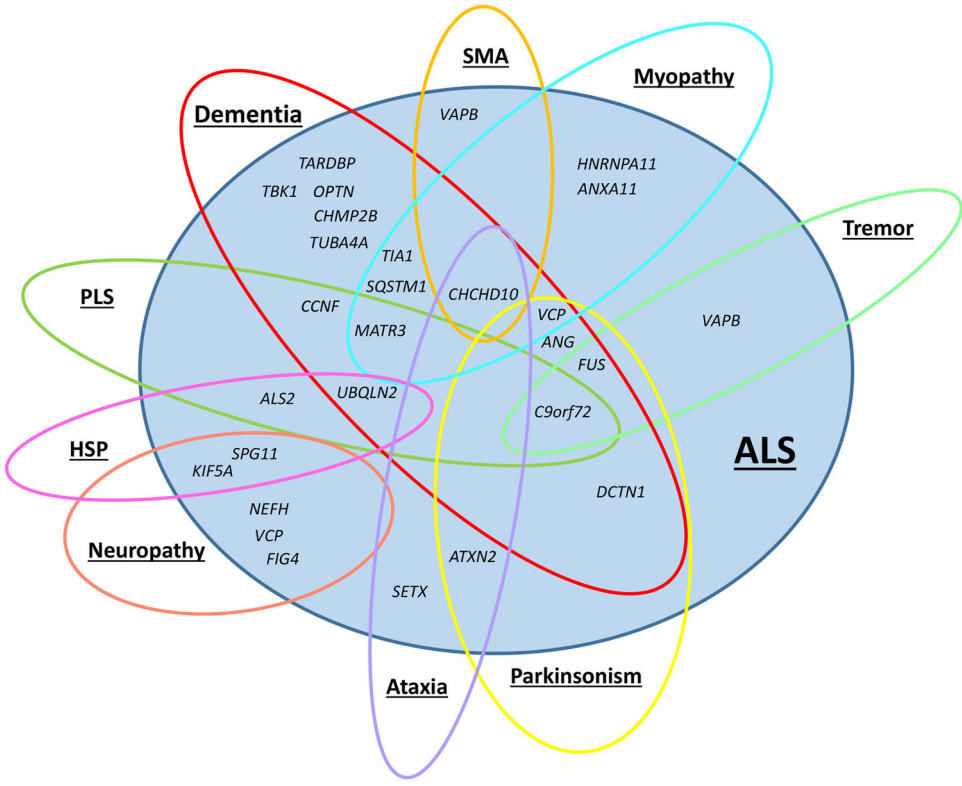

Figure 1. Genetic pleiotropy of amyotrophic lateral sclerosis (ALS) genes. The figure is based on information from Online Mendelian Inheritance in Man (OMIM) (19), GeneReviews (20) and the study by Goutman et al. "Recent advances in the diagnosis and prognosis of amyotrophic lateral sclerosis" (21). Abbreviations: SMA = spinal muscular atrophy; PLS = primary lateral sclerosis; HSP = hereditary spastic paraplegia.

Most identified ALS genes are pleiotropic, i.e., causing more than one disease phenotype, and are involved in other neuromuscular or neurodegenerative disorders (Figure 1). For example, the most common genetic cause of ALS, the *C9orf72* repeat expansion, may also cause frontotemporal dementia (12), parkinsonism (13), and psychiatric diseases including schizophrenia and mania (14). Although ALS and frontotemporal dementia are distinct clinical entities, cognitive impairment occurs in both (15). Furthermore, the *ANG* gene increases the risk of both ALS and Parkinson's disease (PD) (16), whereas *KIF5A* causes ALS, hereditary spastic paraplegia (HSP), and neuropathy (17, 18).

The variable expression of known ALS genes suggests that part of the missing heritability in ALS could be accounted for by genes related to other neuromuscular or neurodegenerative disorders (22). To test this hypothesis, we first investigated the occurrence of neuromuscular or neurodegenerative disorders among the relatives of patients with ALS included in a Norwegian population-based cohort. Second, we explored whether the studied patients with ALS carried possible pathogenic gene variants known to cause other neuromuscular or neurodegenerative phenotypes.

Materials and methods

Study population

Between August 2019 and August 2021, 279 fALS and sALS cases were recruited from all 17 neurological departments in Norway. The clinical characteristics of the study population, inclusion rates and results from the analysis of known ALS genes have previously been described (8). At the time of enrollment, a questionnaire regarding clinical characteristics, and family history of ALS and other neuromuscular or neurodegenerative disorders among first- and second-degree relatives was answered. Patients who reported a family history of ALS among first-degree, second-degree, or other relatives were categorized as fALS. The study was approved by the Regional Committees for Medical and Health Research Ethics (REK) #2018/1916, the Norwegian Center 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.

Genetic analysis

Next-generation sequencing was performed as previously described (8). Sequencing data from all patients were bioinformatically filtered to retrieve

data on 510 genes known to cause neuromuscular or neurodegenerative disorders (Supplementary File 1). The genes were selected using Genomics England PanelApp (https://panelapp.genomicsengland.co.uk/). Genes, in which only expansions contributed to disease development, and genes located in the mitochondrial genome, were excluded. Thirty previously investigated ALS genes were not included, but previous genetic findings (8) were considered during the interpretation. ALS risk genes present in the adult-onset neurodegenerative gene panel from Genomics England were included in the analysis.

The identified variants were filtered based on dominant and recessive inheritance models using gnomAD (https://gnomad.broadinstitute.org/) (23) with a minor allele count of $\leq$ 10 and $\leq$ 250, respectively. Known disease-causing variants registered in the Human Gene Mutation Database (HGMD®) (24) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) were examined regardless of the gnomAD allele count. Intronic regions, untranslated regions, and synonymous variants were discarded before further interpretation unless they had been previously reported as pathogenic. The remaining variants were interpreted using gnomAD, frequencies from a Norwegian in-house hospital database (Department of Medical Genetics, Oslo University hospital) consisting of 12 874 individuals, the *in silico* prediction tool REVEL, Alamut Visual Interface (SOPHiA GENETICS), HGMD®, ClinVar, the Project MinE data browser (http://databrowser.projectmine.com/), and literature (24–28). Special emphasis was given to variants identified in more than one participant, variants previously reported as pathogenic, loss-of-function variants where the loss-of-function is a known disease mechanism or where the gnomAD data predict non-tolerance for loss-of-function variants. An overview of patient inclusion, genetic analysis, and variant interpretation is shown in Figure 2.

Results

Neurodegenerative and neuromuscular disorders among family members

The questionnaires revealed that 30.5% (85/279) of the patients had first- or second-degree relatives with neuromuscular or neurodegenerative disorders, among which dementia and PD were the most common (Figure 3). In our cohort, 32 patients (11.5%) reported a family history of ALS, as specified in our previous report (8). Eight fALS patients (4 first-degree and 4 second-degree) reported relatives with other neurological disorders. Forty-two percent (37/85) did not specify the type of neurological disease reported among relatives. Nearly twice as many patients with ALS reported a family history of neurodegenerative or neuromuscular disorders among first-degree relatives than among second-degree relatives.

Genetic analysis

Our genetic analysis of 510 genes associated with neuromuscular and neurodegenerative disorders revealed 20 potentially pathogenic variants in 25 patients with ALS. All variants had low frequencies both in gnomAD an in the Norwegian hospital database. One patient reported a family history of ALS. Variants were identified in genes causing ataxia, HSP, PD, mitochondrial disorders, sensory neuropathy, spinal muscular atrophy (SMA), and Charcot–Marie–Tooth disease (CMT), and in genes known to increase ALS risk (Table 1). None of the 25 patients had pathogenic variants of the 30 previously investigated ALS genes (8). Four of the 25 patients reported having relatives with neuromuscular or neurodegenerative disorders, one patient reported PD in a first-degree relative, and three patients did not specify the type of disorder or kinship. The clinical characteristics of the patients are shown in Table 2.

Neurodegenerative disorder genes

Case 1 carried a heterozygous nonsense *AFG3L2* variant, which likely has a loss-of-function effect. Pathogenic *AFG3L2* variants cause autosomal recessive spastic ataxia, autosomal-dominant spinocerebellar ataxia, and optic atrophy (19). Loss-of-function *AFG3L2* variants have been reported to cause *AFG3L2*-related disorders (29, 30). Biallelic loss-of-function variants tend to have an earlier onset than heterozygous nonsense variants (29–33). This variant was previously reported as likely pathogenic in ClinVar but without a phenotype.

Case 2, with early disease onset, carried a heterozygous missense *KIF1A* variant. *KIF1A* variants cause both autosomal dominant and recessive HSP as well as recessive hereditary sensory and autonomic neuropathy type 2 (19). Recently, heterozygous *KIF1A* variants were reported in patients with ALS (34). The variant identified in Case 2 is located in the kinesin motor domain close to other pathogenic variants and is predicted to be pathogenic (35, 36). This variant has been reported as a variant of uncertain significance (VUS) for HSP in ClinVar and has been identified in a patient with ALS in Project MinE. Based on the recent study showing a potential link between *KIF1A* and ALS, as well as on the identification of variants located in the C-terminal cargo-binding domain instead of the commonly affected kinesin motor domain (34), we also included variants located in other regions. Two potentially pathogenic heterozygous variants

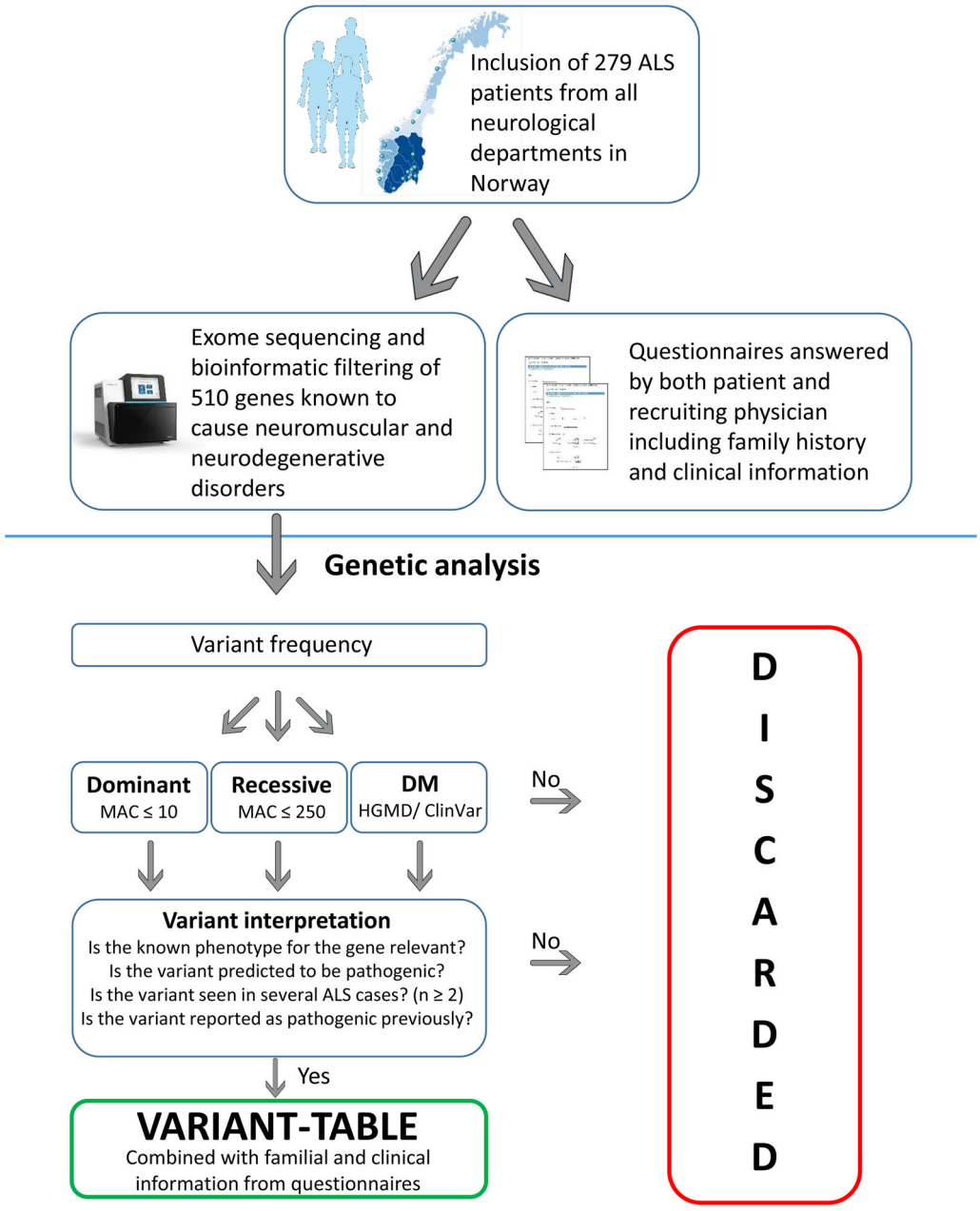

Figure 2. Overview of methods and variant interpretation. DMs = disease-causing mutations, HGMD = the Human Gene Mutation Database, MAC = minor allele count.

were identified (Cases 3 and 4). The variant identified in Case 4 was a VUS in ClinVar.

In Cases 5 and 6, a pathogenic *LRRK2* variant frequently reported to cause PD (37) was identified. This variant has a high carrier frequency and reduced penetrance in Norway (38).

In Cases 7 and 8, an identical heterozygous splice variant in *MSTO1* was identified. *MSTO1* variants cause autosomal recessive mitochondrial and ataxic myopathy with childhood onset (19). Case 7 had bulbar onset in her thirties and reported a first-degree relative with PD, whereas Case 8 reported a negative family history. The identified variant has been reported as pathogenic (biallelic form) in both the literature (39) and ClinVar. Two other *MSTO1* splice variants predicted to be likely deleterious were reported among patients with ALS in Project MinE.

In Cases 9–15, constituting 7/279 (2.5%) of our cohort, one splice variant and two heterozygous loss-of-function *NEK1* variants were identified. Loss-of-function *NEK1* variants increase the risk of ALS (40, 41), but were not included in our previous study (8). Although *NEK1* has not been reported to cause neuromuscular or neurodegenerative disorders other than ALS, it was included in our study because it is a part of the adult-onset neurodegenerative gene panel from Genomics England. *NEK1* variants have been suggested to display reduced penetrance (42) which explains

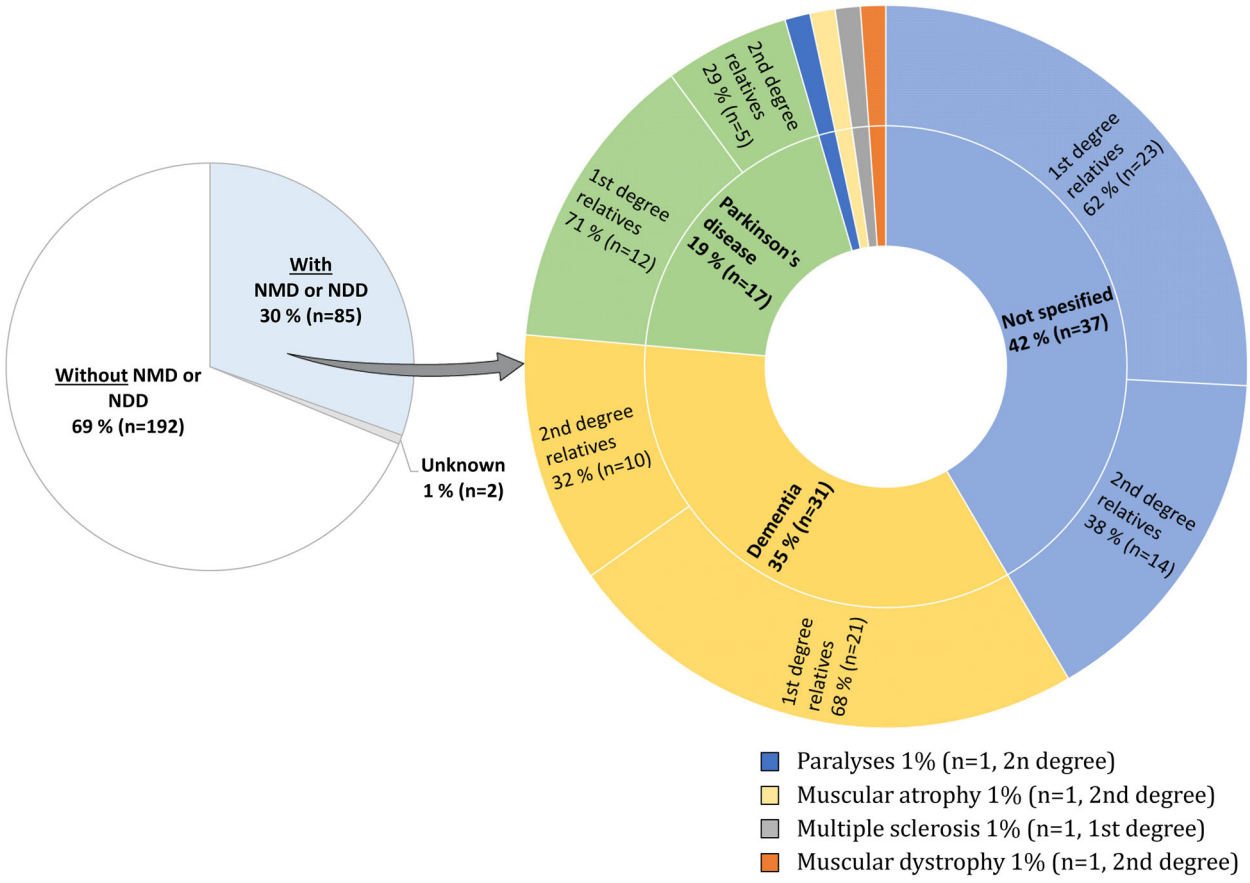

Figure 3. Neuromuscular or neurodegenerative disorders among the relatives of patients with amyotrophic lateral sclerosis (ALS). Left circle: patients with ALS ($n = 279$) reporting relatives with or without other neuromuscular or neurodegenerative disorders. Right circle: neuromuscular and neurodegenerative disorders reported among relatives of the 85 patients with ALS and a positive family history. Four cases had relatives with both PD and dementia; these individuals are shown twice in the right circle. Abbreviation: NMD or NDD = neuromuscular or neurodegenerative disorders.

the relatively high carrier frequency of the p.(Ser1036*) variant identified in four of our cases.

Cases 16 and 17 carried frameshift and splice *SORL1* variants, respectively, both of which are predicted to have a loss-of-function effect. Loss-of-function *SORL1* variants increase the risk of early-onset Alzheimer's disease (43, 44), in which the identified splice variant has been previously reported in early-onset Alzheimer's (45). Neither Case 16 nor Case 17 showed cognitive impairment and did not report relatives with dementia.

Neuromuscular disorder genes. In Case 18, a heterozygous *ATL3* missense variant was identified. *ATL3* variants cause autosomal dominant sensory neuropathy (19). The identified *ATL3* variant was predicted to be pathogenic by *in silico* prediction tools. It is located in the guanylate-binding protein domain and has been reported in ClinVar as a VUS in type 1F hereditary sensory neuropathy. Interestingly, Case 18 showed sensory findings.

Cases 19 and 20 carried hemizygous *ATP7A* variants. *ATP7A* variants are associated with X-linked recessive distal SMA, Menkes disease, and optical horn syndrome. The onset of X-linked SMA usually occurs in the first decade of life; however, adult-onset X-linked SMA can occur as well (19). Case 19 had an early onset in his thirties. A missense *ATP7A* variant was recently reported in an individual with ALS (46). The two variants identified here are highly conserved, predicted to be pathogenic, and located close to other pathogenic variants associated with SMA and Menkes disease, and have been listed as VUS in ClinVar. Case 19 also carried a potentially pathogenic *NEFH* variant (Table 1), which will be discussed later.

Cases 21–23 carried different heterozygous *BICD2* variants. This gene is associated with autosomal dominant SMA, with symptom onset usually in the first decade of life (47). Cases 21 and 22 carried different *BICD2* variants altering the same amino acid at position 265 (Table 1). The p.(Met265Val) substitution has a relatively high carrier frequency, whereas the p.(Met265Leu) variant is absent in gnomAD. The former was previously reported in a patient with HSP (48), but has also been reported as likely benign in ClinVar. Case 23 carried a nonsense variant predicted to have a loss-of-function effect. According to gnomAD, *BICD2* cannot tolerate loss-of-function

Table 1. Variants identified from the genetic analysis of 510 genes known to cause neuromuscular and neurodegenerative disorders.

Case No.	Gene	Associated phenotype	Zyg-osity	cDNA change	Protein change	Frequency				Previous identifications		
						gnomAD (AC)	In-house OUS[1] (AC)	Project Mine cases/control	REVEL[2]	ClinVar Access.	ClinVar Interpret.	HGMD Access.
	Neurodegenerative disorders											
1	*AFG3L2*	Ataxia	Het	NM_006796.3:c.1363C > T	p.(Arg455*)	–	–	–	–	810710	LP	–
2	*KIF1A*	HSP	Het	NM_001244008.2:c.532G > A	p.(Val178Met)	1/248966	–	1/0	0.734	2170690	VUS	–
3	*KIF1A*	HSP	Het	NM_001244008.2:c.1856C > G	p.(Ala619Gly)	–	–	–	0.352	–	–	–
4	*KIF1A*	HSP	Het	NM_001244008.2:c.2131C > T	p.(Arg711Trp)	2/245586	–	–	0.491	850488	VUS	–
5	*LRRK2*	PD	Het	NM_198578.4:c.6055G > A	p.(Gly2019Ser)	138/282542	1/25748	2/0	0.97	1940	P-LP-RF	CM050659
6												
7	*MSTO1*	Mit.dis.	Het	NM_018116.4:c.966 + 1G > A	p.?	2/245702	–	–	–	438834	LP	CS1710302
8												
9	*NEK1*	ALS risk	Het	NM_001199397.3:c.379C > T	p.(Arg127*)	7/222006	–	1/0	–	30428	P	CM110308
10												
11	*NEK1*	ALS risk	Het	NM_001199397.3:c.3107C > G	p.(Ser1036*)	33/280416	23/25748	22/0	–	208600	P-LP	CM173447
12												
13												
14												
15	*NEK1*	ALS risk	Het	NM_001199397.3:c.3715-6G > A	p.?	–	–	–	–	–	–	–
16	*SORL1*	AD	Het	NM_003105.6:c.1016_1020del	p.(Gln339Argfs*7)	–	–	–	–	–	–	–
17	*SORL1*	AD	Het	NM_003105.6:c.4519 + 1G > A	p.?	–	–	–	–	–	–	CS1719333
	Neuromuscular disorders											
18	*ATL3*	Sens.neuro	Het	NM_015459.5:c.484C > G	p.(Leu162Val)	1/248592	–	–	0.831	1046127	VUS	–
19	*ATP7A*	SMA	Hemi	NM_000052.7:c.2957G > A	p.(Arg986Gln)	2/183362	–	–	0.718	245881	VUS	CM078711
20	*ATP7A*	SMA	Hemi	NM_000052.7:c.3007T > C	p.(Ser1003Pro)	–	–	–	0.844	1722297	VUS	–
21	*BICD2*	SMA	Het	NM_001003800.2:c.793A > C	p.(Met265Leu)	–	8/25748	–	0.151	1426770	VUS	–
22	*BICD2*	SMA	Het	NM_001003800.2:c.793A > G	p.(Met265Val)	8/282854	2/25748	–	0.192	1398905	LB	CM1820695
23	*BICD2*	SMA	Het	NM_001003800.2:c.1317C > G	p.(Tyr439*)	–	–	–	–	–	–	–
24	*HARS1*	CMT	Het	NM_002109.6:c.344A > G	p.(Tyr115Cys)	1/251238	–	–	0.811	–	–	–
25	*NEFH*	CMT/ ALS risk	Het	NM_021076.4:c.841C > G	p.(His281Asp)	–	–	–	0.725	1305764	VUS	–
19	*NEFH*	CMT/ ALS risk	Het	NM_021076.4:c.2404C > T	p.(Pro802Ser)	–	–	–	0.213	–	–	–

Abbreviations: 1-allele frequency from a Norwegian in-house hospital database (Department of Medical Genetics, Oslo University hospital) obtained from families referred for genetic analysis of various disorders. 2-REVEL is an *in silico* tool used for predicting the pathogenicity of missense variants. The score can range from 0 to 1, with higher scores indicating a higher possibility that the variant is disease causing (25). AC = allele count; Access. = Accession; AD = Alzheimer's disease; ALS = amyotrophic lateral sclerosis; CMT = Charcot–Marie–Tooth disease; dis = disorder; Het = heterozygous; Hemi = hemizygous; HSP = hereditary spastic paraplegia; Interpret. = interpretation; LB = likely benign; LP = likely pathogenic; Mit. = mitochondrial; P = pathogenic; PD = Parkinson's disease; RF = risk factor; Sens.neuro = sensory neuropathy; SMA = spinal muscular atrophy; VUS = variant of uncertain significance.

Table 2. Patient phenotypes at inclusion.

Case No.	Gender	ALS type	Relatives [w]/NMD or NDD (type)	Relative degree	Age of onset	# years from onset to diagnosis	Site of onset	Bulbar signs	UMN signs	LMN signs	Sensory symptoms	Cognitive affection	Neurological findings consistent with ALS	El Escorial fulfilled
1	F	sALS	–	–	70–80	NA	S	Y	Y	–	–	–	Y	Y
2	M	sALS	–	–	30–40	3	S	–	Y	Y	–	Y	Y	Y
3	F	sALS	–	–	50–60	2	S	–	–	Y	–	–	Y	U
4	F	sALS	–	–	60–70	0	S	–	Y	Y	–	–	Y	Y
5	F	sALS	Y (NA)	NA	70–80	1	S	Y	Y	Y	–	–	Y	Y
6	M	sALS	–	–	60–70	2	Both	Y	Y	Y	Y	–	Y	Y
7	F	sALS	Y (PD)	1st	30–40	1	S	–	Y	Y	–	–	Y	Y
8	F	sALS	–	–	70–80	0	B	Y	–	Y	–	–	Y	Y
9	M	sALS	–	–	60–70	1	S	–	Y	Y	–	–	Y	Y
10	M	sALS	–	–	50–60	1	S	–	U	Y	–	–	U	–
11	M	sALS	Y (NA)	NA	50–60	3	S	–	Y	Y	–	–	Y	Y
12	M	fALS	–	–	70–80	1	S	–	U	Y	–	–	Y	Y
13	M	sALS	–	–	70–80	1	Both	Y	Y	Y	–	–	Y	Y
14	F	sALS	–	–	40–50	1	B	Y	Y	Y	–	–	Y	Y
15	M	sALS	–	–	50–60	0	S	–	Y	Y	–	–	Y	Y
16[A]	F	sALS	–	–	60–70	7	S	Y	Y	–	–	–	–	–
17	F	sALS	–	–	50–60	3	S	Y	Y	Y	–	–	Y	Y
18	M	sALS	–	–	50–60	1	S	–	Y	Y	Y	–	Y	Y
19	M	sALS	–	–	30–40	1	S	–	Y	Y	–	–	Y	Y
20	M	sALS	Y (NA)	NA	60–70	1	Both	Y	Y	Y	–	U	Y	Y
21	M	sALS	–	–	70–80	3	S	–	Y	Y	–	Y	Y	Y
22	M	sALS	–	–	50–60	1	S	–	Y	Y	–	–	Y	Y
23	F	sALS	–	–	40–50	1	Both	Y	Y	Y	–	–	Y	U
24	F	sALS	–	–	60–70	3	S	–	–	Y	–	–	Y	–
25	M	sALS	–	–	60–70	1	B	Y	Y	Y	–	–	Y	Y

Abbreviations: A = Patient diagnosed with PLS; ALS = amyotrophic lateral sclerosis; B = bulbar; D = dementia; F = female; fALS = familial ALS; LMN = lower motor neuron; M = male; NA = not available; NDD = neurodegenerative disorder; NMD = neuromuscular disorder; PD = Parkinson's disease; S = spinal; sALS = simplex ALS; U = uncertain; UMD = upper motor neuron; Y = yes.

variants. To the best of our knowledge, this is the first patient report of a loss-of-function variant in *BICD2*.

Case 24 harbored a heterozygous missense *HARS1* variant. This gene causes autosomal dominant axonal CMT type 2W, which can resemble lower motor neuron ALS (19). The identified variant affects a highly conserved amino acid and is predicted to be pathogenic. Case 24 had only lower motor neuron symptoms.

Heterozygous missense *NEFH* variants were identified in Cases 25 and 19. *NEFH* variants cause autosomal dominant CMT type 2CC, and this gene is believed to be a susceptibility gene for ALS (19). The variant identified in Case 25 was located close to other variants implicated in ALS (24), whereas that identified in Case 19 changed a highly conserved proline residue in the *NEFH* tail domain. This domain contains distinctive regions of lysine-serine-proline repeats (49), and deletion, insertion, and missense variants in this region have been suggested to be causative factors of ALS (50–52).

Discussion

The extensive clinical overlap between ALS and other neurodegenerative or neuromuscular disorders suggests that some of the missing heredity in ALS is caused by genetic variants underlying related disorders (22). Accordingly, a recent genome-wide association study found that the genetic overlap between different neurodegenerative disorders, including ALS, is more extensive than expected (59). To explore this hypothesis further, we examined rare variants in 510 genes implicated in neurodegenerative and neuromuscular phenotypes in 279 patients with ALS and investigated the possible coexistence of a neurodegenerative or neuromuscular family history and rare variants in genes causing similar disorders.

Nearly one-third of the patients with ALS reported relatives with related neuromuscular or neurodegenerative disorders. Unfortunately, nearly half of them did not specify the disorder type, making it difficult to compare our results with those of similar studies. Among the patients that specified the phenotype, neurodegenerative disorders, particularly dementia and PD, were much more common than neuromuscular disorders. Some (53, 54), but not all previous studies (55, 56) have found an increased prevalence of PD and Alzheimer's disease among relatives of patients with ALS. Furthermore, twice as many patients with ALS in our study reported other neurological disorders among their first-degree relatives than among their second-degree relatives. This may reflect a lack of knowledge about second-degree relatives.

Among the 510 neurodegenerative and neuromuscular genes, we identified 20 possible pathogenic variants. Fifteen patients had variants in genes causing other neurological phenotypes, whereas nine patients had variants in genes increasing ALS risk. One patient had a variant in both *ATP7A* and *NEFH*, the latter being an ALS risk gene. Intriguingly, only three of the 15 patients with possible pathogenic variants in other neurodegenerative or neuromuscular genes reported relatives with such disorders, and one patient with a *NEK1* variant reported a family history of ALS. Whether this was because of the absence of a causal relationship, reduced penetrance, or missing information regarding disorders among family members is unknown.

Among the neurodegenerative genes, *AFG3L2*, *KIF1A*, and *MSTO1* have previously been directly or indirectly linked to ALS, making their variants particularly interesting. *AFG3L2* is an important *SPG7* paralogue. Together, they encode an m-AAA protease that degrades damaged proteins and regulates mitochondrial ribosome synthesis (57). Heterozygous *SPG7* variants have recently been associated with increased ALS risk (58). *KIF1A* is part of the anterograde transport machinery (59), and was recently proposed as an ALS gene (34). *KIF5A*, another kinesin motor gene involved in anterograde transport, is known to increase ALS risk (60). *MSTO1*, a gene important for mitochondrial dynamics, has been implicated in pathways perturbed in ALS (61). *MSTO1* has not yet been linked to monoallelic disease; however, it is not uncommon for neurodegenerative genes to cause both a severe early-onset recessive phenotype and a late-onset dominant phenotype, as observed for the ALS genes *FIG4* and *GLE1* (19). To our knowledge, neither *SORL1* nor *LRRK2* have been implicated in ALS pathology; thus, it is uncertain whether the variants identified in our study are relevant to ALS or merely an incidental finding.

Among the neuromuscular genes, variants were identified in two genes, namely *ATP7A* and *BICD2*, known to cause SMA or distal motor neuropathy. These disorders may resemble spinal ALS, but usually have a much earlier onset (19, 62). Notably, one of our patients carrying an *ATP7A*-variant had ALS onset in his thirties. *BICD2* variants usually cause childhood-onset SMA; however, its onset in adulthood has recently been reported (47, 63). In our study, two patients with *BICD2* had spinal-onset ALS. Furthermore, *BICD2* is involved in retrograde transport along axon microtubules (19, 59) together with two other motor neuron associated genes, namely *DYNC1H1* (64–66) and *DCTN1* (19, 67), suggesting a plausible role in ALS pathogenesis. Two patients carried variants in genes involved in peripheral neuropathy, namely *ATL3* and *HARS1*

(19). To our knowledge, these genes have not been associated with ALS. Notably, *ATL3* is a sensory neuropathy gene, and Case 18 had spinal ALS with sensory neuropathy; in contrast, *HARS1* is an axonal neuropathy gene, and Case 24 reported spinal ALS with pure lower motor signs at the time of diagnosis.

We identified variants in two genes that increase the ALS risk: *NEFH* and *NEK1* (19). *NEFH* was first proposed as an ALS risk gene because it encodes the heavy neurofilament protein that is a biomarker of neuronal damage, as the neurofilament accumulation may play a causal pathogenic role in ALS development (68, 69). However, reports confirming this relationship have been lacking until recently, when especially variants in the *NEFH* tail domain that disturb distinctive lysine-serine-proline repeats, have been reported to be disease-causing (70, 71). Case 19 carried an *NEFH* variant that changed a highly conserved proline residue in the tail domain, making this variant particularly interesting. This patient had ALS onset in his thirties and he also carried a potentially pathogenic *ATP7A* variant; therefore, it could be speculated in a possible digenic effect.

Seven (2.5%) patients with ALS in our cohort carried *NEK1* variants predicted to cause loss-of-function. Loss-of-function *NEK1* variants have been reported to increase ALS risk (40, 41). In a meta-analysis involving 8603 cases from five European and three Asian studies, loss-of-function variants were reported in 1% of the patients from Europe and 0.7% from Asia (72). This indicates a high prevalence of *NEK1* loss-of-function variants in our Norwegian cohort compared with other populations.

The main limitation of the present study is that family history was based on self-reported information; thus, it is possible that the numbers are falsely low, either caused by early death, unknown family history, misunderstandings, forgetfulness, denial, or, in some cases misdiagnosis because of unclear symptoms. The lack of a matched control group makes it difficult to interpret whether there is an enrichment of these disorders among relatives of our ALS patients. Furthermore, it cannot be ruled out that some variants have not been called or that our strict bioinformatics filtering discarded some relevant variants with higher frequency and reduced penetrance. The relationship between the genes identified in this study and ALS pathogenesis is unclear, and a causal relationship needs to be further confirmed. It should also be stressed that although genetic variants are rare, they are mostly not disease-causing. Several studies have shown that rare genetic variants are much more common than previously thought (73, 74), and the minor allele frequencies of 99% of the variants in the ExAC frequency database are lower than 1% (74). Nevertheless, we believe that several variants and genes identified in the present study are good candidates for further studies. The low frequencies of these variants in the Norwegian hospital database indicates that they are not part of the normal genetic background in Norway.

In conclusion, a substantial proportion (9%) of Norwegian ALS patients carry rare genetic variants associated with other neurodegenerative or neuromuscular disorders, regardless of whether they report a family history of such disorder or not. ALS risk variants in *NEK1* seems to be relatively frequent in the Norwegian population.

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Disclosure statement

The authors report there are no competing interests to declare.

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Data availability statement

Data supporting the findings of this study are available from the corresponding author upon request.

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