



# Revisiting the complex architecture of ALS in Turkey: Expanding genotypes, shared phenotypes, molecular networks, and a public variant database

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**Abstract**

The last decade has proven that amyotrophic lateral sclerosis (ALS) is clinically and genetically heterogeneous, and that the genetic component in sporadic cases might be stronger than expected. This study investigates 1,200 patients to revisit ALS in the ethnically heterogeneous yet inbred Turkish population. Familial ALS (fALS) accounts for 20% of our cases. The rates of consanguinity are 30% in fALS and 23% in sporadic ALS (sALS). Major ALS genes explained the disease cause in only 35% of fALS, as compared with ~70% in Europe and North America. Whole exome sequencing resulted in a discovery rate of 42% (53/127). Whole genome analyses in 623 sALS cases and 142 population controls, sequenced within Project MinE, revealed well-established fALS gene variants, solidifying the concept of incomplete penetrance in ALS. Genome-wide association studies (GWAS) with whole genome sequencing data did not indicate a new risk locus. Coupling GWAS with a coexpression network of disease-associated candidates, points to a significant enrichment for cell cycle- and division-related genes. Within this network, literature text-mining highlights *DECR1*, *ATL1*, *HDAC2*, *GEMIN4*, and *HNRNPA3* as important genes. Finally, information on ALS-related gene variants in the Turkish cohort sequenced within Project MinE was compiled in the GeNDAL variant browser ([www.gendal.org](http://www.gendal.org)).

**KEYWORDS**

ALS, ALS variant database, genetics, clinical exome sequencing, coexpression network analysis, genome-wide association study, motor neuron disease, next generation sequencing, Turkish peninsula

**1 | INTRODUCTION**

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease of upper and lower motor neurons, leading to muscle wasting. The average age of onset (AO) is 55–60 years, however juvenile cases exist (Ghasemi & Brown, 2018). Two-thirds of patients present with spinal-onset, the rest shows bulbar-onset with dysarthria and/or swallowing problems. Cognitive and behavioral changes are seen in almost 50% of ALS cases (van Es et al., 2017). The disease results in death within 2–5 years due to respiratory failure, which can only be slightly extended with exceptional medical care (Brown & Al-Chalabi, 2017; Kiernan et al., 2011).

ALS genetics is complex; the familial form (fALS) is rare (5–10%), sporadic cases or isolated (singlet) patients (sALS) constitute 90% of cases. Familial and sporadic ALS are clinically indistinguishable and well-established fALS genes are implicated in sporadic disease, pointing to “apparently” sporadic cases with incomplete penetrance (Brown & Al-Chalabi, 2017).

In populations in which consanguinity is common, juvenile atypical disease accompanies classical, resulting in a more heterogeneous genetic background that makes the differential diagnosis in the clinic challenging.

The Human Genome map, advances in next generation sequencing (NGS), genome-wide association studies (GWAS), and applications of whole exome sequencing (WES) changed paradigms in identifying ALS-associated alleles even with low power, both in family-based studies and in large admixed populations (Brenner et al., 2018; Cirulli et al., 2015; Smith et al., 2017). Moreover, GWAS performed using single nucleotide polymorphisms (SNPs) from whole genome sequencing (WGS) data, enabled association of rare variants with rare diseases, such as ALS (Nicolas et al., 2018; van Rheenen et al., 2016). Project MinE Sequencing Consortium, a large multinational ALS collaboration, is established based on this purpose, to define new disease-causing genes and risk loci associated with true sporadic disease to target novel therapeutics (van Rheenen et al., 2018).

## 2 | DATA SPECIFICATIONS

Data type	Tables and figures
Data acquisition method	Sanger sequencing, NGS
Data format	Filtered and analyzed
Experimental factors	None
Experimental features	Pathogenic genomic variation analysis in a large Turkish ALS cohort using conventional and next generation sequencing methods. Variant interpretation through in silico tools and clinician-researcher collaboration. Genotype-phenotype correlations in ALS and ALS-like disease. Data collection and sharing in public databases for ALS.
Data source location	Suna and İnan Kıraç Foundation Neurodegeneration Research Laboratory, Koç University Hospital, Davutpaşa Street No. 4, 34010, Istanbul, Turkey
Data accessibility	Data in this paper is published within the paper and deposited to ClinVar public database ( <a href="https://www.ncbi.nlm.nih.gov/clinvar/?term=SUB7287039">https://www.ncbi.nlm.nih.gov/clinvar/?term=SUB7287039</a> ).

## 3 | IMPACT OF DATA

The last decade has seen an unprecedented and exponential progress in data output, based on advances in genetics/genomics and on large international collaborations. Consequently, our knowledge of the genetic factors behind ALS has improved in an unparalleled fashion and the scientific scenario of ALS has dramatically changed. Today, the disease is accepted to be part of a continuum with other neurological diseases and a crossroads between genetic, neurometabolic and environmental factors.

This manuscript, five years apart from our previous publication on ALS in Turkey (Özoğuz et al., 2015), is not only an update with a triple increase in patient numbers, but it supersedes our earlier work by reflecting a whole new picture, being much more comprehensive in its scope, upgraded in cutting edge techniques, applying genome-wide and bioinformatic approaches to extract candidate disease genes and pathways, followed by a population-specific database.

Our understanding of the etiopathology of neurological diseases stem from the identification of disease genes and pathways. With its well-selected and large cohort, this study not only represents a distinct resource for ALS in Turkey, it also reveals the genetic variation in a highly inbred and admixed population, is thus expected to contribute to human disease at large.

## 4 | EXPERIMENTAL DESIGN, MATERIALS AND METHODS

This study includes 1,200 Turkish patients recruited from hospitals across Turkey between 2002 and 2019; 246 cases with a family history of ALS, plus 80 affected family members and 954 isolated ALS cases. Sample collection was approved by Boğaziçi University Ethics Committee. Genetic counseling was given to patients at the local institutions during blood collection and signed informed consent was obtained from all subjects. DNA samples from healthy relatives were obtained for research purposes only with their written approval. Genomic DNA was isolated from whole blood using the MagNa Pure Compact System (Roche, Switzerland).

### 4.1 | Screening for common ALS genes

Conventional screening for the hexanucleotide repeat expansion in the *C9orf72* gene was performed in all patients with or without family history of ALS, whereas *SOD1*, *TARDBP*, and *FUS* were screened only in familial cases. The *C9orf72* repeat expansion was tested using repeat-primed polymerase chain reaction (PCR) and flanking PCR was performed to identify the zygosity and the size of the repeats within the normal range. All five exons of *SOD1* were analyzed in fALS. Additionally, exon 4 of the *SOD1* gene was screened in all cases with consanguineous parents, independent of family history. Genomic variant analyses in *TARDBP* and *FUS* genes were restricted to their hotspots, exon 5 for *TARDBP* and exons 14 and 15 for *FUS*. Genotyping experiments were performed with GoTaq® Flexi DNA Polymerase (Promega), MyTaq™ DNA Polymerase (Bioline), FastStart Universal Master Mix (Roche, Switzerland) and One Taq® 2× Master Mix (New England Biolabs). The sequences of primers are available upon request. Sanger sequencing was outsourced (Macrogen Inc., Korea) and CLC Main software (Qiagen, Germany) was used for analysis.

#### 4.1.1 | Bisulfite sequencing in the promoter region of *C9orf72*

The 5mC levels of the *C9orf72* promoter regions harboring 26 CpG sites were detected using direct bisulfite sequencing assay (BST-PCR). EZ DNA Methylation-Gold Kit (Zymo Research) was used for bisulfite conversion of genomic DNA according to the manufacturer's protocol. The converted genomic DNA was amplified using nested PCR with primers targeting the converted sequence in the promoter region. ZymoTaq Premix was used for these consecutive amplifications. Methylation levels were detected by direct evaluation of Sanger sequencing results. Commercially available human methylated (100%) and nonmethylated (0%) standards were used as controls, 50% control was prepared by mixing equal amounts of commercial standards (Zymo Research). The number of methylated

CpG sites was calculated for each individual and two-tailed Fisher's exact test was used to assess the association between promoter hypermethylation for the expansion carriers. The maximum number of methylated CpG sites among controls (2/26) was considered as the threshold for hypermethylation.

## 4.2 | WES

WES was applied in 250 individuals; 127 probands, 32 affected, and 91 healthy family members (Macrogen Inc., Korea). Selection criteria of the patients subjected to WES were (a) close consanguinity in the parents of the affected individual, (b) atypical clinical features, and (c) early/juvenile disease-onset. In addition, WES was also applied to cases with a positive family history of disease in the upper generations, if screening in four common dominant genes did not reveal any disease-associated variants. Suspected inheritance pattern was autosomal dominant for 25, autosomal recessive for 79 families, and in 23 cases the inheritance pattern could not be certified. Clinical information of the cases subjected to WES, their suspected inheritance patterns and initial clinical diagnoses are listed in Appendix (Table A1).

Bioinformatic analyses of the samples were initially performed using in-house Burrows-Wheeler Aligner (BWA) (H. Li & Durbin, 2009) and Genome Analysis Toolkit (GATK; McKenna et al., 2010) pipeline. More recently, the online SEQ Platform, a cloud-based genomics software, was used and all samples have been retrospectively analyzed with this platform (Genomize Inc., Turkey). The SEQ Platform enables calculation of real-time minor allele frequency (MAF) for variants using NDAL- and SEQ-specific cohorts.

For the in-house pipeline, paired-end sequencing reads obtained from sequencing platforms were aligned to the human reference genome GRCh37 plus the decoy using BWA-MEM algorithm. Quality control and variant calling from binary sequence alignment/map format files were performed with HaplotypeCaller tool of GATK. The ANNOVAR software was used for structural and functional annotation of variants (Wang, Li, & Hakonarson, 2010). MAFs were recruited from 1000 Genomes Project (1000G) and National Heart, Lung and Blood Institute Exome Sequencing Project (NHLBI-ESP6500; Auton et al., 2015; EVS, 2014). Functional consequences of variants were predicted via several sources (e.g., SIFT, PolyPhen2, and GERP++) and DANN scores were assigned to each variant.

For variant prioritization, the association of candidate genes with known human phenotypes was obtained from the OMIM database. Annotated variants were filtered using the VarSifter software (version 1.7) or SEQ Platform according to the inheritance mode and MAF (>0.01; Teer, Green, Mullikin, & Biesecker, 2012). Functional predictions were used for evaluation, but not for filtration. Center (NDAL)-specific MAF lower than 0.01 was used as a parameter during variant prioritization in the WES data of 600 Turkish patients and healthy family members. American College of Medical Genetics (ACMG) guideline verdict was determined for each candidate for further evaluation. Segregation analysis for candidate variants was

performed by Sanger sequencing in the index case and in all available family members.

## 4.3 | WGS

Whole genome sequencing of 632 Turkish sALS cases and 151 neurologically healthy controls was performed within the scope of Project MinE. Samples were selected on the basis of definitive, late-onset ALS diagnosis, without a family history. The mean AO for the patients included in Project MinE was 51 years, in agreement with 52-year-old mean AO of the total sALS cohort; control subjects had a mean age of 55 years. *C9orf72* hexanucleotide repeat expansion was excluded in all patients before WGS. Project MinE guidelines were followed for sample selection and preparation (van Rheenen et al., 2018). PCR-based library free paired-end sequencing was performed on the Illumina HiSeq 2000 platform with an average of 40× coverage per sample (Illumina FastTrack Services, San Diego). Alignment to the hg19 reference genome and variant calling were performed using the Isaac pipeline and provided by Illumina as aligned reads in BAM files and individual-based gVCF files containing the single nucleotide variations (SNVs), short indels and structural variations (Raczy et al., 2013).

Protein coding variants in all ALS-causing and -associated genes reported (Ghasemi & Brown, 2018) were screened in annotated variant files. Candidate variants identified in sALS patients were further analyzed for pathogenicity using prediction tools, VarSome software (Kopanos et al., 2019) and our in-house exome database.

### 4.3.1 | WGS sample processing and quality control

All variants across the individuals were merged with AGG tool (Illumina). Individual/variant-level quality control was performed using PLINK (version: 1.9; Purcell et al., 2007) and VCF-tools (Danecek et al., 2011) (version:0.1.16). Samples with a deviated inbreeding coefficient (>3 SD) from the mean of the distribution, as well as related/duplicate samples and those with missingness rate higher than 10% were not included for further analyses (623 cases and 142 healthy controls remaining). A pruned set of high-quality SNPs were prepared using missingness rate (<10%), MAF (>5%) and Hardy-Weinberg equilibrium ( $p < 1 \times 10^{-6}$  for controls and  $p < 1 \times 10^{-12}$  for cases) thresholds. SNPs within the *MHC* or *LCT* loci or, the inversions on chromosome 8/17 were excluded. Principal components (PCs) for each individual were calculated using PLINK.

### 4.3.2 | Genome-wide association study

Variants with MAF > 5% in the whole cohort were tested for association using a binary logistic regression in PLINK. First 10 PCs and gender were used as covariates.

**TABLE 1** Clinical characteristics of the Turkish ALS cohort under study

	Total ALS	fALS	sALS
Number			
Probands	1,200	246 (20%)	954 (80%)
Affected family members	80	80	–
Male:female ratio	1.5	1.2	1.6
Consanguinity	301 (25%)	75 (30%)	226 (24%)
Dementia	30 (2.5%)	13 (5%)	17 (2%)
Age of onset			
Juvenile (<25 years)	101 (8%)	33 (13%)	68 (7%)
Middle (25–45 years)	292 (24%)	61 (25%)	231 (24%)
Late (>45 years)	718 (60%)	123 (50%)	595 (62%)
Not available	89	29	60
Mean age of onset (total ±SD)	50 ± 15.4	47 ± 16.9	51 ± 15.1
Site of onset			
Limb	773 (64%)	163 (66%)	610 (64%)
Bulbar	212 (18%)	36 (15%)	176 (18%)
Limb + bulbar	84 (7%)	12 (5%)	72 (8%)
Not available	131	35	96

Abbreviations: ALS, amyotrophic lateral sclerosis; fALS, familial ALS; sALS, sporadic ALS; SD, standard deviation.

### 4.3.3 | Gene-based burden testing

All variants were annotated using Ensembl Variant Effect Predictor version 92 (McLaren et al., 2016) and classified into two functional groups for gene-based association testing: (a) disruptive variants (stop-gained, stop-loss, start-loss, splice sites, and frameshift indels with high confident according to loftee prediction), and (b) missense variants predicted to be damaging using REVEL and MetaLR algorithms, on the basis of a combining approach (Dong et al., 2015; Ioannidis et al., 2016). ClinVar-benign variants were excluded. ClinVar-pathogenic variants were kept regardless of other elimination criteria (Landrum et al., 2014). In the remaining list, variants with MAF ≥ 1% in any public population databases (ExAC, gnomAD, 1000 G, and ESP6500; Auton et al., 2015; EVS, 2014; Exome Aggregate Consortium, 2016; Karczewski et al., 2019) and variants with MAF ≥ 5% in our cohort were excluded.

Gene-based burden testing was performed using the R-package of SKAT-O by aggregating disruptive and possibly damaging variants (missense variants) on genic regions and pathways (Ionita-Laza, Lee, Makarov, Buxbaum, & Lin, 2013). Pathway and associated lists were downloaded from the Broad Institute GSEA site (<http://software.broadinstitute.org/gsea>) using all canonical pathway dump (version 6.2). Tests were adjusted for gender and first 10 PCs.

### 4.3.4 | Gene coexpression network analysis

The ST-Steiner algorithm (Norman & Cicek, 2019) was used, which searches for a connected component (a tree) on a gene coexpression network or a cascade of gene-coexpression networks. The algorithm

aims at maximizing the prizes of the selected genes and minimizes the cost of edges that are used to connect these genes.

To construct the gene coexpression network, we utilized the full BrainSpan microarray data set of the Allen Brain Atlas (Sunken et al., 2013). This data set contains gene expression measurements of 524 brain samples from various brain regions obtained from 42 individuals that represent various time points in neurodevelopment, starting from embryonic period up to adulthood. We used correlation threshold of 0.7 (Pearson correlation,  $r^2$ ). That is, an edge was added to a graph if two genes' correlation exceeded this threshold. This is a common threshold choice in the literature (Çiçek, 2017; Liu et al., 2014; Liu, Lei, & Roeder, 2015). After pruning for the genes that do not exist in the data, the final network contains 547,056 edges and 8,499 nodes.

As our edge cost  $1-r^2$  was used. For the node (gene) prizes, we used the negative  $\log_{10}$  transformed  $p$ -values derived from gene-based SKAT-O analysis. The ST-Steiner algorithm also inputs a list of terminal nodes, which have to be included in the tree (due to a very large artificial prize) and joined by other nodes. These are the genes with high level of risk confidence for ALS: *SOD1*, *TARDBP*, *SQSTM1*, *HNRNPA1*, *FUS*, *VCP*, *OPTN*, *PFN1*, *ATXN2*, *NEFH*, *SETX*, *ALS2*, *DCTN1*, *ANG*, *ELP3*, *FIG4*, *TAF15*, *SPG11*, *NEK1*, *PON1*, *PON3*, *TBK1*, *DAO*, *CHRNA3*, *CHRNA4*, *CREST* (*SS18L1*), *CHRNA4*, *NTE* (*PNPLA6*) (Ghasemi & Brown, 2018).

There are four hyperparameters to set in the ST-Steiner Algorithm. The first parameter is  $\omega$ , which is the number of trees in an estimated forest. We set this parameter to 0 to obtain a single connected component with the assumption of a single functional cluster of genes as done in Norman and Cicek (2019).  $\lambda$  and  $\alpha$  were set to zero, since the algorithm was run on a single network and

these parameters are used when a cascade of networks is employed. The third parameter  $\beta$  was used to put the node prizes and the edge costs on the same scale and adjust the size of the predicted sub-network. After a line search to obtain a network, which includes approximately three predictions for every ground truth (terminal) gene, it was set to 0.17. Edge thickness denotes the correlation threshold (thicker = higher correlation).

Functional annotation clustering of the candidate genes predicted in the coexpression network was performed using DAVID Functional Annotation Tool (version 6.8; Huang et al., 2007). All 8,499 nodes (genes) used to create the coexpression network were given to the algorithm as background. Literature-mining for association between the predicted genes in the network and ALS was evaluated by screening GeneRif and DisGeNet databases (all species considered; Jimeno-Yepes, Sticco, Mork, & Aronson, 2013; Piñero et al., 2017). We determined the number of occurrences of genes in association with ALS based on specific search matching restricted words: "ALS," "fALS," "sALS," and "amyotrophic lateral sclerosis." The output counts were used as a score to denote the strength of the evidence for each gene with ALS in GeneRIF and DisGeNet databases (Table A2).

## 5 | DATA

### 5.1 | 45% of fALS and 10% of sALS are explained by known ALS genes in the Turkish cohort, indicating genetic heterogeneity in fALS and incomplete penetrance among sALS patients

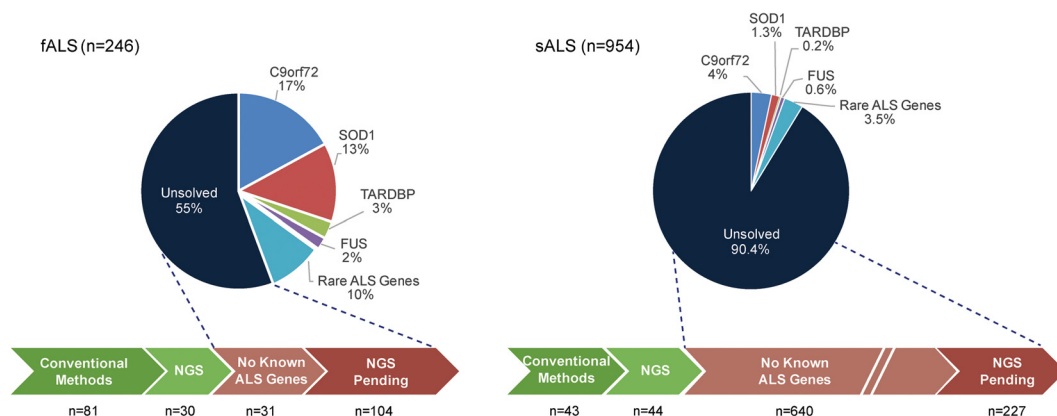
A total of 1,200 Turkish patients diagnosed with ALS or ALS-like motor neuron disease were analyzed within the scope of this study, adopting a combination of conventional and NGS approaches. The clinical summary of the study cohort is compiled in Table 1. Sixty percent of the Turkish ALS cohort under study had an age of disease

onset beyond 45 years and the mean ages of onset were 47 for fALS and 51 for sALS patients. In 64% of our cases, spinal symptoms were detected as the initial clinical features, 18% reported to suffer from bulbar symptoms and 7% showed mixed site of onset. The male to female ratio in the present cohort was 1.5.

Four common ALS genes (*C9orf72*, *SOD1*, *TARDBP*, and *FUS*) contribute to 35% of fALS and 6.1% of sALS in Turkey and analysis of pathogenic exonic variants obtained from WES and WGS data increases these numbers to 45% in fALS and 10% in sALS (Figure 1). This 10% of sALS cases explained by genomic variants in well-established and highly penetrant ALS genes, like *C9orf72*, *SOD1*, *TARDBP*, *FUS*, *OPTN* and *VCP*, are "apparently" sporadic, who are either (a) the only affected child of consanguineous couples, or (b) cases with low penetrance of the variant in the upper generations, or (c) carriers of de novo variants.

The GGGGCC hexanucleotide repeat expansion in the *C9orf72* gene was detected in 42 families (plus 8 affected family members) and in 38 sporadic cases in 1,200 ALS patients (Table A3). Mean age of disease onset among the expansion carriers was 54.5, representing classical ALS. A higher frequency of bulbar-onset ALS was observed among *C9orf72* cases (23%), compared with 18% in all cases. Intrafamilial phenotypic variability was present among family members manifesting either ALS, ALS accompanied by frontotemporal dementia (ALS-FTD) or solely FTD symptoms. Dementia was reported in nine expansion carriers and two affected family members (13%).

In ALS cases with or without the expansion and in controls, two, five, and eight repeats were found to be the predominant allelic variants in the Turkish population for the nonexpanded allele of the *C9orf72* gene. In three cases the intermediate repeat sized GGGGCC<sub>(30-35)</sub> was detected, which did not segregate with the disease in the two families tested. Bisulfite sequencing assay of the 26 CpG sites, located in the promoter region of *C9orf72*, revealed a significant increase in promoter hypermethylation for the expansion carriers ( $n = 52$  expansion carriers and 31 age- and sex-matched controls, Student's  $t$  test  $p < .0023$ ); no significant correlation was



**FIGURE 1** Frequency of ALS gene variants in the Turkish cohort. The four major ALS genes account for 35% of fALS, NGS increases this number to 45% (left pie). The same four ALS genes solve 6.1% of sALS, NGS increases it to almost 9% (right pie). The dark blue areas in the pies, represent unsolved cases and also samples not yet analyzed by NGS. ALS, amyotrophic lateral sclerosis; fALS, familial ALS; NGS, next generation sequencing; sALS, sporadic ALS

observed between number of CpGs methylated and the AO of patients (Hamzeiy et al., 2018).

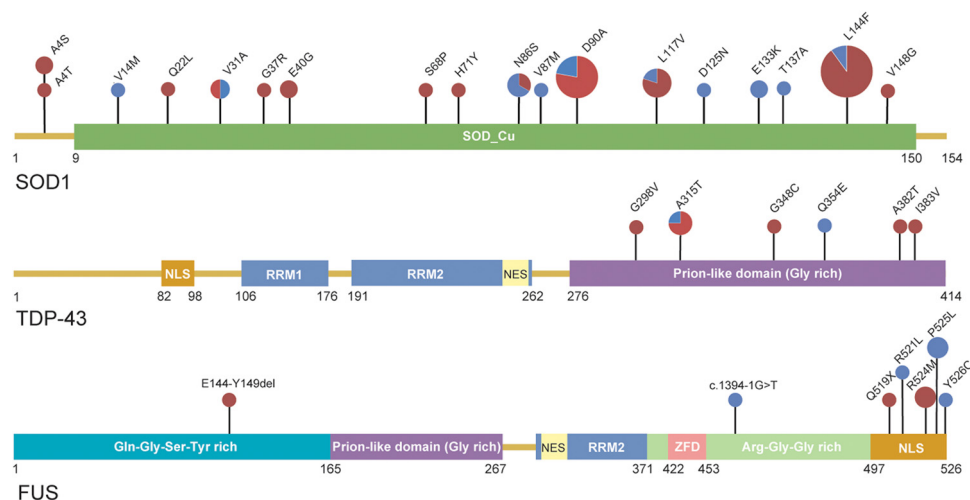
Eighteen distinct pathogenic genomic variants in the *SOD1* gene were identified in 57 patients (32 fALS index cases plus 13 affected family members and 12 sporadic cases; Figure 2; Table 2). The human reference transcript NM\_000454.4 was used for the nucleotide numbering of *SOD1*; as an exception, the old nomenclature (excluding the initiation codon) was used for the amino acid changes. The SOD1-p.(Leu144Phe; c.435G>T) Balkan variant (Battistini, Benigni, Ricci, & Rossi, 2013) was observed in 10 probands and seven affected family members, being the predominant *SOD1* variant in the study cohort. The highly characterized SOD1-p.(Asp90Ala; c.272A>C) genomic variant with a dual inheritance pattern, very common among Scandinavian populations in recessive form (Andersen et al., 1995), explained the disease in nine consanguineous Turkish cases. The dual inheritance pattern known for the SOD1-p.(Asp90Ala), was also true for three additional rare changes in *SOD1*; p.(Asn86Ser) (c.205T>C), p.(Leu117Val; c.352G>C), and p.Glu133Lys (c.400G>A), which were detected in 10 different pedigrees with or without family history of ALS (Table 2). Apart from the highly penetrant and frequent pathogenic *SOD1* variations, others identified in our cohort are present in relatively small families with few affected children in the same generation (Table 2). Examples of *SOD1* variants with evidence of reduced penetrance are the p.(Glu22Leu) (c.68A>T), p.(Glu40Gly) (c.122A>G), p.(His71Tyr) (c.214C>T), p.(Val87Met) (c.262G>A) and p.(Thr137Ala) (c.412A>G) variations with asymptomatic carriers in the families.

Pathogenic *TARDBP* (NM\_007375.3) and *FUS* (NM\_004960.3) genomic variants explained the disease in 20 probands and four affected family members (Figures 1 and 2; Table 2). The heterozygous *FUS*-p.(Pro525Leu) (c.1574C>T) and *FUS*-p.(Tyr526Cys) (c.1577A>G) variations were detected in four isolated juvenile cases without a family history, in whom de novo occurrence of the variants was

shown via variation-negative parents. Additionally, the intermediate CAG repeat expansions in the *ATXN2* gene, associated with an increased ALS risk, were reanalyzed in an extended cohort of 519 sALS cases as compared with 236 fALS and sALS patients in a previous study from our laboratory (Elden et al., 2010; Lahut et al., 2012). Analysis, using the control cohort of Lahut et al. (2012) ( $n = 420$ ), confirmed increased ALS risk in carriers with (27–33) CAGs (21/519; Fisher's exact test  $p = .0086$ ).

Analysis of exonic variants in the WES ( $n = 127$ ) and WGS ( $n = 623$ ) data revealed the presence of pathogenic gene variants in 74 cases, out of which, 19 were previously published by our group (Tables 2 and 3; Akçimen et al., 2019; Özoğuz et al., 2015; Tunca et al., 2018). Variant information and clinical features of the cases solved are compiled in Table 3 and in Appendix (Table A4). Homozygous *OPTN* variants were observed in a total of eight cases in the current Turkish cohort. In five families out of eight, the homozygous AA deletion in the *OPTN* gene leading to a premature stop codon (p.(Lys360Valfs\*18), c.1078\_1079delAA, NM\_021980.4) was identified. *OPTN*-based disease in our cohort, presents as classical ALS with an earlier onset at 38 years on average (Table 3). *SPG11* and *ALS2* genomic variants are the second most frequent causes of autosomal recessive ALS in the cohort, with average ages of onset of 14 and 1, respectively.

Rare autosomal dominant ALS genes predominating in our cohort include *VCP*, *ANG*, and *TBK1*, identified in both familial and “apparently sporadic” cases without any reported disease history in the family (Table 3). The novel heterozygous *ERBB4* (p.Arg1096Cys, c.3286C>T, NM\_001042599.1) and *KIF5A* (p.Asp1002Gly, c.3005A>G, NM\_004984.2) pathogenic genomic variations were detected in two distinct families, in which the causative variants segregated with the disease in at least three affected family members. Among the ALS gene variants with unknown pathogenicity identified through WGS, two variations in the *PON1* and *PON3* genes, were



**FIGURE 2** Amino acid changes identified in *SOD1*, *TDP-43*, and *FUS* proteins. Variant-specific pie charts represent the variant's proportion in fALS (red) or sALS (blue) cases, smallest circle corresponding to one case and the largest to 11 cases. fALS, familial ALS; NES, nuclear export signal; NLS, nuclear localization signal; RRM, RNA recognition motif; sALS, sporadic ALS; ZFD, Zinc finger domain

**TABLE 2** Clinical data of patients with *SOD1*, *TARDBP*, *FUS* genomic variants

Gene	ALS ID	Nucleotide change	Protein change <sup>c</sup>	Gender	Age of onset	Site of onset	Gene dosage	Family history	Phenotype
<i>SOD1</i> (NM_000454.4)	1398	c.13G>A	p.(Ala4Thr)	F	25	B	het	Yes	Juvenile ALS
	221	c.13G>T	p.(Ala4Ser)	M	20	L	het	Yes	Juvenile ALS
	308			F	44	L			ALS
	907			F	NA	NA			ALS
	1167 <sup>a</sup>	c.43G>A	p.(Val14Met)	M	42	L	het	No	ALS
	960	c.68A>T	p.(Gln22Leu)	F	30	L	het	Yes	ALS
	1327	c.95T>C	p.(Val31Ala)	M	45	B	het	Yes	ALS
	1547 <sup>a</sup>			F	64	L		No	ALS
	1453	c.112G>C	p.(Gly37Arg)	M	41	L	het	Yes	ALS
	802	c.122A>G	p.(Glu40Gly)	F	39	L	het	Yes	ALS
	816			F	32	L		Yes	ALS
	1450	c.205T>C	p.(Ser68Pro) <sup>n</sup>	M	54	L	het	Yes	ALS
	226	c.214C>T	p.(His71Tyr) <sup>n</sup>	M	19	L	het	Yes	Juvenile ALS
	707			F	57	L			ALS
	191	c.260A>G	p.(Asn86Ser)	M	28	L	hom	No	ALS
	623 <sup>a</sup>			F	42	L	het	No	ALS
	1207			M	48	L		Yes	ALS
	102 <sup>a</sup>	c.262G>A	p.(Val87Met)	F	29	L	het	No	ALS
	147	c.272A>C	p.(Asp90Ala)	M	49	L	hom	Yes	Lower limb
	310			M	55	L		Yes	dominant
	429			F	45	L		Yes	stereotyped
	741			F	32	L		Yes	Scandinavian
	810			F	29	L		Yes	phenotype
	1256 <sup>b</sup>			M	44	L		Yes	
	1359			M	35	L+B		Yes	
	1545			F	64	L		No	
	1579			M	51	L		No	
	561	c.352C>G	p.(Leu117Val)	F	62	L	het	Yes	ALS
	1527			F	38	L			ALS
	1396			F	62	L		Yes	ALS
	1412			F	40	L			ALS
	1472 <sup>a</sup>			F	36	L		No	ALS
	1888			M	50	L		Yes	ALS
	1882			F	58	L			ALS
	1439			F	24	L	hom	Yes	juvenile ALS
	355 <sup>a</sup>	c.376G>A	p.(Asp125Asn)	M	50	L	het	No	ALS
	1716 <sup>b</sup>	c.400G>A	p.(Glu133Lys)	F	37	L	hom	No	ALS
	1064 <sup>a</sup>			M	34	L	het	No	ALS
	1655 <sup>b</sup>	c.412A>G	p.(Thr137Ala)	F	49	L	het	No	ALS
	61	c.435G>T	p.(Leu144Phe)	F	52	L	het	Yes	ALS
	281 <sup>a</sup>			M	57	L		No	ALS
	607			F	45	L		Yes	ALS
713			F	53	L			ALS	
724			M	52	L			ALS	
727			F	NA	L			ALS	
1773			M	60	L			ALS	
635			F	54	L		Yes	ALS	
772			M	49	L		Yes	ALS	
1059			F	51	L			ALS	
1063			F	56	L			ALS	
1235			F	59	L			ALS	



**TABLE 2** (Continued)

Gene	ALS ID	Nucleotide change	Protein change <sup>c</sup>	Gender	Age of onset	Site of onset	Gene dosage	Family history	Phenotype
	<b>935</b>			M	37	L		Yes	ALS
	<b>1036</b>			F	60	L		Yes	ALS
	<b>1633</b>			M	64	L		Yes	ALS
	<b>1691</b>			M	60	L		Yes	ALS
	<b>1715</b>			F	34	L		Yes	ALS
	<b>97<sup>b</sup></b>	c.446T>G	p.(Val148Gly)	F	46	L	het	Yes	ALS
<i>TARDBP</i> (NM_007375.3)	<b>1082</b>	c.893G>T	p.(Gly298Val) <sup>n</sup>	F	66	L	het	Yes	ALS
	<b>356<sup>b</sup></b>	c.943G>A	p.(Ala315Thr)	M	58	L	het	Yes	ALS
	<b>357</b>			F	59	L		Yes	ALS (man in barrel)
	<b>600</b>			F	57	L		Yes	ALS
	<b>1448</b>			M	62	L		Yes	ALS
	<b>408<sup>a</sup></b>			M	48	L		No	ALS
	<b>910</b>	c.1042G>T	p.(Gly348Cys)	M	37	L	het	Yes	ALS
	<b>919</b>			M	42	L			ALS
	<b>911</b>			M	NA	L			ALS
	<b>660</b>	c.1060C>G	p.(Gln354Glu) <sup>n</sup>	F	42	L	het	No	ALS
	<b>976</b>	c.1144G>A	p.(Ala382Thr)	M	39	L	het	Yes	ALS
	<b>277<sup>b</sup></b>	c.1147A>G	p.(Ile383Val)	M	67	B	het	Yes	ALS
	<b>311</b>			F	42	NA			ALS
<i>FUS</i> (NM_004960.3)	<b>264<sup>b</sup></b>	c.430_447del	p.(Glu144_-Tyr149del)	M	16	L	het	No	Juvenile ALS
	<b>1034</b>	(NC_000016.10) c.1394-1G>T <sup>n</sup>	p.(=)	F	47	L	het	Yes	ALS
	<b>1208</b>	c.1555C>T	p.(Gln519*)	F	42	L		Yes	ALS
	<b>485<sup>a</sup></b>	c.1562G>T	p.(Arg521Leu)	M	39	L	het	No	ALS
	<b>227<sup>b</sup></b>	c.1571G>T	p.(Arg524Met)	F	32	L	het	Yes	ALS
	<b>581</b>			M	53	L		Yes	ALS
	<b>1647</b>			F	29	L		Yes	ALS
	<b>549</b>	c.1574C>T	p.(Pro525Leu)	M	14	L	het (de novo)	No	Juvenile ALS with fast progression
	<b>1610<sup>b</sup></b>			M	17	L	het (de novo)	No	
	<b>377<sup>a</sup></b>			F	16	L	het (de novo)	No	
	<b>1423<sup>b</sup></b>	c.1577A>G	p.(Tyr526Cys)	M	12	L	het (de novo)	No	

Note: Bold indicates index cases.

Abbreviations: B, bulbar; het, heterozygous; hom, homozygous; L, limb; n, novel; NA, not available; WES, whole exome sequencing; WGS, whole genome sequencing.

<sup>a</sup>Identified in the framework of Project MinE (WGS).

<sup>b</sup>Identified with WES.

<sup>c</sup>The old nomenclature of the *SOD1* gene is adopted here for amino acid changes.

detected in Turkish sALS cases as compared to none of the controls (*PON1*, rs755475189, 2 patients/0 controls; *PON3*, rs147006695, 5 patients/0 controls; Table A5).

Apart from classical ALS genes, WES revealed variants in rare genes associated with diverse motor neuron phenotypes either with upper or lower motor neuron predominance (e.g., *ZFYVE26*, *DNAJB2*, *PLEKHG5*, *TRPV4*, *FBXO38*, and *VRK1*; Table 3 and Table A4). The *C19orf12* genomic variant, implicated in neurodegeneration with brain iron accumulation, mimicking ALS, was detected in three cases with an autosomal recessive inheritance (AO: 9–24), who were initially diagnosed with a probable juvenile ALS (Deschauer et al., 2012).

From the clinical exome sequencing perspective, WES-only success rate of NDAL is calculated as 42% for patients diagnosed with ALS and ALS-like disease in the Turkish cohort (Tables 2 and 3, Table A4). This rate increases to almost 50% in familial cases and in cases with consanguineous parents, regardless of family history.

## 5.2 | GeNDAL, a web-based variant browser for ALS-related genes

Fully anonymized information regarding ALS-related variants with known or unknown pathogenicity identified in WGS analysis are

presented in the Genome Browser of NDAL, GeNDAL (<http://www.gendal.org>). GeNDAL is a platform which allows the users to query variants by dbSNP ID, amino acid change, gene symbol, Human Genome Variant Server ID, transcript or sequence ontology defined by the Sequence Ontology Consortium (<http://www.sequenceontology.org/>). Detailed variant annotations and graphical representations of variant-related information from public databases (ClinVar, gnomAD, etc.) can be visualized (Figure 3). In addition, the phenotypes can be distinguished as ALS or unaffected control. The GeNDAL database currently constructed for ALS-related gene variants will be complemented in future for other phenotypes in NDAL's cohort.

### 5.3 | Whole genome sequencing analysis of 623 Turkish sALS cases and 142 neurologically healthy controls did not reveal significant risk loci

A joint cohort of ALS patients and control samples worldwide were analyzed by Project MinE Sequencing Consortium, which included 224 Turkish samples (van der Spek et al., 2019; van Rheenen et al., 2016). Analysis of WGS data of an expanded Turkish cohort consisting of 623 Turkish sALS patients and 142 neurologically healthy controls, revealed 47,971,649 novel variants which are not represented in gnomAD. Out of all variants detected, 23,410,513 had a MAF smaller than 0.1% in the cohort (Table A6). GWAS analysis and gene-based burden testing (SKAT-O) on this cohort did not reveal a significant risk/protective variant or gene (Figure 4). The top 25 SNPs detected in GWAS and the top ten genes from SKAT-O are listed in Tables A7 and A8 in the Appendix. Even though there is a lack of association with new variants or genes from the GWAS, we scanned the literature for the candidate genes and none of them was associated with ALS or similar phenotypes.

On the basis of the hypothesis that ALS genes are working as a functional cluster, we conducted a gene coexpression network analysis (a) to search for other candidate genes which might also confer ALS risk, and (b) to investigate the function of the predicted cluster and its relation to ALS. The "guilt-by-association principle"-based gene discovery approach has been applied on many complex neurologic/psychiatric disorders to discover more risk genes as in the autism spectrum disorder (De Rubeis et al., 2014; Sanders et al., 2015) and schizophrenia (Torkamani, Dean, Schork, & Thomas, 2010). For this purpose, the ST-Steiner algorithm (Norman & Cicek, 2019) was used to create a network around established ALS genes using the SKAT-O *p*-values as the prize for the network analysis. The resulting subnetwork contains 98 newly predicted genes around the 28 ALS-associated terminal genes (Figure 5). Ninety-five of 98 predicted genes had higher variation rates in patients and were significantly enriched for cell cycle (DAVID enrichment score: 15,03) and cell division genes (DAVID enrichment score: 6,8; Table A9; Huang et al., 2007). Coexpression network analysis, coupled with literature text-mining using GeneRIF and DisGeNet databases, pointed to *DECR1* (case count: 3 and control count: 0), *ATL1* (case count: 1 and control count: 0), *HDAC2* (case count: 1 and control count: 0), *GEMIN4* (case count: 1 and control count: 0) and *HNRNPA3*

(case count: 1, control count: 0) genes which are marked in Figure 5. Finally, accumulation of all variants in WGS to canonical disease pathways obtained from Broad Institute also did not suggest a significant association (Figure A1); top ranking pathways are listed in Table A10. Even with a Turkish sample size four times larger than the two initial Project MinE studies, neither GWAS nor network analysis point to any known or new significant association with ALS, indicating the crucial requirement for larger sample sizes.

## 6 | DISCUSSION

### 6.1 | Clinical presentation of ALS in Turkey

For almost two decades, well-established patient registers operating in European countries gather organized patient data to understand the epidemiology of ALS (Hardiman et al., 2017). These registers work countrywide and are unbiased in terms of origin, socioeconomic status and the disease stage of the patient in contrast to a local clinic. The incidence reported for ALS worldwide is argued to be misleading in the absence of long-running patient registers which are more efficient in recognizing family history and hidden symptoms like FTD (Hardiman et al., 2017). Turkey's ALS patient registry operated by the Turkish ALS-MND Association ([www.als.org.tr](http://www.als.org.tr)) since 2001 with headquarters in Istanbul and Izmir, is relatively recent and may not represent the whole country. Hence, information regarding the incidence and prevalence of ALS in Turkey and survival rates are still restricted and scattered. NDAL, being the only reference center for the molecular analysis of ALS in Turkey, has been recruiting patients from across the country for over 20 years and gathers available patient data to investigate the clinical and molecular basis of this complex neurodegenerative disease in an admixed population inhabiting the Turkish peninsula in the crossroads of many civilizations since several centuries.

This study, with 1,200 probands, offers an update on the phenotypic and genetic landscape of ALS in Turkey. The fALS percentage of 20% (246/1,200), exceeding North American and European populations (5–10%; Ghasemi & Brown, 2018), is explained by population-specific and social factors, such as extensive kindreds consisting of many generations and offspring. A unique aspect in Turkey, common to countries in the Near and Middle East, is the high proportion of close consanguineous marriages, approaching 50% in the eastern parts of the country. Consanguinity in the ALS cohort under study is calculated as 30% in fALS and 23% in sALS. This suggests even a higher percentage for Mendelian inheritance in yet unexplained cases that are classified originally as sALS due to singlet patients in the family. Thus, familial ALS, harboring a simplex genetic component, seems to be above 20% among Turkish cases.

### 6.2 | Impact of common genes on ALS in Turkey

The *C9orf72* hexanucleotide repeat expansion and pathogenic missense variants in *SOD1* together explain 30% of familial cases in our

**TABLE 3** Variants identified via WES and WGS in rare genes

Gene	Transcript ID	Variation		Gene dosage	AO	Family history	Consanguinity	Phenotype
		DNA change	Protein change					
ERBB4	NM_001042599.1	c.3286C>T	p.(Arg1096Cys)	Het	48	Yes	No	ALS
KIF5A	NM_004984.2	c.3005A>G	p.(Asp1002Gly) <sup>n</sup>	Het	50	Yes	No	ALS
TBK1	NM_013254.4	c.922C>T	p.(Arg308*) <sup>na</sup>	Het	46	No	No	ALS
		c.1436_1437delTG	p.(Val479Glufs*4)	Het	20	No	No	Juvenile ALS
VCP	NM_007126.5	c.463C>T	p.(Arg155Cys) <sup>a</sup>	Het	62	No	No	ALS
		c.475C>T	p.(Arg159Cys) <sup>a</sup>	Het	52	No	No	ALS
		c.572G>C	p.(Arg191Pro)	Het	60	Yes	No	ALS-FTD
UBQLN2	NM_013444.3	c.1516C>T	p.(Pro506Ser)	Hemi	26	Yes	No	ALS
		c.1573C>T	p.(Pro525Ser)	Hemi	22	Yes	Yes	ALS
TFG	NM_001007565.2	c.854C>T	p.(Pro285Leu)	Het	47	Yes	No	ALS with sensory neuropathy
ANG	NM_001145.4	c.208A>G	p.(Ile70Val) <sup>a</sup>	Het	52	No	No	ALS
		c.208A>G	p.(Ile70Val) <sup>a</sup>	Het	40	No	No	ALS
		c.208A>G	p.(Ile70Val)	Het	28	No	No	ALS
CHCHD10	NM_213720.2	c.476C>T	p.(Ser59Leu) <sup>a</sup>	Het	51	No	No	ALS
FBXO38	NM_001271723.1	c.1577G>A	p.(Arg526Gln) <sup>n</sup>	Het	congenital	No	Yes	Juvenile MND
TRPV4	NM_147204.2	c.943C>T	p.(Arg315Trp)	Het	infancy	Yes	Yes	Juvenile MND
TRPM7	NM_017672.6	c.4445C>T	p.(Thr1482Ile)	Het	teenage	Yes	No	Juvenile ALS
SETX	NM_015046.7	c.5839G>A	p.(Ala1947Thr) <sup>n</sup>	Het	11	Yes	No	Juvenile ALS
ERLIN1	NM_006459.4	c.281T>C	p.(Val94Ala) <sup>n</sup>	Hom	15	Yes	Yes	Juvenile ALS
SPG11	NM_025137.4	c.1432C>T	p.(Gln478*) <sup>n</sup>	Hom	20	No	Yes	Juvenile ALS
		c.1966_1967delAA	p.(Lys656Valfs*11)	Hom	16	Yes	Yes	Juvenile ALS
		c.2250delIT	p.(Phe750Leufs*3) <sup>n</sup>	Hom	16	Yes	Yes	Juvenile ALS
		c.7155T>G	p.(Tyr2385*) <sup>n</sup>	Hom	23	Yes	Yes	Juvenile ALS
OPTN	NM_021980.4	c.76delC	p.(His26Thrfs*19) <sup>a</sup>	Hom	35	No	Same village	ALS
		c.875dupC	p.(Glu293Glyfs*19) <sup>n</sup>	Hom	33	Yes	Yes	ALS
		c.1078_1079delAA	p.(Lys360Valfs*18)	Hom	32	Yes	Yes	ALS
		c.1078_1079delAA	p.(Lys360Valfs*18)	Hom	43	Yes	Yes	ALS
		c.1078_1079delAA	p.(Lys360Valfs*18)	Hom	31	Yes	Yes	ALS
		c.1078_1079delAA	p.(Lys360Valfs*18) <sup>a</sup>	Hom	42	No	No	ALS
		c.1078_1079delAA	p.(Lys360Valfs*18) <sup>a</sup>	Hom	42	Yes <sup>b</sup>	No	ALS
		c.1217delC	p.(Thr406Lysfs*5) <sup>na</sup>	Hom	42	No	No	ALS

(Continues)

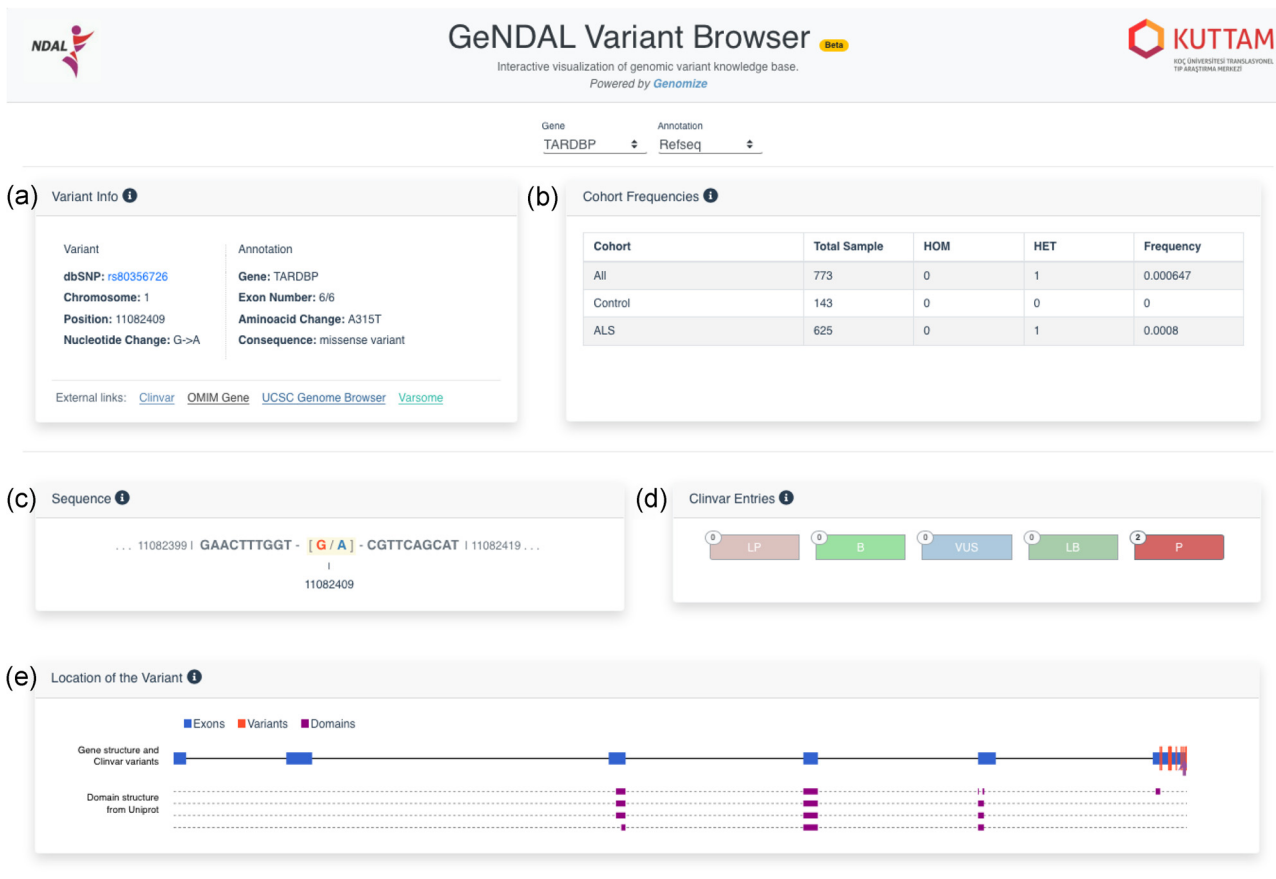
TABLE 3 (Continued)

Gene	Transcript ID	Variation		Gene dosage	AO	Family history	Consanguinity	Phenotype
		DNA change	Protein change					
ALS2	NM_020919.4	c.1718C>A	p.(Ala573Glu)	Hom	2,5	No	Yes	Juvenile ALS
		c.2761C>T	p.(Arg921*)	Hom	1	No	Yes	Juvenile ALS
		c.4381C>T	p.(Arg1461*) <sup>n</sup>	Hom	1	Yes	Yes	Juvenile ALS
		c.4808C>T	p.(Pro1603Leu)	Hom	1	No	Yes	Juvenile ALS
C19orf12	NM_001031726.3	c.32C>T	p.(Thr11Met)	Hom	24	No	Yes	Juvenile ALS
		c.194G>T	p.(Gly65Val)	Hom	10	Yes	Yes	Juvenile ALS
		c.194G>T	p.(Gly65Val)	Hom	9	No	Yes	Juvenile ALS
SYNE1	NM_182961.3	c.22930C>T	p.(Gln7644*) <sup>n</sup>	Hom	21	Yes	Yes	Juvenile ALS
		c.23524C>T	p.(Arg7842*)	Hom	17	Yes	Yes	Juvenile ALS
ZFYE26	NM_015346.3	c.2074delC	p.(Leu692Serfs*52) <sup>n</sup>	Hom	17	No	Yes	Juvenile MND
		c.2615-2617delGCTinsTGAA	p.(Arg872Hisfs*17) <sup>n</sup>	Hom	22	No	Yes	Juvenile MND
DNAJB2	NM_006736.5	c.14A>G	p.(Tyr5Cys)	Hom	31	No	Yes	Juvenile ALS
		c.757G>A	p.(Glu253Lys)	Hom	22	No	Yes	Juvenile MND
PLEKHG5	NM_198681.3	c.1648C>T	p.(Gln550*)	Hom	20	Yes	Yes	Juvenile ALS
		c.2120C>A	p.(Pro707His)	Hom	14	No	Yes	Juvenile ALS
SIGMAR1	NM_147157.2	c.355G>A	p.(Glu119Lys)	Hom	2	No	Yes	Juvenile ALS
		c.358A>G	p.(Thr120Ala) <sup>n</sup>	Hom	17	No	Yes	Juvenile ALS
VRK1	NM_003384.3	c.961C>T	p.(Arg321Cys)	Hom	22	Yes	Yes	Juvenile ALS
		c.1135_1136delCA	p.(Gln379Aspfs*23) <sup>n</sup>	Hom	17	No	Yes	Juvenile MND
DJ1	NM_007262.5	c.133C>T	p.(Gln45*)	Hom	24	Yes	Yes	ALSPDC
IGHMBP2	NM_002180.2	c.638A>G	p.(His213Arg)	Hom	9	Yes	Yes	Juvenile MND
SLC52A3	NM_033409.4	c.802C>T	p.(Arg268Trp)	Hom	1,5	Inconclusive	Yes	Madras MND

Abbreviations: ALS, amyotrophic lateral sclerosis; ALSPDC, ALS-Parkinsonism-Dementia Complex; AO, age of onset; fALS, familial ALS; Het, heterozygous; Hom, homozygous; MND, motor neuron disease; sALS, sporadic ALS.

<sup>a</sup>Cases solved in the framework of Project MinE (WGS).

<sup>b</sup>Patient status changed from sALS to fALS upon diagnosis of a younger sister with ALS; <sup>n</sup>novel variant.



**FIGURE 3** Representation of the GeNDAL variant database. (a) Information regarding the variant and its annotation with complementary links to external databases. (b) Phenotype-dependent frequencies of the variant of interest in the internal WGS cohort of NDAL. (c) Genomic location of the nucleotide change and its surrounding sequence. (d) The number of pathogenicity verdict and detailed information on the variant in ClinVar. (e) Display of pathogenic and likely pathogenic variants reported in ClinVar aligned to the current transcript and protein domains, allowing visualization of variational hotspots. WGS, whole genome sequencing

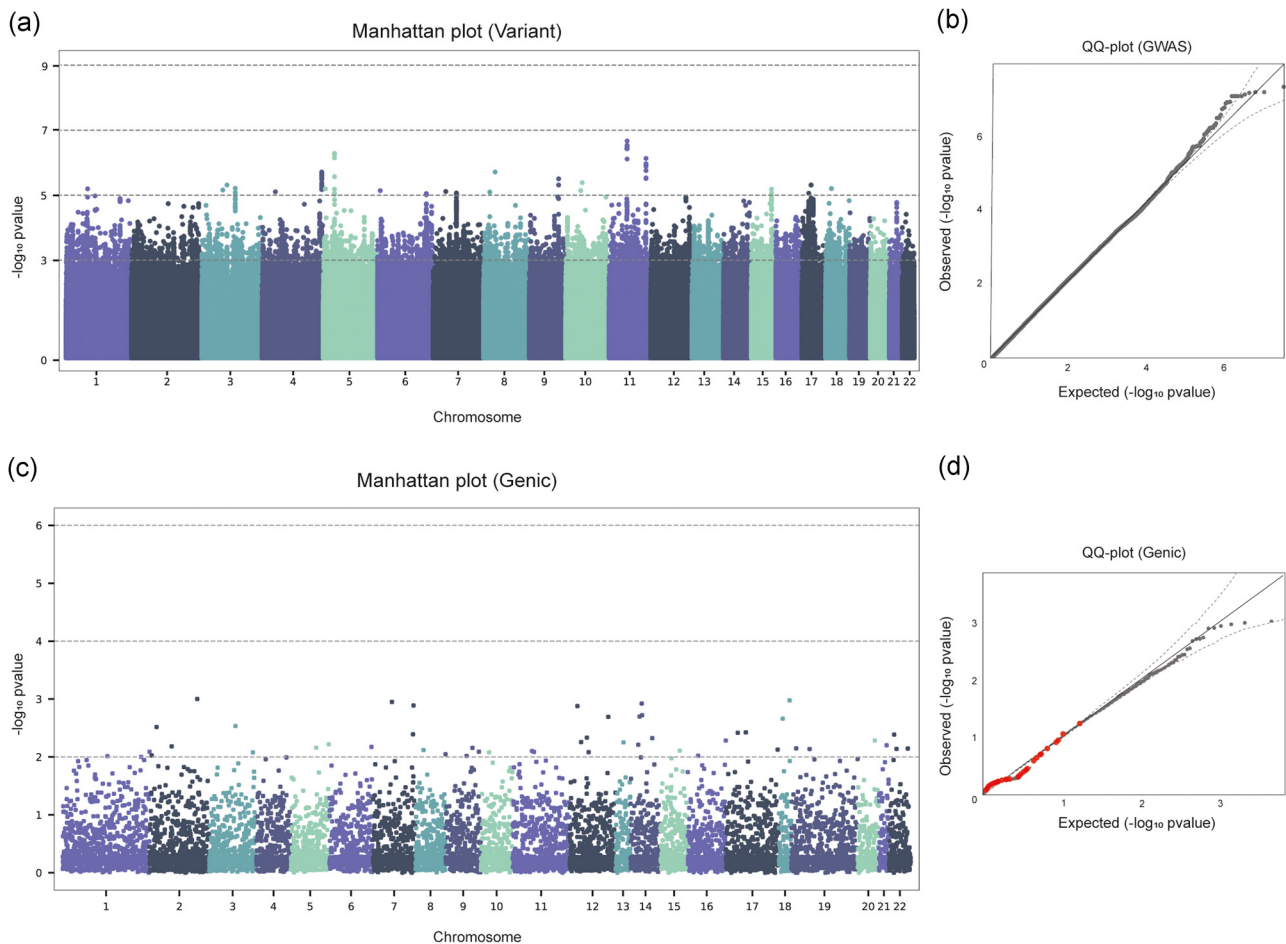
cohort, with *TARDBP* and *FUS* solving another 5%. NGS data of “apparently sporadic/isolated cases, unraveled genomic variants in common ALS genes that contribute to 2.1% (*SOD1*: 1.3%, *TARDBP*: 0.2% and *FUS*: 0.6%), to total 6.1% with sporadic cases carrying the *C9orf72* expansion. These results not only support the evidence of incomplete penetrance but also the de novo occurrence of variants in these genes, leading to genetic misclassification of patients as sporadic. Thus, the distinction between the clinically indistinguishable familial and sporadic disease, becomes unclear and should be handled with care in diagnostic settings and particularly during genetic counseling.

The hexanucleotide repeat expansion in the *C9orf72* gene is the most common genetic cause of ALS worldwide, with an exception of Japan (Ogaki et al., 2012). Although lower in frequency compared to Northern European countries (50% of fALS and 20% of sALS; Majounie et al., 2012), this expansion is the most abundant genomic variation both in fALS (17%) and sALS (4%) also in the present Turkish cohort. *SOD1* variants, the second most frequent genetic causes of ALS in the Turkish cohort contribute to high allelic

heterogeneity (Figure 2). Evidence for the reduced penetrance of *SOD1* variants was obvious in the Turkish population, such as (a) the genomic variants detected in sporadic patients, (b) the families with asymptomatic carriers, and (c) dual inheritance patterns observed in *SOD1*-p.(Asn86Ser), p.(Asp90Ala), p.(Leu117Val), and p.(Glu133Lys). This variability may stem from modifier genes/variants, an upcoming research field.

The fact that an ample number of people with Turkish origin migrated to Turkey from the Balkans many generations ago, rationalizes the predominance of the common Balkan variant, *SOD1*-p.(Leu144Phe) in our cohort. The average AO for p.(Leu144Phe) carriers is 52 years without any gender bias and all of them have limb-onset disease. This variation results in classical ALS and appears to be highly penetrant in large families consisting of several branches. The only exception is an apparently sporadic male patient (AO:57), with deceased parents who were not available for analysis (Table 2).

The biallelic p.(Asp90Ala) variation is the second most common pathogenic *SOD1* variant in our cohort. No affected individuals have been detected carrying the heterozygous variant and the presence of



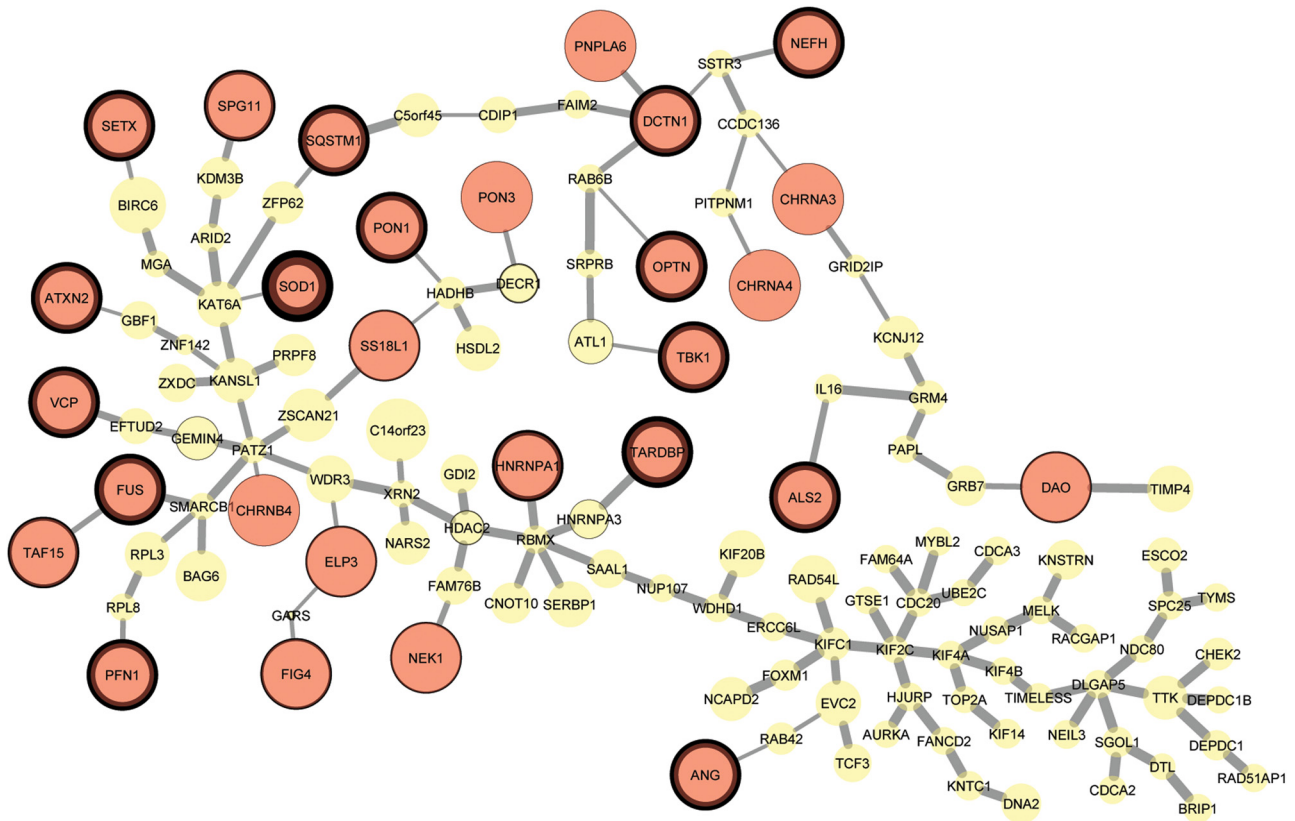
**FIGURE 4** Manhattan plots and Quantile-quantile plots of GWAS and SKAT-O analysis using logistic regression. (a) Approximately six million SNPs ( $MAF \geq 0.05$ ) are displayed in the Manhattan plot. (b) Quantile-quantile plot of the GWAS  $p$  values. (c)  $p$  values derived from the gene-based SKAT-O analysis are displayed in the Manhattan plot. Each of  $\sim 10,000$  genes in SKAT-O analysis is represented by a single dot. (d) Quantile-quantile plot of genic association  $p$  values. Known ALS-related genes are highlighted in red. Dashed curves correspond to the 95% confidence limits. ALS, amyotrophic lateral sclerosis; GWAS, Genome-wide association studies

the Scandinavian founder haplotype in Turkish recessive p.(Asp90Ala) cases was previously shown (Özoğuz et al., 2015). Except for one patient with a mixed site of onset, limb-onset disease predominates in p.(Asp90Ala) patients. The disease progression is very slow and in accordance with the stereotyped Scandinavian phenotype (Andersen et al., 1995). The average AO of the recessive p.(Asp90Ala) carriers is 10 years earlier than the p.(Leu144Phe) patients.

SOD1 is a ubiquitously expressed protein that acts as a superoxide radical scavenger in the cell. Its pathogenicity in ALS is explained by the misfolding of the mutant product which leads to accumulation within the cell in aggregates (Paré et al., 2018). There is not enough evidence in the literature to comment on the mechanism behind the phenotypic heterogeneity of different SOD1 variants as well as their acting mechanisms that show both dominant and recessive inheritance. Although most evidence supports the gain-of-function mechanism, loss-of-function of the mutant allele may still have a role in the presentation of SOD1-based disease. Reduction in overall activity of the mutant form has been shown in blood and

fibroblast samples, but the two specific genomic variants, p.(Asp90Ala) and the heterozygous p.(Leu117Val), had only slight reductions in enzymatic activity. This might explain the milder phenotype in patients carrying these variants and the low penetrance observed in parents of homozygous individuals (Saccon, Bunton-Stasyshyn, Fisher, & Fratta, 2013). On the contrary, the homozygous p.(Leu117Val) genomic variation was reported to result in a more severe reduction in enzymatic activity than the heterozygous variant which is also concordant with the early AO (AO:24) and the fast disease progression of the patient with the biallelic genomic variation reported in this study (Table 2; Synofzik et al., 2012). Although the function of the mutant protein is not completely lost, the activity may be reduced by aggregation; thus, different SOD1 variants with different aggregation propensities may have variable enzymatic activity and this may act on the phenotypic representation of the disease.

ALS-associated TDP-43 and FUS variants are known to accumulate in the C-termini and although the pathogenicity behind these two RNA/DNA binding proteins is not yet clear, nuclear clearance and cytoplasmic accumulation of both proteins are observed in ALS.



**FIGURE 5** The predicted subnetwork of genes by ST-Steiner on ALS GWAS data. The predicted subnetwork contains 126 genes. Red denotes ground truth (terminal) genes ( $n = 28$ ) used to build the network, and yellow denotes the newly predicted genes ( $n = 98$ ). The node size represents ALS risk, based on  $-\log_{10}$  transformed  $p$  values from SKAT-O analysis. The border thickness depicts the GeneRIF and DisGeNet scores for each gene, and edge thickness the strength of gene expression correlation between a pair of genes according to the BrainSpan database. ALS, amyotrophic lateral sclerosis; GWAS, Genome-wide association studies

In fact, TDP-43-positive cytoplasmic inclusions are a common hallmark of fALS and sALS, regardless of an ALS-associated genetic variation in patients. Unlike FUS variants gathered in the nuclear localization signal domain, TDP-43 variants are found in the prion-like domain of the protein (Figure 2). Our results regarding TARDBP and FUS variants are restricted to screening of C-terminal hotspots for these two genes with the exception of the heterozygous deletion in the N-terminus of the protein detected via WES. There are rare variants reported in the N-terminal region of TDP-43, like the p.Ala90Val, however with the lack of segregation analysis, the presence of the variant in healthy individuals and with mild abnormal cytoplasmic localization, the pathogenicity of the variant remains questionable (Winton et al., 2009; Wobst et al., 2017). Despite the importance of nuclear import and export signals on the transport of a protein, studies showed different cytoplasmic accumulation levels for different N-terminal FUS variants and also a critical role for C-terminal deletions in FUS in formation of stress granules, all suggesting a complicated mechanism for both proteins, ranging from loss of nuclear function to gain of toxic function through aggregates (Guerrero et al., 2016).

Juvenile ALS (AO < 25) was observed in 68 isolated/sporadic cases (7%) in our cohort. This form of ALS most frequently occurs due to consanguinity and has a rather slow disease progression compared

to classical ALS. However, four de novo FUS cases with non-consanguineous parents, ages of onset ranging from 12 to 17, had an aggressive disease progression, resulting in the retirement of the children from all daily activities. Severe bulbar symptoms in addition to initial limb-onset disease, eventually lead to death almost within a year. De novo FUS gene variants are reported in juvenile cases in populations where consanguinity is not common. We also suggest the screening of FUS as the initial step in isolated juvenile patients with a fast disease progression and asymptomatic parents (Hübers et al., 2015; Leblond et al., 2016; Therrien, Dion, & Rouleau, 2016).

In the cohort under study, common ALS genes contribute to 35% of fALS, which increases to 45% with the addition of rare genes. According to this picture, more than 50% of Turkish fALS cases remain unsolved as compared to 30% in Caucasian populations (Ghasemi & Brown, 2018); this result points towards an expected higher locus heterogeneity in the Turkish population. The Turkish peninsula, geographically located at the intersection of many civilizations, has a heterogeneous ethnic and genetic background. This complexity in the population leads to the dilution of pathogenic variants in common ALS genes like *C9orf72* or *SOD1*. In this sense, the frequencies observed in Turkey are concordant with the common notion of decreasing north-south gradient for these genes (Andersen, 2006; Lamp et al., 2018; Smith et al., 2013). Novel coding variants, as well as

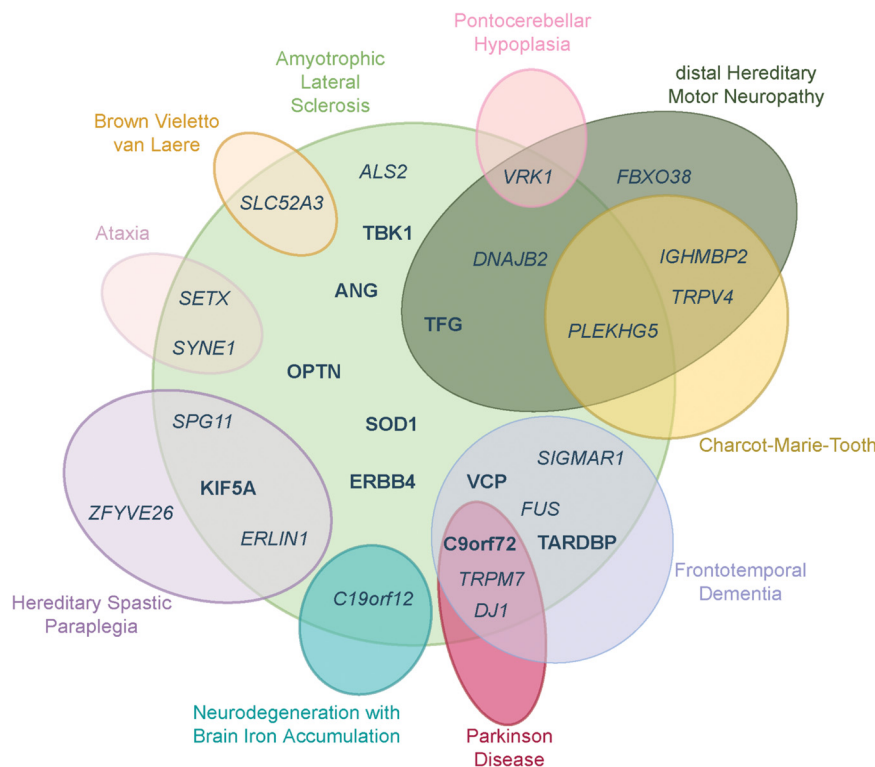
chromosomal changes (large rearrangements, copy number variations, repeat expansions, indels), and variants in regulatory, intronic and intergenic regions, not covered by WES, are expected to unravel the missing heritability in the present cohort.

### 6.3 | Clinical exome sequencing in the differential diagnosis of ALS and ALS-like phenotypes

The majority of inherited diseases are caused by genomic variations in protein-coding regions; thus, exome sequencing unravels the causative variants in a considerable number of cases allowing data interpretation, less dependent of the initial clinical diagnosis, which is to the benefit of both the clinician and the patient. This unbiased candidate variant prioritization approach allows to differentially diagnose cases with uncertain clinical phenotypes due to overlapping features between diseases and their progressive nature with absence of full-grown symptoms in juvenile cases. Some examples to this common problem are reported here in non-ALS MND genes like *ZFYVE26*, *DNAJB2*, *PLEKHG5*, *TRPV4*, and *FBXO38*, in which early

disease-onset or intrafamilial phenotypic heterogeneity, ranging from neuropathy to motor neuron disease, lead to uncertain clinical diagnoses (Figure 6).

Identification of new genetic players implicated in ALS and ALS-like disease opens new opportunities for understanding the converging mechanisms in neurodegeneration and/or motor neuron loss. Moreover, these also contribute to the development of more specific, even personalized, therapeutic targets like gene-specific antisense oligonucleotides. Thus, today, it is important to define the genetic causes of even yet untreatable diseases, to drive pharmaceutical/gene-editing research and to offer hope to patients and their families. Clinical exome sequencing, for which the diagnosis success rate increased exponentially in clinical settings, includes several beneficial outcomes: (a) treatment of patients with syndromic diseases like enzyme deficiencies, (b) using genetic information for reproductive genetic counseling and family planning and (c) recruitment of patients with specific genomic variants into clinical trials. Most importantly, WES shortens the diagnostic delay of at least 1 year in ALS, which may include several invasive and expensive procedures, and these should be taken into account while considering its cost-effectiveness



**FIGURE 6** Genetic heterogeneity behind motor neuron diseases in our cohort. Individuals carrying the genomic variations represented in bold had clear/definitive initial diagnosis of classical ALS with an average age of onset of 46. Genes represented in italics are identified in patients presenting with juvenile motor neuron disease or nonclassical ALS-like disease with expanding phenotypes in the index or in affected family members (average AO: 13). This picture emphasizes pleiotropy in genes in addition to the expansion of phenotypes, calling for a gene-based disease classification. Venn diagram is drawn according to the disease-gene associations obtained from the literature (Akçimen et al., 2019; Al-Saif, Al-Mohanna, & Bohlega, 2011; Annesi et al., 2005; Brenner et al., 2018; Chen et al., 2004; Cirulli et al., 2015; Cottenie et al., 2014; Daoud et al., 2012; Deschauer et al., 2012; Frasquet, Va, & Sevilla, 2017; Greenway et al., 2006; Grohmann et al., 2001; Hermosura et al., 2005; Hughes et al., 2001; Ishiura et al., 2012; Iskender et al., 2015; Kimonis, Fulchiero, Vesa, & Watts, 2008; Maruyama et al., 2010; Maystadt et al., 2007; Nalini, Pandraud, Mok, & Houlden, 2013; H. P. Nguyen, Van Broeckhoven, & van der Zee, 2018; Stoll et al., 2016; Sumner et al., 2013; Synofzik et al., 2016; Takahashi et al., 2013; Tunca et al., 2018; Velilla et al., 2019; Yang et al., 2001). ALS, amyotrophic lateral sclerosis; AO, age of onset



(Fogel et al., 2014; Fogel, Satya-Murti, & Cohen, 2016; Trujillano et al., 2017).

## 6.4 | Project MinE to understand sporadic ALS: Impact of Turkish WGS data

Currently, the Turkish population is one of the highly represented cohorts in Project MinE in terms of sample size. Our results from screening for pathogenic exonic variants in the Turkish Project MinE cohort revealed marked incomplete penetrance for the three common ALS genes (*SOD1*, *TARDBP*, and *FUS*). In recent years, many studies showed that the frequency of ALS patients carrying more than one variant is higher than expected by chance, providing evidence that ALS may result from multiple rare variants with additive effects on disease development and presentation, for example, age of disease onset, progression and severity (van Blitterswijk et al., 2012). This “oligogenic model” of ALS may solve a portion of unexplained sporadic cases. The variations in *PON1* and *PON3* detected in our cohort might also act in such a manner (Table A5). The PON variants can lead to oligomerization of the native protein through N-terminal HDL particles, as previously reported, and further decrease its own hydrolytic activity (Josse et al., 2002). Disease pathology caused by paraoxonase genes, intensely studied for their role in ALS, may arise with reduced ability of PON enzymes, responsible of detoxifying organophosphates, which are neurotoxins associated with an increased ALS risk (Cronin, Greenway, Prehn, & Hardiman, 2007; Landers et al., 2008; Menini & Gugliucci, 2014; Merwin, Obis, Nunez, & Re, 2017; Ticozzi et al., 2010; Verde et al., 2019; Wills et al., 2009).

Genome-wide association study and gene-based burden testing for population-specific local signals with WGS data of 623 sporadic patients and 142 Turkish controls, a four times larger Turkish cohort than the one from van Rheenen et al. (2016), did not reveal any significant loci neither in variant-based GWAS, nor in gene-based disease burden analyses. Yet, the resulting network, which combined gene-based burden analysis with coexpression information, pointed to a significantly high enrichment (~15-fold) of cell cycle-related genes. Accordingly, changes in expression levels and subcellular localization of cell cycle proteins and their transcriptional regulators have been linked to neuronal death in the literature in terms of ALS and other neurodegenerative diseases (M. D. Nguyen et al., 2003; Ranganathan & Bowser, 2003).

Network analysis combined with literature text-mining suggested *DECRC1*, *ATL1*, *HDAC2*, *GEMIN4*, and *HNRNPA3* as disease susceptibility candidates although these had no significant burden for ALS in the SKAT-O analysis. Previous studies show increased levels of the mitochondrion-related  $\beta$ -oxidation enzyme *DECRC1* at disease onset in *SOD1*-G93A mice spinal cords (Q. Li et al., 2010; Pharaoh et al., 2019). *Atlastin-1* (*ATL1*), associated with upper motor neuron syndromes (De Bot et al., 2013), is a protein effective in structural and functional integrity of the endoplasmic reticulum (Muriel et al., 2009). *Histone deacetylase 2* (*HDAC2*) is important for the nervous system and was shown to be upregulated in ALS patients (Janssen et al., 2010). The increased

HDAC activity in neurodegeneration and positive effects of HDAC inhibition, including HDAC2, on motor symptoms are reported in the literature (Lazo-Gómez, Ramírez-Jarquín, Tovar-y-Romo, & Tapia, 2013; Rossaert et al., 2019). Finally, *GEMIN4* and *HNRNPA3* are involved in RNA processing and interact closely with ALS-causative proteins in this machinery. *GEMIN4* acts in the survival motor neuron complex formation, disrupted in lower motor neuron disease, and is in the *FUS* interactome together with *HNRNPA3*, which is detected in the spinal cords of *C9orf72*-positive ALS and FTD patients (Chi et al., 2018; Davidson et al., 2017; Fifita et al., 2017). Altogether, pathogenic variants in these genes, which are not yet detected in familial ALS, should be further investigated in larger cohorts for their possible contribution to sporadic ALS.

Although there is a wide variability in disease incidence and manifestation across populations and geographical regions, we acknowledge the shortcoming of our analyses to catch a significant population-specific signal considering the insufficient number of ALS patients and controls, which calls for far more Turkish samples to be sequenced. On the other hand, our WGS data with 900 samples and expanding, is expected to exemplify a unique population with a heterogeneous gene pool that will support to study the combinatorial effects of diverse SNPs in manifestation of sALS. In this respect, we are confident that this report will encourage local clinicians for recruitment of new patients. Only then, it will be possible to overcome power limitations, currently faced even by Project MinE with >9,000 DNA samples analyzed (Dekker et al., 2019; van Rheenen et al., 2018). The answer to the question of how to move forward at this point will be the collection of larger case and control cohorts in the framework of national and international collaborations and making all genomic data publicly available to increase the power of datasets. This will allow us to understand and interpret how a variant causes disease within the context of the larger population.

## 7 | CONCLUSION

Genetics offers a means to dissect the heterogeneity of ALS and to understand the cellular mechanisms resulting in motor neuron degeneration. The recent genetic findings driven by the NGS technology have not only expanded our knowledge of the wealth of genes giving rise to motor neuron degeneration, but also on the pleiotropic effects and extensive phenotypic spectra associated with specific ALS genes. Since the road from family pedigrees to clinical interpretation of variants is challenging, deep phenotyping of the patient, comprehensive analysis of the candidate variants with advanced bioinformatic tools and most importantly a tight researcher-clinician relationship are indispensable parts of the whole process. Ultimately, the discovery of all ALS genes will help to better define the multifaceted nature of ALS, which is accepted no more as a monolithic disease, but recognized as a spectrum of diseases converging into common clinical features. This allows a subclassification of patients into more precise clinical categories in which a common genetic cause is more likely to be identified.

Data presented in this study compiles the molecular analysis results of ALS patients at NDAL in Istanbul, Turkey for 18 years and is an expanded update and upscale of our 2015 results from 443 to 1,200 probands (Özoğuz et al., 2015). The excessive number of rare and novel variants reported here once more show the power of clinical exome sequencing. GWAS conducted with 800 WGS samples and coupled with a coexpression network analysis identifies disease-risk gene candidates. The results on Turkey presented here will hopefully contribute to the diversity of genetic and mechanistic factors underlying ALS, further driving research. Moreover, they are expected to shed light on the multilevel heterogeneity of the disease, as an important factor in a precision medicine approach towards the development of molecular therapies for stratified patient subgroups. Finally, the GeNDAL variant browser, a novel tool to observe Turkish population-specific allele frequencies, is expected to be a unique and valuable resource for disease gene identification studies for the neurogenetics community.

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## CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

## AUTHOR CONTRIBUTIONS

C. T., A. N. B., A. E. Ç., and E. K. designed the study. C. T., T. Ş., F. A., C. C., E. B., R. P., S. Z., C. K., E. K., N. E. Ş., H. H., A. O. E., U. N., O. K., and G. O. performed the experiments. T. A., H. D., E. Ş., A. Ç., A. B. G., G. B. Y., B. İ., K. U., H. H., B. B., N. T., F. A., M. E., C. B., D. K., H. İ., A. S., N. U. A., M. A. A., F. K., E. T., P. O., F. D., and Y. P. performed neurological examination on the patients and provided detailed clinical information. C. T. and A. N. B.

wrote the manuscript. F. A., A. E. Ç., E. K., Ö. T., and Y. P. were instrumental in the compilation of parts of the manuscript.

## DATA AVAILABILITY STATEMENT

Data in this paper is published within the paper and deposited to ClinVar public database (<https://www.ncbi.nlm.nih.gov/clinvar/?term=SUB7287039>). Additionally, ALS-related variants identified in the WGS data are available in GeNDAL variant browser ([www.gendal.org](http://www.gendal.org)).

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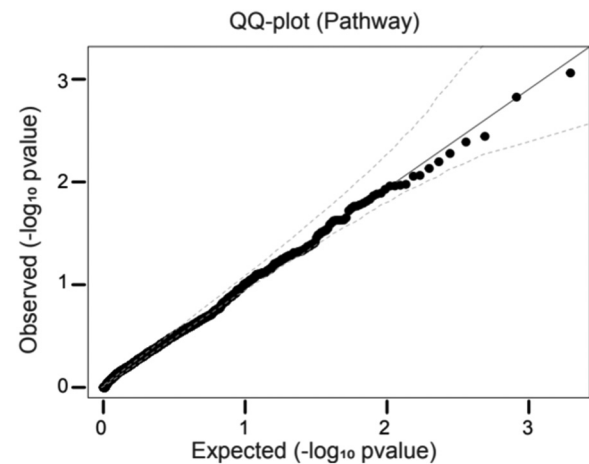
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## APPENDIX

Figure A1. Table A1–A10.



**FIGURE A1** The quantile-quantile plot of pathway based SKAT-O  $p$  values. Each dot represents a pathway entry in gene set enrichment analysis

**TABLE A1** Clinical data of cases investigated with WES

Family #	Gender	Age of onset	Consanguinity	Family history	Inheritance	Initial diagnosis																																																																																																																																																																																																																																																															
Fam1	M	50	No	Yes	AD	ALS																																																																																																																																																																																																																																																															
	M	45	No				Fam2	M	20	No	No	Inconclusive	Juvenile ALS	Fam3	M	17	No	No	Inconclusive	Juvenile ALS	Fam4	M	23	Yes	Yes	AR	Early onset ALS	Fam5	M	14	Yes	Yes	AR	Juvenile ALS	Fam6	F	17	Yes	Yes	AR	Juvenile ALS	Fam7	F	15	Yes	Yes	AR	Juvenile ALS	M	49	Yes	M	32	Yes	Fam8	F	17	Yes	No	AR	Juvenile MND	Fam9	M	22	Yes	No	AR	Juvenile ALS	Fam10	M	17	Yes	No	AR	Juvenile ALS	Fam11	F	37	Yes	No	AR	ALS	Fam12	F	22	Yes	Yes	AR	Juvenile ALS	Fam13	F	47	No	Yes	AD	ALS/sensory neuropathy	Fam14	M	21	No	No	Inconclusive	MND	Fam15	M	40	Yes	No	AR	ALS	Fam16	F	45	Yes	No	AR	ALS	Fam17	M	27	Yes	No	AR	ALS	Fam18	M	25	Yes	No	AR	ALS	Fam19	F	37	Yes	No	AR	ALS	Fam20	M	14	Yes	No	AR	Juvenile MND	Fam21	M	17	Yes	No	AR	Juvenile MND	Fam22	M	61	Yes	No	AR	ALS	Fam23	M	60	Yes	Yes	AR	ALS	Fam24	F	49	No	Yes	AD	ALS	Fam25	M	44	Yes	Yes	AR	ALS	Fam26	F	17	Yes	No	AR	Juvenile ALS/disferlinopathy	Fam27	M	60	No	Yes	AD	ALS	Fam28	F	52	Yes	Yes	Inconclusive	ALS	F	32	Yes	AR	Fam29	M	23	Yes	No	AR	MND	Fam30	M	26	Yes	No	AR	ALS	Fam31	F	31	Yes	No	AR	MND	Fam32	M	9	Yes	No	AR	Juvenile ALS	Fam33	F	10	Yes	No	AR	Juvenile ALS	Fam34	M	21	Yes	Yes	AR	Juvenile ALS	Fam35	F	1	Yes	Yes	AR	Juvenile MND	M	41	Yes	ALS	Fam36	F	20
Fam2	M	20	No	No	Inconclusive	Juvenile ALS																																																																																																																																																																																																																																																															
Fam3	M	17	No	No	Inconclusive	Juvenile ALS																																																																																																																																																																																																																																																															
Fam4	M	23	Yes	Yes	AR	Early onset ALS																																																																																																																																																																																																																																																															
Fam5	M	14	Yes	Yes	AR	Juvenile ALS																																																																																																																																																																																																																																																															
Fam6	F	17	Yes	Yes	AR	Juvenile ALS																																																																																																																																																																																																																																																															
Fam7	F	15	Yes	Yes	AR	Juvenile ALS																																																																																																																																																																																																																																																															
	M	49	Yes																																																																																																																																																																																																																																																																		
	M	32	Yes																																																																																																																																																																																																																																																																		
Fam8	F	17	Yes	No	AR	Juvenile MND																																																																																																																																																																																																																																																															
Fam9	M	22	Yes	No	AR	Juvenile ALS																																																																																																																																																																																																																																																															
Fam10	M	17	Yes	No	AR	Juvenile ALS																																																																																																																																																																																																																																																															
Fam11	F	37	Yes	No	AR	ALS																																																																																																																																																																																																																																																															
Fam12	F	22	Yes	Yes	AR	Juvenile ALS																																																																																																																																																																																																																																																															
Fam13	F	47	No	Yes	AD	ALS/sensory neuropathy																																																																																																																																																																																																																																																															
Fam14	M	21	No	No	Inconclusive	MND																																																																																																																																																																																																																																																															
Fam15	M	40	Yes	No	AR	ALS																																																																																																																																																																																																																																																															
Fam16	F	45	Yes	No	AR	ALS																																																																																																																																																																																																																																																															
Fam17	M	27	Yes	No	AR	ALS																																																																																																																																																																																																																																																															
Fam18	M	25	Yes	No	AR	ALS																																																																																																																																																																																																																																																															
Fam19	F	37	Yes	No	AR	ALS																																																																																																																																																																																																																																																															
Fam20	M	14	Yes	No	AR	Juvenile MND																																																																																																																																																																																																																																																															
Fam21	M	17	Yes	No	AR	Juvenile MND																																																																																																																																																																																																																																																															
Fam22	M	61	Yes	No	AR	ALS																																																																																																																																																																																																																																																															
Fam23	M	60	Yes	Yes	AR	ALS																																																																																																																																																																																																																																																															
Fam24	F	49	No	Yes	AD	ALS																																																																																																																																																																																																																																																															
Fam25	M	44	Yes	Yes	AR	ALS																																																																																																																																																																																																																																																															
Fam26	F	17	Yes	No	AR	Juvenile ALS/disferlinopathy																																																																																																																																																																																																																																																															
Fam27	M	60	No	Yes	AD	ALS																																																																																																																																																																																																																																																															
Fam28	F	52	Yes	Yes	Inconclusive	ALS																																																																																																																																																																																																																																																															
	F	32	Yes		AR																																																																																																																																																																																																																																																																
Fam29	M	23	Yes	No	AR	MND																																																																																																																																																																																																																																																															
Fam30	M	26	Yes	No	AR	ALS																																																																																																																																																																																																																																																															
Fam31	F	31	Yes	No	AR	MND																																																																																																																																																																																																																																																															
Fam32	M	9	Yes	No	AR	Juvenile ALS																																																																																																																																																																																																																																																															
Fam33	F	10	Yes	No	AR	Juvenile ALS																																																																																																																																																																																																																																																															
Fam34	M	21	Yes	Yes	AR	Juvenile ALS																																																																																																																																																																																																																																																															
Fam35	F	1	Yes	Yes	AR	Juvenile MND																																																																																																																																																																																																																																																															
	M	41	Yes			ALS																																																																																																																																																																																																																																																															
Fam36	F	20	Yes	Yes	AR	Juvenile ALS																																																																																																																																																																																																																																																															

(Continues)

TABLE A1 (Continued)

Family #	Gender	Age of onset	Consanguinity	Family history	Inheritance	Initial diagnosis
	M	13	Yes			SBMA
	F	20	Yes			Juvenile ALS
Fam37	F	25	Yes	No	AR	MND
Fam38	M	17	Yes	No	AR	Juvenile MND
Fam39	M	20	Yes	No	AR	Juvenile MND
Fam40	M	2	Yes	No	AR	Juvenile ALS
Fam41	F	Childhood	No	Yes	AD	CMT
	F		No			Juvenile MND
Fam42	M	52	No	No	Inconclusive	ALS
Fam43	F	60	Yes	Yes	Inconclusive	ALS/FTD
	F	60	Yes			
Fam44	F	48	No	Yes	AD	ALS
	F	48	No			
	M	47	No			
Fam45	F	16	No	Inconclusive	Inconclusive	ALS/Madras MND
Fam46	M	17	Yes	No	Inconclusive	ALS
Fam47	F	10	Yes	Yes	AR	Juvenile MND
	F		Yes			
Fam48	M	20	Yes	No	AR	MND
Fam49	M	35	Yes	No	AR	MND
Fam50	M	25	Yes	No	AR	Early onset ALS
Fam51	F	~3 months	Yes	Yes	AR	Juvenile MND
	F		Yes			
Fam52	M	25	No	No	Inconclusive	MND
Fam53	F	57	Yes	Yes	AR	ALS
	M	44	Yes			
Fam54	M	29	Yes	No	AR	MND
Fam55	F	58	No	Yes	AD	ALS
Fam56	F	76	No	Inconclusive	Inconclusive	ALS
Fam57	M	51	No	Yes	AD	ALS
	F	NA	No			
Fam58	F	40	No	Yes	AR	ALS
	M	45	No			
	F	NA	No			
Fam59	M	46	No	Yes	Inconclusive	ALS
Fam60	M	40	No	Yes	AD	ALS
	F	67	No			
Fam61	M	52	No	Yes	AD	ALS
Fam62	M	46	No	Yes	AD	ALS
Fam63	M	65	No	Yes	Inconclusive	ALS
Fam64	M	41	Yes	No	AR	ALS
Fam65	M	24	Yes	No	AR	Early onset ALS



**TABLE A1** (Continued)

Family #	Gender	Age of onset	Consanguinity	Family history	Inheritance	Initial diagnosis
Fam66	M	6	Yes	Yes	AR	Juvenile MND
Fam67	M	14	Yes	No	AR	MND
Fam68	F	22	Yes	No	AR	Juvenile MND
Fam69	M	22	Yes	Yes	AR	ALS
Fam70	F	20	Yes	No	AR	MND
Fam71	F	Congenital	Yes	No	AR	MND
Fam72	M	14	Yes	Yes	AR	Juvenile ALS
Fam73	F	16	Yes	Yes	AR	ALS
Fam74	F	8	Yes	Yes	AR	Juvenile MND
	F	8	Yes			
	F	9	Yes			
Fam75	F	54	No	Yes	AD	ALS
	F	58	No			
	F	54	No			
Fam76	F	58	No	Yes	Inconclusive	ALS
Fam77	F	16	No	No	Inconclusive	ALS/Madras MND
Fam78	M	12	Yes	Yes	AR	Juvenile ALS
Fam79	M	39	No	Yes	Inconclusive	MND
	F	24	No			
Fam80	M	66	No	No	Inconclusive	ALS
Fam81	M	29	Yes	No	AR	ALS
Fam82	F	45	No	Yes	AD	ALS
Fam83	M	14	No	No	Inconclusive	Juvenile ALS
Fam84	F	32	No	Yes	AD	ALS
Fam85	M	67	No	Yes	AD	ALS
	F	42	No			
Fam86	M	58	No	Yes	AD	ALS
	F	59	No			
Fam87	F	46	No	Yes	AD	ALS
Fam88	M	44	Yes	Yes	AR	ALS
Fam89	F	37	Yes	No	AR	ALS
Fam90	M	57	Yes	No	AR	ALS
Fam91	M	24	Yes	No	AR	ALS
Fam92	M	51	No	Yes	AD	ALS
	F	NA	No			
Fam93	F	11	No	Yes	AD	Juvenile ALS
Fam94	F	38	Yes	No	AR	ALS
Fam95	M	31	No	No	AR	MND
Fam96	M	27	No	No	Inconclusive	ALS
Fam97	M	20	No	Yes	AR	MND
Fam98	F	52	Yes	No	AR	ALS

(Continues)

**TABLE A1** (Continued)

Family #	Gender	Age of onset	Consanguinity	Family history	Inheritance	Initial diagnosis																																																																																																																																																																																																															
Fam99	M	61	No	Yes	AR	ALS																																																																																																																																																																																																															
	F	65	No				Fam100	M	28	No	No	Inconclusive	MND	Fam101	F	31	Yes	Yes	AD	ALS	Fam102	F	54	No	Yes	AD	ALS	Fam103	F	49	Yes	No	AR	ALS	Fam104	M	43	Yes	No	AR	ALS	Fam105	F	29	No	No	Inconclusive	ALS	Fam106	M	45	Yes	No	AR	ALS	Fam107	F	1	Yes	No	AR	Juvenile ALS	Fam108	M	Teenage	No	Yes	Inconclusive	Juvenile ALS	M	Teenage	No	Fam109	M	33	Yes	Yes	AR	ALS	M	36	Yes	Fam110	M	21	Yes	Yes	AR	Juvenile ALS	Fam111	F	58	No	Yes	AD	ALS	Fam112	F	32	Yes	Yes	AR	ALS	Fam113	F	57	Yes	No	AR	ALS	Fam114	M	19	Yes	Yes	Inconclusive	ALS	Fam115	F	58	No	Yes	AD	ALS	Fam116	M	42	Yes	Yes	AR	ALS	F	43	Yes	Fam117	M	26	No	Yes	AD	ALS	Fam118	F	9	Yes	No	AR	ALS	Fam119	M	37	No	Yes	AD	ALS	M	NA	No	M	42	No	Fam120	F	21	No	No	Inconclusive	ALS	Fam121	F	38	Yes	No	AR	ALS	Fam122	M	58	Yes	No	AR	ALS	Fam123	M	1	Yes	No	AR	Juvenile ALS	Fam124	M	1	Yes	Yes	AR	Juvenile ALS	Fam125	F	2,5	Yes	No	AR	Juvenile ALS	Fam126	M	16	Yes	No	AR	ALS	Fam127	M	24
Fam100	M	28	No	No	Inconclusive	MND																																																																																																																																																																																																															
Fam101	F	31	Yes	Yes	AD	ALS																																																																																																																																																																																																															
Fam102	F	54	No	Yes	AD	ALS																																																																																																																																																																																																															
Fam103	F	49	Yes	No	AR	ALS																																																																																																																																																																																																															
Fam104	M	43	Yes	No	AR	ALS																																																																																																																																																																																																															
Fam105	F	29	No	No	Inconclusive	ALS																																																																																																																																																																																																															
Fam106	M	45	Yes	No	AR	ALS																																																																																																																																																																																																															
Fam107	F	1	Yes	No	AR	Juvenile ALS																																																																																																																																																																																																															
Fam108	M	Teenage	No	Yes	Inconclusive	Juvenile ALS																																																																																																																																																																																																															
	M	Teenage	No																																																																																																																																																																																																																		
Fam109	M	33	Yes	Yes	AR	ALS																																																																																																																																																																																																															
	M	36	Yes																																																																																																																																																																																																																		
Fam110	M	21	Yes	Yes	AR	Juvenile ALS																																																																																																																																																																																																															
Fam111	F	58	No	Yes	AD	ALS																																																																																																																																																																																																															
Fam112	F	32	Yes	Yes	AR	ALS																																																																																																																																																																																																															
Fam113	F	57	Yes	No	AR	ALS																																																																																																																																																																																																															
Fam114	M	19	Yes	Yes	Inconclusive	ALS																																																																																																																																																																																																															
Fam115	F	58	No	Yes	AD	ALS																																																																																																																																																																																																															
Fam116	M	42	Yes	Yes	AR	ALS																																																																																																																																																																																																															
	F	43	Yes																																																																																																																																																																																																																		
Fam117	M	26	No	Yes	AD	ALS																																																																																																																																																																																																															
Fam118	F	9	Yes	No	AR	ALS																																																																																																																																																																																																															
Fam119	M	37	No	Yes	AD	ALS																																																																																																																																																																																																															
	M	NA	No																																																																																																																																																																																																																		
	M	42	No																																																																																																																																																																																																																		
Fam120	F	21	No	No	Inconclusive	ALS																																																																																																																																																																																																															
Fam121	F	38	Yes	No	AR	ALS																																																																																																																																																																																																															
Fam122	M	58	Yes	No	AR	ALS																																																																																																																																																																																																															
Fam123	M	1	Yes	No	AR	Juvenile ALS																																																																																																																																																																																																															
Fam124	M	1	Yes	Yes	AR	Juvenile ALS																																																																																																																																																																																																															
Fam125	F	2,5	Yes	No	AR	Juvenile ALS																																																																																																																																																																																																															
Fam126	M	16	Yes	No	AR	ALS																																																																																																																																																																																																															
Fam127	M	24	Yes	Yes	AR	ALS-Parkinson-Dementia Complex																																																																																																																																																																																																															

Abbreviations: ALS, amyotrophic lateral sclerosis; MND, motor neuron disease.

**TABLE A2** Gene-based scores from GeneRIF and DisGeNet databases

	Gene	Count	
GeneRIF Scores	SOD1	216	
	TARDBP	106	
	C9ORF72	91	
	FUS	66	
	ATXN2	19	
	OPTN	18	
	PFN1, VAPB	17	
	ANG, TBK1	15	
	VCP	13	
	VEGFA, UBQLN2	11	
	IGFALS, PON1	10	
	HFE, NEFH, CHCHD10	8	
	SQSTM1	7	
	APOE, CHGB, GRN, NEFL, MATR3, UNC13A, ZNF512B	6	
	DPP6, SIGMAR1, SETX, FGGY	5	
	APP, DCTN1, IGF1, MMP9, MTHFR, KIFAP3, CHMP2B, ALS2	4	
	CCNF, CST3, HNRNPA1, MAPT, SLC1A2, TIA1, TUBA4A, FIG4, TREM2, RTN4, LOC643387	3	
	AR, CASP3, CYBB, DAO, ELAVL1, ERBB4, EWSR1, GPX3, GRIA2, GSK3B, IGFBP3, ITPR2, LGALS3, NFE2L2, NOS1, P2RX7, P4HB, PON2, PRPH, CCL2, SMN1, SMN2, TLR4, TNF, XRCC1, TAF15, RAB29, GPNMB, PPARGC1A, PARK7, SS18L1, KCNIP1, VPS54, SUSD2, CAMK1G, ARHGEF28, BTBD10, LRRK2, NIPA1, RBM45, MIR206	2	
	ADARB1, ADCYAP1, ADCYAP1R1, ADH5, ADORA2A, PARP1, AGER, ANXA11, APEX1, ATP5F1D, BDNF, BRCA1, BSG, C3, CHGA, CHI3L1, CHI3L2, CHRNA4, TBCB, CNTF, CSF2, CSPG4, CTSD, CX3CR1, CYP27A1, ACE, DDIT3, EPHA3, EPO, FXN, FTL, GABRA1, GJA1, GLE1, GRIA1, GSK3A, GSTP1, HTT, HDAC2, NRG1, HIF1A, HK1, HLA-DRA, HLA-DRB5, HLA-F, HMGB1, HNMT, HNRNPA2B1, HNRNPH1, HSF1, HTR2B, IDI1, IFNG, IGF1R, IGF2, IGFBP2, IGHMBP2, IL6, IL10, IL18, ITIH4, KARS, KCNA2, KDR, KIF5C, KIFC3, KIR3DL2, KRT18, LAMC1, LOX, SMAD2, SMAD3, MAP1B, MBL2, MEF2C, MEF2D, MET, MMP2, MYO6, MYOD1, NAIP, NEK1, NGFR, SLC11A2, OGG1, PAX7, PGR, ABCB1, SERPINA1, PIN1, PLA2G4A, PON3, POU5F1, PPIA, MAP2K5, PSEN1, PTGS2, PURA, RAB1A, RAB5A, RET, RRAD, SLC16A2, SNCA, SNRPC, SOX2, SOX5, STAT3, TERT, TFAM, TIAM1, TLR2, TNFRSF1A, TNFRSF1B, TNNT2, TP53, TXNRD1, VDAC1, VDR, VSNL1, SLC30A3, TNFSF14, AIFM1, VAPA, ABCG2, KEAP1, CCS, HDAC6, SH2B3, TNIP1, YWHAQ, KIF3A, DCTN3, SIRT2, KIF1B, FAM120A, SIRT3, SIRT1, CABIN1, HSPB8, PABPC1, COQ2, STK39, HTRA2, UBQLN1, CHCHD2, IL23A, BCL11A, EQTN, OXR1, ELP3, RHOT1, MFN1, SLC30A6, PDGFC, UBQLN4, GJD2, SORCS2, PINK1, FTO, HDAC11, IFT74, SPG11, POLDIP3, TTBK1, IDI2, NLRP3, FBXO32, GRIN3B, TTBK2, NEWENTRY, HNRNPA3, ALS2CL, MIR125B1, MIR125B2, MIR141, MIR200A, MIR338, MIR424, CCR2, WASHC1, MIR1825	1	
	DisGeNet Scores	SOD1	1,291
		TARDBP	396
		C9ORF72	282
		FUS	199
		IGFALS	55
		VCP	54
		ATXN2	46
		OPTN	41
		ALS2	36
		ANG	35
		VEGFA, UBQLN2	32
		SLC1A2	29
		RBMS3, VAPB, PTBP1, SRRM2	28
		PNO1	27
PFN1, SQSTM1		23	
SETX		21	
GRN		18	
PRPH		17	
GDNF, NEFH		16	
SMN2, SMN1		14	

(Continues)

TABLE A2 (Continued)

Gene	Count
NEFL, MATR3, CSF2, TBK1	13
PON1, UNC13A, SIGMAR1, DCTN1, LAMC2	12
NUP62, DCTN4, MAPT, GFAP, KHDRBS1, STMN1, GTF2H1, HFE	11
BCL2, SNRPN, SNURF, GRIA2	10
NFE2L2, MAK16, APOE	9
TREM2, SPG11, CASP3, CHCHD10, RIMS2, HNRNPA1, GABPA, CNTF	8
DPP6, GRM2, PTGS2, PPARGC1A, AR, IGF1, TNF, APP, CHMP2B	7
TP53, SOD2, CCL2, TAF15, CST3	6
NUP98, ADARB1, C3	5
ROPN1L, ASPM, APEX1, RNASE4, A1CF, P2RX7, ASPA, CAT, GPNMB, CNR2, ATG5, TUBA4A, CDK5, RAN, MOB3B, ASIP, EPHA4, KIFAP3, EPO, CASP1, NTF3, MST1, XBP1, SIRT1, ADA	4
ZNF569, COX2, CYP27A1, TMEM189-UBE2V1, DECR1, DES, S100A6, GRM5, BDNF, FIG4, TNFRSF1B, LIF, ELP3, GARS, TRPM7, NOS2, NOS1, NFKB1, PPARG, TMEM106B, TMPPRS13, RREB1, FMR1, ADAR, ZNF436, MMP9, THY1, XBP1P1, TLR4, PARP1, XIAP, ALAD, MSMB, HSPA4, P2RX4, MIR206, ZNF253, HSF1, BCL2L1, TMEM189, CCS, ZNF763, LMLN, CHGB, TNPO1, NEK1, UBE2V1, ZNF629, CYBB	3
CAST, PLA2G4A, PRNP, RBM8A, DNMT1, DAO, CUX1, CTSD, PTPA, RTN4, VDR, PIN1, VGF, PDC, VPS54, CREBBP, NELFE, FMO1, KIF1B, P2RX2, ATF6, HDAC6, OXR1, CTF1, P2RY1, TANK, RNF19A, SARM1, SS18L1, SUMO1, PHGDH, P2RY2, P2RX1, PRKN, P2RX3, C5AR1, P2RX5, TXN, MAPK8, TRPM2, OSBP, NIPA1, KCNA3, KHSRP, AKT1, POMC, AKT3, CS, EPHA3, FGF2, CASP12, PSEN1, TIAM1, IL2RB, AQP4, SRR, TUSC2, NRG1, GRIK1, SUGP1, KCNJ10, SIGLEC7, NGF, TIA1, APRT, TGM2, TFAM, NEFM, NAIP, HNRNPA2B1, CCNF, GRIA3, GRIA1, LSM2, GPX3, LGALS1, HNRNPH2, LGALS3, ATXN3, BSCL2, INA, MOBP, PSIP1, UBQLN1, HCLS1, HTT, PIGL, P2RX6, HNRNPA1P10, KEAP1, CHGA, ATXN2-AS, HMOX1, RAG2, HNRNPH1, RNASE1, SLC3A2, HSPB1, PINK1, RRAD, IFNG	2
RBM45, COX8A, PPARGC1B, SLC06A1, MAP3K8, CNR1, TPPP, COL4A2, DBX1, LRRK2, CRP, CP, SH2B3, CRYAB, CRYM, MAPK14, CSF3, TTBK2, RBFOX3, LGALS16, CTNND2, CPNE4, RIPK3, YWHAQ, PNPLA6, CLU, SERPINA3, MECP2, ARAP2, MYBPH, MAP1B, MB, MDH1, CHAF1A, MAP3K5, MET, KITLG, MMP14, MPZ, MIR338, MT1A, MYH6, GRIN3B, MYO6, MAOB, DDX19A, ANKRD1, LAT, EGLN3, SLC52A3, NLRP3, CARD16, FBXO32, ADCYAP1, MIR1825, SPAG11B, MIR4299, GJB1, DDIT3, SIRT3, GRIP1, ZFYVE26, CABIN1, FOS, FPR2, SMUG1, DDX58, SLC7A11, ALOX5, MTOR, ABL1, ALS3, GABRA1, SIRT5, SUN3, RAB3GAP2, ALS2CL, GAP43, GAPDH, TIPARP, OSBP3, GART, SPAG8, CLVS1, CLEC10A, OCLN, CDKN2A, AFF1-AS1, ERVK-2, MIR4649, ERVK-12, ERVK-22, ERVK-11, LINC00351, PPIF, CDK2, ALYREF, OLIG2, KCNMB2, NES, CHL1, MASP2, SIX2, OLFM4, ADARB2, CX3CR1, KCNMB2-AS1, CFDP1, KDR, SEMA3A, CDKN2D, TNIP1, MARCKS, XRN1, MIR23A, JAG2, JUN, IGF1R, HLA-DRB1, HLA-B, ANXA11, TNFRSF21, AMPH, HPGDS, GLE1, EIF3K, UBE2S, GLG1, HTRA2, ADGRD1, KRT18P55, SLC9A9, GPX1, ANK3, GRIA4, ANPEP, GRIN2A, GRM1, GRM3, GSR, GSTP1, JAK3, ITPR2, MIR155, ITGA9, HSPB8, HNRNPDL, OTOG, HSPB2, HSPA8, DNAJB2, HRES1, PRMT1, IDE, HNRNPK, HNRNPC, APAF1, HMOX2, HES1, TGM6, IGF2R, HLA-F, IGFBP7, FAS, IL1B, IL4, IL10, IL13, IL17A, CXCL10, UBN1, SLC40A1, HDAC2, HEXA, PFN2, PKLR, PLCD1, ATP7A, TMED9, PPIA, PPID, FGGY, RHOT1, NGFR, NFIL3, NEUROG1, NCAM1, LAMA3, LAMC1, LCN2, LDLR, LGALS4, LIG4, FADS3, LMNB1, LTBR, LUM, LY6E, MIR146A, PENK, SERPINF1, IL23A, NTF4, HGF, PIK3R4, HIF1A, UBE2K, GSTK1, MNX1, KCNK2, KIF3C, STK36, NOTCH1, NRF1, OPCML, PDGFA, P4HB, GEMIN4, PAWR, PCBP1, FOXP3, ATL1, APH1A, DBR1, PCSK2, SLC25A37, PRRX2, ANKRD2, DCTN5, GBX2, CDK5R1, PROM1, HSP90B2P, TRN-GTT2-1, TLE3, TTR, TNFRSF10B, CCR2, TXNRD1, RAB11A, UCP2, UCP3, UGCG, TNFSF14, RIPK1, VDACL1, ELP1, MAP4K3, VIM, INPP4B, GTPBP1, POLDIP3, DDX46, RARA, RASGRF1, ABCG1, TP73, ARHGEF7, TFPI2, TNS1, MSTN, IFT74, BRD3, TTBK1, CASR, PABPN1, CAMK4, DHX16, TRIM8, CAPN1, CDC7, ARHGAP24, BTBD10, C19ORF12, CASP9, XRCC1, WNT7A, CLIP2, VSNL1, RAB29, TLR2, TMSB4X, CLDN5, BCL10, TNFRSF1A, REG1A, PRMT8, RELA, RANGAP1, SUSD2, MFN2, SORT1, PKN1, RXRA, TDP1, SLC12A5, PTPRN, MAPK1, MAPK3, CLIP1, CD40LG, RRM2, PRDX6, MAPK9, MAP2K5, EIF2AK2, S100A8, S100A9, KIAA0513, ZNF704, ATXN1, PTPRZ1, BCYRN1, CCDC88A, GDA, CAMK1G, NUP153, CD59, UPF1, WASHC5, PTS, MIR524, CD68, HDAC4, TRIM27, RET, CTR9, ZNF512B, BAX, RAB5A, PVALB, KIAA0040, RAB1A, PTEN, BAG1, SLC17A6, PTGER2, PSMD2, RALGDS, LXN, BBS2, JPH3, HDAC11, ATAT1, CALB2, SIRT2, CBLL2, AAK1, FCGR3B, FCGR3A, PTK2B, F9, EWSR1, ETS2, FGF6, PRF1, A1BG, SLC30A6, SNAP25, SNCA, VAPA, SLC33A1, SOS1, SPAST, BRCA1, SST, STAT5A, SULT1E1, UNC119, BSG, CALB1, FNDC3A, SCFD1, TAC1, CAMTA1, GDI1, PABPC1, SUN2, ICE1, PSD3, BICD2, GSX2, DHFR, DNM2, DNMT3A, DPYD, EDN1, EGF, CELSR3, EGFR, EIF4G2, ELAVL1, ENG, EPHA1, EPHB2, AK4, ERBB4, EREG, MGRN1, FMO3, SYT1, SYP, HSPB3, SI, TACR1, ST3GAL3, BCL11B, SLC1A1, NRXN1, WNK1, CD163, SLC6A1, SLC6A3, SPAG11A, SLC12A2, CD14, SLC12A4, MSC, CHST2, SCD, TIMP3, MGAM, GEMIN2, PHF5A, CFAP410, SLC30A3, CACNA1S, SCG2, DERL1, TIMP2, MTHFS, GORASP1, SUMO2, TAF12, TAT, MIR582, PRDX2, MFN1, TEAD1, TGFB1, TGFB2, TSP0, SHC1, SNAI1, TIMP1, SUMO3, ARHGEF28, SGK1, SIL1, CD7, COP1, LOC643387, SFPQ, NPEPPS, SELPLG, PTGES, SCN8A	1

**TABLE A3** Clinical data of patients with C9orf72 repeat expansion

	Total ALS	fALS	sALS
<b>#</b>			
Probands	80	42	38
Family members	8 + 7 asymptomatic	8	–
Male	45	23	22
Female	35	19	16
Male:female	1.3	1.2	1.4
<b>AO</b>			
Juvenile (<25 yrs)	1	1	–
Middle (25–45 yrs)	9	5	4
Late (>45 yrs)	66	36	32
Range (years)	32–80	32–80	40–71
<b>MAO</b>			
Total $\pm$ SD	54.5 $\pm$ 9.9	54.0 $\pm$ 11.0	55.0 $\pm$ 7.8
Male $\pm$ SD	52.7 $\pm$ 11.2	51.14 $\pm$ 13.3	54.4 $\pm$ 8.1
Female $\pm$ SD	56.7 $\pm$ 7.5	57.3 $\pm$ 7.5	56.0 $\pm$ 7.3
	9	5	4
<b>Dementia SO</b>			
Limb	50	26	24
Bulbar	18	12	6
Limb + bulbar	5	2	3
Not available	5	1	4

Abbreviations: #, numbers; ALS, amyotrophic lateral sclerosis; AO, age of onset; fALS, familial ALS; MAO, mean age of onset; sALS, sporadic ALS; SD, standard deviation; SO, site of onset.

**TABLE A4** Information on variants identified by WES and WGS

Gene	Chromosomal location	Amino acid change	dbSNP ID	GERP++ score (version 2010)	DANN score (version 2014)	ACMG verdict	ExAC
ERBB4	2:212251725	p.(Arg1096Cys)	rs144311212	5.59	0.99	Likely pathogenic	-
KIF5A	12:57976397	p.(Asp1002Gly)	-	5.47	0.99	Likely pathogenic	-
TBK1	12:64875731	p.(Arg308*)	rs1284582102	5.15	0.99	Pathogenic	-
	12:64882360	p.(Val479Gluufs*4)	rs876657405	5.25	-	Likely pathogenic	-
VCP	9:35065361	p.(Arg155Cys)	rs121909330	6.02	0.99	Likely pathogenic	-
	9:35065349	p.(Arg159Cys)	rs387906789	6.07	0.99	Likely pathogenic	-
	9:35065252	p.(Arg191Pro)	-	5.64	0.99	Likely pathogenic	-
UBQLN2	X:56591822	p.(Pro506Ser)	rs387906711	4.17	0.93	VUS	-
	X:56591879	p.(Pro525Ser)	rs369947678	3.92	0.84	VUS	-
TFG	3:100467026	p.(Pro285Leu)	rs2074822230	6.16	1	Likely pathogenic	-
ANG	14:21161931	p.(Ile70Val)	rs121909541	4.73	0.5	VUS	0.0006095
CHCHD10	22:24109646	p.(Ser59Leu)	-	3.66	0.99	Likely pathogenic	-
FBXO38	5:147796726	p.(Arg526Gln)	rs376255193	5.51	0.99	Likely pathogenic	0.07485
TRPV4	12:110236628	p.(Arg315Trp)	rs267607143	0.22	0.99	Likely pathogenic	-
TRPM7	15:50878630	p.(Thr1482Ile)	rs8042919	5.25	0.98	VUS	0.08703
SETX	9:135172384	p.(Ala1947Thr)	rs141440621	5.53	0.99	VUS	0.00008318
ERLIN1	10:101937913	p.(Val94Ala)	-	5.23	1	Pathogenic	-
SPG11	15:44943713	p.(Gln478*)	-	6.05	0.99	Pathogenic	-
	15:44943713	p.(Lys656Valfs*11)	-	5.71	-	Likely pathogenic	-
	15:44914992	p.(Phe750Leufs*3)	-	2.64	-	Pathogenic	-
	15:44855496	p.(Tyr2385*)	rs778305085	3.09	0.99	Pathogenic	-
OPTN	10:13151192	p.(His26Thrfs*19)	rs766608795	5.44	-	Pathogenic	-
	10:13164479	p.(Glu293Glyfs*19)	-	5.82	-	Likely pathogenic	-
	10:13167494	p.(Lys360Valfs*18)	-	5.44	-	Likely pathogenic	-
	10:13168014	p.(Thr406Lysfs*5)	-	5.57	-	Pathogenic	-
ALS2	2:202617888	p.(Ala573Glu)	-	5.16	0.99	Likely pathogenic	-
	2:202593315	p.(Arg921*)	rs587777132	5.82	0.99	Pathogenic	-
	2:202572614	p.(Arg1461*)	rs374047961	5.92	0.99	Pathogenic	-
	2:202569207	p.(Pro1603Leu)	-	5.53	0.99	Likely pathogenic	-
C19orf12	19:30199322	p.(Thr11Met)	rs397514477	-11.2	0.89	Likely pathogenic	0.00000831
	19:30193884	p.(Gly65Val)	-	4.57	0.99	Likely pathogenic	0.0000165

TABLE A4 (Continued)

Gene	Chromosomal location	Amino acid change	dbSNP ID	GERP++ score (version 2010)	DANN score (version 2014)	ACMG verdict	ExAC
SYNE1	6:152527392	p.(Gln7644*)	-	5.51	1	Pathogenic	-
	6:152497632	p.(Arg7842*)	rs775935265	5.75	1		0.000008237
ZFYVE26	14:68264904	p.(Leu692Serfs*52)	-	5.71	-	Pathogenic	-
	14:68257427	p.(Arg872Hisfs*17)	-	5.05	-	Likely pathogenic	-
DNAJB2	2:220144569	p.(Tyr5Cys)	rs730882140	2.34	1	Likely pathogenic	-
	2:220149491	p.(Glu253Lys)	-	4.48	0.99	Likely pathogenic	-
PLEKHG5	1:6530920	p.(Gln550*)	-	4.1	0.99	pathogenic	-
	1:6529648	p.(Pro707His)	-	5.67	0.97	Likely pathogenic	-
SIGMAR1	9:34635853	p.(Glu119Lys)	rs757260058	4.32	0.99	Likely pathogenic	-
	9:34635850	p.(Thr120Ala)	-	4.67	0.99	Likely pathogenic	-
VRK1	14:97326965	p.(Arg321Cys)	rs772731615	5.13	0.99	Likely pathogenic	0.0002071
	14:97342428	p.(Gln379Aspfs*23)	-	5.4	-	Pathogenic	-
DJ1	1:8025426	p.(Gln45*)	-	5.61	0.99	Pathogenic	-
IGHMBP2	11:68678998	p.(His213Arg)	rs137852666	4.7	0.99	Likely pathogenic	-
SLC52A3	20:744413	p.(Arg268Trp)	rs145498634	4.6	0.99	Likely pathogenic	0.00004945

Note: DANN score ranges is from 0 to 1, 1 predicted to be the most damaging, GERP++ score ranges from -12.3 to 6.17, 6.17 being the most conserved. Abbreviations: VUS, variant of unknown significance; WES, whole exome sequencing; WGS, whole genome sequencing.

**TABLE A5** ALS gene variants with unknown significance identified in WGS

Chromosome location	Gene	Transcript	DNA change	Protein change	Zygoty	rsID	No. of controls	No. of cases
chr9:35067907	VCP	NM_007126.5	c.283C>T	p.(Arg95Cys)	het	rs121909332	0	1
chr9:35068336	VCP	NM_007126.5	c.41C>T	p.(Thr14Ile)	het	-	1	0
chr2:212295795	ERBB4	NM_005235.3	c.2518G>A	p.(Val840Ile)	het	rs369248674	0	1
chr2:212989553	ERBB4	NM_005235.3	c.158A>G	p.(Tyr53Cys)	het	rs756650586	0	1
chr2:212589887	ERBB4	NM_005235.3	c.655G>A	p.(Gly219Ser)	het	rs757597004	0	1
chr2:212812268	ERBB4	NM_005235.3	c.308G>A	p.(Arg103His)	het	rs754487821	0	1
chr14:21162046	ANG	NM_001145.4	c.324dupT	p.(Gly109Trpfs*24)	het	-	0	1
chr9:135163699	SETX	NM_015046.7	c.6248G>T	p.(Arg2083Ile)	het	rs751252138	0	1
chr9:135172398	SETX	NM_015046.7	c.5825T>C	p.(Ile1942Thr)	het	rs773379832	1	0
chr4:170428877	NEK1	NM_012224.3	c.1816G>T	p.(Glu606*)	het	-	0	1
chr5:179251221	SQSTM1	NM_001142298.2	c.319G>A	p.(Gly107Arg)	het	rs781478225	0	1
chr17:4851653	PFN1	NM_005022.4	c.37G>A	p.(Ala13Thr)	hom	rs763837842	0	2
chr12:109278828	DAO	NM_001917.5	c.46G>A	p.(Ala16Thr)	het	rs778735604	0	1
chr12:109281243	DAO	NM_001917.5	c.212C>T	p.(Thr71Ile)	het	rs138277420	0	1
chr12:109286795	DAO	NM_001917.5	c.490G>T	p.(Val164Leu)	het	-	1	0
chr12:109294229	DAO	NM_001917.5	c.962G>A	p.(Gly321Glu)	het	-	0	1
chr2:74593112	DCTN1	NM_004082.4	c.2794C>T	p.(Arg932Cys)	het	rs373818927	1	1
chr2:74593597	DCTN1	NM_004082.4	c.2617G>A	p.(Ala873Thr)	het	rs764492372	1	0
chr2:74594488	DCTN1	NM_004082.4	c.2244C>G	p.(Asp748Glu)	het	rs751069902	0	1
chr2:74594495	DCTN1	NM_004082.4	c.2237T>C	p.(Leu746Pro)	het	-	0	1
chr2:74605312	DCTN1	NM_004082.4	c.94C>T	p.(Arg32Cys)	het	rs751177222	0	1
chr8:28017873	ELP3	NM_018091.6	c.1385G>A	p.(Arg462His)	hom	rs190129217	0	1
chr6:110036336	FIG4	NM_014845.5	c.122T>C	p.(Ile41Thr)	het	rs121908287	0	1
chr6:110056402	FIG4	NM_014845.5	c.547C>T	p.(Arg183*)	het	rs121908288	0	1
chr6:110086229	FIG4	NM_014845.5	c.1448G>A	p.(Arg483Gln)	het	rs749233172	0	1
chr6:110106223	FIG4	NM_014845.5	c.1940A>G	p.(Tyr647Cys)	het	rs150301327	1	0
chr19:7605129	PNPLA6	NM_006702.5	c.532G>T	p.(Gly178Cys)	het	-	0	1
chr19:7620584	PNPLA6	NM_006702.5	c.2914G>A	p.(Gly972Arg)	het	rs768107851	0	1
chr19:7626175	PNPLA6	NM_006702.5	c.365del	p.(Ala122Glnfs*3)	het	-	1	0
chr7:94935670	PON1	NM_000446.7	c.707A>G	p.(Tyr236Cys)	het	rs755475189	0	2
chr7:95024007	PON3	NM_000940.3	c.94C>T	p.(Arg32*)	het	rs147006695	0	5
chr12:49689173	PRPH	NM_006262.4	c.190C>T	p.(Arg64*)	het	-	0	2
chr15:78894232	CHRNA3	NM_000743.5	c.752C>G	p.(Pro251 Arg)	het	-	0	1
chr15:78894258	CHRNA3	NM_000743.5	c.725del	p.(Leu242Cysfs*32)	het	-	1	2
chr15:78894275	CHRNA3	NM_000743.5	c.708_709insG	p.(Ile237Aspfs*35)	het	-	1	2
chr15:78910978	CHRNA3	NM_000743.5	c.247_248insG	p.(Thr83Serfs*11)	het	-	1	0
chr15:78911138	CHRNA3	NM_000743.5	c.1A>G	p.?	het	rs745905590	0	3
chr20:61981766	CHRNA4	NM_000744.6	c.997C>T	p.(Arg333Cys)	het	rs761631713	1	0
chr20:61981784	CHRNA4	NM_000744.6	c.979G>A	p.(Val327Met)	het	rs201841018	0	1



**TABLE A5** (Continued)

Chromosome location	Gene	Transcript	DNA change	Protein change	Zygoty	rsID	No. of controls	No. of cases
chr20:61982107	CHRNA4	NM_000744.6	c.656A>C	p.(Asn219Thr)	het	rs201645533	0	1
chr20:61982321	CHRNA4	NM_000744.6	c.442C>T	p.(Arg148Trp)	het	rs121912243	0	1
chr15:78921343	CHRN4	NM_000750.5	c.1304C>T	p.(Ala435Val)	het	rs56317523	1	1
chr15:78922149	CHRN4	NM_000750.5	c.498C>G	p.(Asn166Lys)	het	rs148540431	1	1
chr15:78927869	CHRN4	NM_000750.5	c.116G>T	p.(Arg39Leu)	het	–	0	1

Abbreviations: ALS, amyotrophic lateral sclerosis; WGS, whole genome sequencing.

**TABLE A6** The number of variants from WGS filtered according to minor allele frequency in gnomAD

	Total	<0.1% in TR cohort	<0.5% in TR cohort	<1% in TR cohort	<5% in TR cohort	≥5% in TR cohort
No. of novel variants	47971649	23339705	10652389	2166448	3648946	8164161
No. of existing variants in gnomAD	135851	70808	35459	6164	8483	14937

Abbreviation: WGS, whole genome sequencing.

**TABLE A7** The top ranked variants from GWAS

Variant	Odds ratio	p value	dbSNP ID(s)
Odds ratio (case/control) > 1			
chr9:115956718:T:A	2.589	3E-06	rs10817455
chr18:19728805:T:A	2.414	6.1E-06	rs72879076
chr18:19728806:C:A	2.414	6.1E-06	rs62092220
chr5:4478547:A:C	2.150	6.3E-06	rs10066908
chr7:67216370:T:A	2.977	8.4E-06	rs6977845
chr17:20215352:T:G	1.984	8.6E-06	rs7223476
chr6:152561045:C:T	2.012	8.8E-06	rs1830820
chr7:67228631:A:G	2.068	9E-06	rs13307299
chr7:67231257:A:G	2.068	9E-06	rs9691826
chr6:152560478:C:A	2.010	9.3E-06	rs4869757
chr6:152559389:C:A	2.009	9.6E-06	rs9397089
chr6:152559605:A:G	2.009	9.6E-06	rs6928675
chr6:152559803:G:A	2.009	9.6E-06	rs6905741
chr7:67233851:T:C	2.027	1.2E-05	rs34912838
chr9:115974044:T:G	2.437	1.3E-05	rs4989078
chr17:39695278:C:T	2.100	1.3E-05	rs7212439
chr1:240672803:T:G	4.385	1.4E-05	rs61832588
chr7:67235252:T:G	2.068	1.5E-05	rs6946808
chr7:67235264:A:G	2.068	1.5E-05	rs6942794
chr17:39691789:G:A	2.087	1.5E-05	rs963478
chr17:19657847:C:G	2.194	1.5E-05	rs56191443

(Continues)

TABLE A7 (Continued)

Variant	Odds ratio	p value	dbSNP ID(s)
chr7:67236543:T:C	2.072	1.5E-05	rs35629450
chr7:67237915:A:C	2.059	1.6E-05	rs6964106
chr17:39677699:T:C	2.079	1.6E-05	rs56389952
chr17:39684410:G:A	2.079	1.6E-05	rs11550883
Odds ratio (case/control) < 1			
chr11:56460941:TTTG:T	0.403	2.1E-07	rs146003833, rs3071452, rs567274730
chr11:56466099:C:T	0.409	2.9E-07	rs1397048
chr11:56467085:T:C	0.409	2.9E-07	rs10896513
chr11:56465305:T:A	0.410	3E-07	rs1509995
chr11:56451517:A:T	0.411	3.3E-07	rs4340069
chr11:56441125:T:C	0.412	3.6E-07	rs7130569
chr11:56453126:G:T	0.412	3.6E-07	rs7109249
chr11:56453323:C:T	0.412	3.6E-07	rs1588387
chr11:56454288:G:A	0.412	3.6E-07	rs7113794
chr11:56457140:T:C	0.412	3.6E-07	rs55848395
chr5:32087228:C:T	0.472	5.1E-07	rs157494
chr5:32087802:A:G	0.473	5.1E-07	rs157497
chr5:32101168:A:T	0.475	5.1E-07	rs2279232
chr5:32094654:A:G	0.465	5.5E-07	rs245154
chr5:32103496:C:T	0.480	7E-07	rs4867419
chr11:121301819:C:A	0.429	7.2E-07	rs17125331
chr11:56455965:TAA:T	0.415	7.5E-07	rs3071436, rs71058026, rs762762596
chr11:121301299:TA:T	0.435	1.1E-06	rs34153159
chr11:121306462:G:A	0.439	1.1E-06	rs4146874
chr11:121302634:T:A	0.441	1.3E-06	rs55736743
chr11:121302646:A:G	0.441	1.3E-06	rs17125333
chr11:121303206:G:A	0.441	1.3E-06	rs17125336
chr11:121303952:CAAA:C	0.441	1.3E-06	rs10616182
chr11:121303574:T:G	0.446	1.7E-06	rs12417885
chr8:24329635:C:CA	0.281	1.9E-06	rs370799783

Abbreviation: GWAS, Genome-wide association studies.

**TABLE A8** The top ranked genes from SKAT-O analysis

Symbol	Gene ID	# of controls (# of variants)	# of cases (# of variants)	Odds ratio	p value
Odds ratio > 1					
<i>RALGAPA1</i>	ENSG00000174373	3 (3)	15 (15)	1.14	.0020094
<i>C16orf3</i>	ENSG00000221819	0 (0)	2 (6)	-	.0052194
<i>C14orf23</i>	ENSG00000186960	3 (3)	32 (32)	2.43	.0061734
<i>RET</i>	ENSG00000165731	2 (2)	13 (13)	2.48	.0083422
<i>DENND2C</i>	ENSG00000175984	1 (1)	7 (7)	1.6	.0096899
<i>RPL10L</i>	ENSG00000165496	0 (0)	2 (2)	-	.0101959
<i>C17orf96</i>	ENSG00000179294	1 (1)	6 (6)	1.37	.0120467
<i>ACSM5</i>	ENSG00000183549	0 (0)	3 (3)	-	.0139554
<i>BIRC6</i>	ENSG00000115760	1 (1)	7 (7)	1.6	.014459
<i>TRIM49</i>	ENSG00000168930	0 (0)	2 (4)	-	.015285
Odds ratio < 1					
<i>IKZF2</i>	ENSG00000030419	7 (7)	12 (12)	0.39	.0009985
<i>RTTN</i>	ENSG00000176225	6 (7)	6 (6)	0.23	.0010553
<i>CDK14</i>	ENSG00000058091	2 (2)	1 (1)	0.11	.001121
<i>ATP5S</i>	ENSG00000125375	2 (2)	1 (1)	0.11	.001198
<i>WDR86</i>	ENSG00000187260	2 (2)	3 (4)	0.34	.0012955
<i>GPRC5D</i>	ENSG00000111291	1 (1)	3 (3)	0.68	.0013205
<i>FRMD6</i>	ENSG00000139926	3 (3)	2 (2)	0.15	.0019035
<i>TPCN1</i>	ENSG00000186815	1 (1)	4 (4)	0.91	.0020293
<i>KCTD1</i>	ENSG00000134504	4 (4)	3 (4)	0.17	.0021836
<i>CCDC80</i>	ENSG00000091986	2 (2)	0 (0)	0	.0029323

**TABLE A9** Enriched gene clusters based on coexpression network analysis

Category/term	List Count	Fold enrichment	%	p value	Bonferroni	Benjamini	FDR	Genes	
Cell Cycle-Related Cluster-Enrichment Score: 15.03									
UP_KEYWORDS/cell cycle	28	92	9	30	7.66E-19	1.26E-16	9.29E-16	ENSG00000010292, ENSG000000161800, ENSG000000123485, ENSG000000184661, ENSG000000129195, ENSG000000237649, ENSG000000111665, ENSG000000144554, ENSG000000111602, ENSG000000171320, ENSG000000137804, ENSG000000152253, ENSG000000129810, ENSG000000184445, ENSG000000183765, ENSG000000111206, ENSG000000099956, ENSG000000138182, ENSG000000080986, ENSG000000126787, ENSG000000142945, ENSG000000117399, ENSG000000186871, ENSG000000115760, ENSG000000128944, ENSG000000165304, ENSG000000175063, ENSG000000087586	
UP_KEYWORDS/mitosis	20	92	17	21	2.79E-18	4.57E-16	2.28E-16	3.38E-15	ENSG000000010292, ENSG000000184661, ENSG000000138182, ENSG000000129195, ENSG000000237649, ENSG000000080986, ENSG000000111665, ENSG000000142945, ENSG000000117399, ENSG000000186871, ENSG000000115760, ENSG000000111602, ENSG000000128944, ENSG000000137804, ENSG000000152253, ENSG000000129810, ENSG000000184445, ENSG000000175063, ENSG000000087586, ENSG000000183765
UP_KEYWORDS/cell division	21	92	11	22	5.53E-16	9.10E-14	3.03E-14	6.77E-13	ENSG000000010292, ENSG000000161800, ENSG000000184661, ENSG000000138182, ENSG000000129195, ENSG000000237649, ENSG000000080986, ENSG000000111665, ENSG000000142945, ENSG000000117399, ENSG000000186871, ENSG000000115760, ENSG000000111602, ENSG000000128944, ENSG000000137804, ENSG000000129810, ENSG000000152253, ENSG000000129810, ENSG000000184445, ENSG000000175063, ENSG000000087586, ENSG000000183765
GOTERM_BP_DIRECT/GO:0051301~cell division	20	88	11	21	4.59E-15	2.36E-12	2.36E-12	6.59E-12	ENSG000000010292, ENSG000000118193, ENSG000000184661, ENSG000000138182, ENSG000000129195, ENSG000000237649, ENSG000000080986, ENSG000000111665, ENSG000000142945,

TABLE A 9 (Continued)

Category/term	List Count	Fold enrichment %	Bonferroni	Benjamini	FDR	Genes	
GOTERM_BP_DIRECT/GO:0007067~mitotic nuclear division	14	15	1.41E-10	7.32E-08	3.66E-08	2.04E-07	ENSG00000117399, ENSG00000186871, ENSG00000115760, ENSG00000111602, ENSG00000128944, ENSG00000152253, ENSG00000129810, ENSG00000184445, ENSG00000175063, ENSG00000087586, ENSG00000183765
GOTERM_BP_DIRECT/GO:0007777~condensed chromosome kinetochore	10	11	1.41E-10	7.32E-08	3.66E-08	2.04E-07	ENSG00000080986, ENSG0000011665, ENSG00000142945, ENSG00000117399, ENSG00000186871, ENSG00000115760, ENSG00000111602, ENSG00000152253, ENSG00000129810, ENSG00000184445, ENSG00000087586
Chromosome Structure Related Cluster-Enrichment Score: 6.8							
GOTERM_CC_DIRECT/GO:0000777~condensed chromosome kinetochore	10	90	4.44E-10	7.41E-08	2.47E-08	5.40E-07	ENSG00000120071, ENSG00000123485, ENSG00000128944, ENSG00000111581, ENSG00000080986, ENSG00000152253, ENSG00000129810, ENSG00000184445, ENSG00000186871, ENSG00000142945
UP_KEYWORDS/chromosome	15	92	8.64E-10	1.42E-07	2.36E-08	1.05E-06	ENSG0000010292, ENSG00000120071, ENSG00000123485, ENSG00000123485, ENSG0000090889, ENSG00000080986, ENSG00000142945, ENSG00000186871, ENSG00000143476, ENSG00000128944, ENSG00000111581, ENSG00000171320, ENSG00000137804, ENSG00000152253, ENSG00000129810, ENSG00000184445
UP_KEYWORDS/centromere	10	92	4.17E-09	6.84E-07	9.77E-08	5.06E-06	ENSG00000120071, ENSG00000123485, ENSG00000128944, ENSG00000111581, ENSG00000080986, ENSG00000152253, ENSG00000129810, ENSG00000184445, ENSG00000186871, ENSG00000142945
UP_KEYWORDS/kinetochore	9	92	1.49E-08	2.45E-06	3.06E-07	1.81E-05	ENSG00000120071, ENSG00000128944, ENSG00000111581, ENSG00000080986, ENSG00000152253, ENSG00000129810, ENSG00000184445, ENSG00000142945
GOTERM_BP_DIRECT/GO:0007059~chromosome segregation	8	88	2.87E-07	1.49E-04	4.97E-05	4.16E-04	ENSG00000131747, ENSG00000123485, ENSG00000184661, ENSG00000128944, ENSG00000080986,

(Continues)

TABLE A9 (Continued)

Category/term	Count	List total	Fold enrichment	% p value	Bonferroni	Benjamini	FDR	Genes	
GOTERM_BP_DIRECT/GO:0007062~sister chromatid cohesion	8	88	16	9	5.79E-07	3.01E-04	7.52E-05	8.39E-04	ENSG00000171320, ENSG00000152253, ENSG00000129810
GOTERM_CC_DIRECT/GO:0000776~kinetochore	6	90	13	6	8.29E-05	0.013743	0.002764	0.100739	ENSG00000128944, ENSG00000111581, ENSG000000080986, ENSG00000112742, ENSG00000129810, ENSG00000142945
GOTERM_CC_DIRECT/GO:0000775~chromosome, centromeric region	4	90	18	4	0.001218	0.184197	0.020152	1.471814	ENSG00000123485, ENSG000000080986, ENSG00000129810, ENSG00000142945

**TABLE A10** The top ranked pathways in SKAT-O analysis

Pathway	# of variant (control)	# of variant (case)	Odds ratio	p value (SKAT-O)
Odds ratio > 1				
BIOCARTA_FEEDER_PATHWAY	15	54	0.82	.0008682
REACTOME_FGFR4_LIGAND_BINDING_AND_ACTIVATION	16	58	0.83	.0014919
KEGG_GALACTOSE_METABOLISM	60	200	0.76	.0035946
BIOCARTA_CARDIACEGF_PATHWAY	7	23	0.75	.0040825
KEGG_ETHER_LIPID_METABOLISM	12	34	0.65	.0052787
REACTOME_FGFR2C_LIGAND_BINDING_AND_ACTIVATION	4	6	0.34	.0063526
REACTOME_PI_METABOLISM	41	121	0.67	.0086292
REACTOME_POL_SWITCHING	2	1	0.11	.0087933
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_LACTO_AND_NEOLACTO_SERIES	19	54	0.65	.0105438
REACTOME_LAGGING_STRAND_SYNTHESIS	4	11	0.63	.0107531
Odds ratio < 1				
REACTOME_GLYCOGEN_BREAKDOWN_GLYCOGENOLYSIS	19	125	1.50	.0073818
KEGG_INOSITOL_PHOSPHATE_METABOLISM	47	235	1.14	.0160813
PID_RET_PATHWAY	7	62	2.02	.0166805
KEGG_DNA_REPLICATION	5	30	1.37	.0222791
KEGG_ARGININE_AND_PROLINE_METABOLISM	25	130	1.19	.0234903
REACTOME_GLUCOSE_METABOLISM	65	324	1.14	.0322741
SA_B_CELL_RECEPTOR_COMPLEXES	4	22	1.25	.0327217
REACTOME_DNA_REPAIR	36	179	1.13	.0396380
PID_NETRIN_PATHWAY	15	74	1.12	.0404352
PID_BCR_5PATHWAY	6	43	1.63	.0405759