**eMaterials**

**Patients and controls characteristic**

Patients were included in PARALS if they met definite, probable, or probable laboratory-supported ALS diagnosis according to the revised El Escorial criteria[1]. Control samples were composed of 350 females and 405 males. The 755 controls were chosen randomly from the same population and matched to the cases by sex, age (±5 years), and geographic location. Control subjects were excluded from the study if they had a first-degree relative with a neurological or psychiatric disorder.

**Standard protocol approvals and patient consents**

All subjects participating in the study gave written informed consent. Ethical approval was obtained from the medical ethical review board of the A.O.U Citta della Salute e della Scienza di Torino, Italy.

**Whole-genome sequencing**

Whole-genome sequencing was performed at The American Genome Center at the Uniformed Services University on the Walter Reed National Military Medical Center campus in Bethesda, MD, USA. Briefly, libraries were prepared using TruSeq DNA PCR-Free High Throughput Library Prep Kit (Illumina Inc.) as per the manufacturer's instructions. Sequencing was performed on an Illumina HiSeq X10 sequencer using paired-end 150 base pair reads, and the data were processed according to Genome Analysis Toolkit's (GATK) best practices (https://software.broadinstitute.org/gatk/best-practices/). Variant quality control was performed using the GATK variant quality score method with default filters using Genome Reference Consortium Human Build 38 as the reference.

**ALS-related genes**

We extracted variant information from the data for the following genes: *ALS2, ANXA11, ATXN2, C21orf2, C9orf72, CCNF, CHCHD10, CHMP2B, DAO, DCTN1, DNAJC7, ERBB4, EWSR1, FIG4, FUS, GLE1, GRN, HRNPA1, HRNPA2B1, HNRNPD, KIF5A, MAPT, MATR3, NEFH, NEK1, OPTN, PFN1, PRNP, PRPH, SETX,*

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*J Neurol Neurosurg Psychiatry*

*SIGMAR1, SOD1, SPG11, SPTLC1, SQSTM1, SS18L1, TAF15, TARDBP, TBK1, TUBA4A, UBQLN2, VAPB,* and *VCP*. Prognostic genes (*UNC13A, CAMTA1*) were also extracted.

**Variant annotation**

Annotation was then performed using ANNOVAR v2020-06-07 (https://annovar.openbioinformatics.org) and KGGseq v1.0 (http://pmglab.top/kggseq/) using the gnomAD database (version 2.1.1) to determine minor allele frequency (MAF) in the European-derived population. The current study was focused on coding variants due to the limitations in interpreting non-coding variants.

**Expansion screening**

The samples were screened for *C9orf72* expansions and the microsatellite repeat in *ATXN2* using repeat-primed PCR methodology as previously described.[2,3] A cut-off of 30 repeated expansion and the characteristic sawtooth pattern was considered pathogenic for *C9orf72*[2]. *ATXN2* CAG expansions were deemed intermediate if they were within the range of 30-33 repeats[3]. ExpansionHunter - Targeted software (version 0.3) was used to estimate repeat lengths of known, disease-causing expansions in samples undergoing whole-genome sequencing. This algorithm has been validated using experimentally confirmed samples carrying expansions[4]. The performance of whole-genome sequencing in terms of sensitivity for repeated expansion has been validated in the same ALS cohort [5,6].

**Variant interpretation**

We set a conservative MAF frequency threshold of <0.01% based on the epidemiology of ALS We defined Loss of Function (LoF) variants when the sequence changes were predicted to be a premature stop codon, a frameshift causing insertion/deletion (indel), or a splice-site disrupting variant located in the canonical splice sites (+1 and +2, –1 and –2) that cause the premature termination codon falling < 50–55 nucleotides upstream of the 3′ most exon–exon junctions. Loss of function variants was considered deleterious unless they exceeded the 0.01% MAF threshold. For all other type of variants, the combination of different sets of algorithms was

considered using recommended treshold [MutationTaster, Mendelian Clinically Applicable Pathogenicity (M-CAP), CADD, Variant Effect Scoring Tool (VEST3), Rare Exome Variant Ensemble Learner (REVEL), Meta-analytic Support Vector Machine (Meta-SVM)][7]. hose variants underwent an independent review by clinical and genetic ALS experts and were confirmed to be clinically reportable if agreed by consensus. When available, gene-specific information data was used in the classification

**Statistical Analysis**

A 2-tailed Fisher exact test was used to evaluate the genetic association between *ATXN2* CAG repeat sizes and ALS (significance set at $P < 0.05$). The burden of multiple variant carriers was assessed by a binomial test[8]. Age at disease onset and disease progression were also assessed across oligogenic and monogenic with analysis of variance (ANOVA). The analyses were performed in R (version 3.6.0).

**Data availability statement**

The individual-level sequence data are available on dbGaP (accession number: phs001963.v1.p1) upon motivated request by interested researchers.

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**eFigure 1. Study Flowchart.** Simplified flowchart describing the steps taken to filter and prioritize variants in ALS genes. MAF, minor allele frequency; NFE, non-Finnish Europeans; ACMG, American College of Medical Genetic.
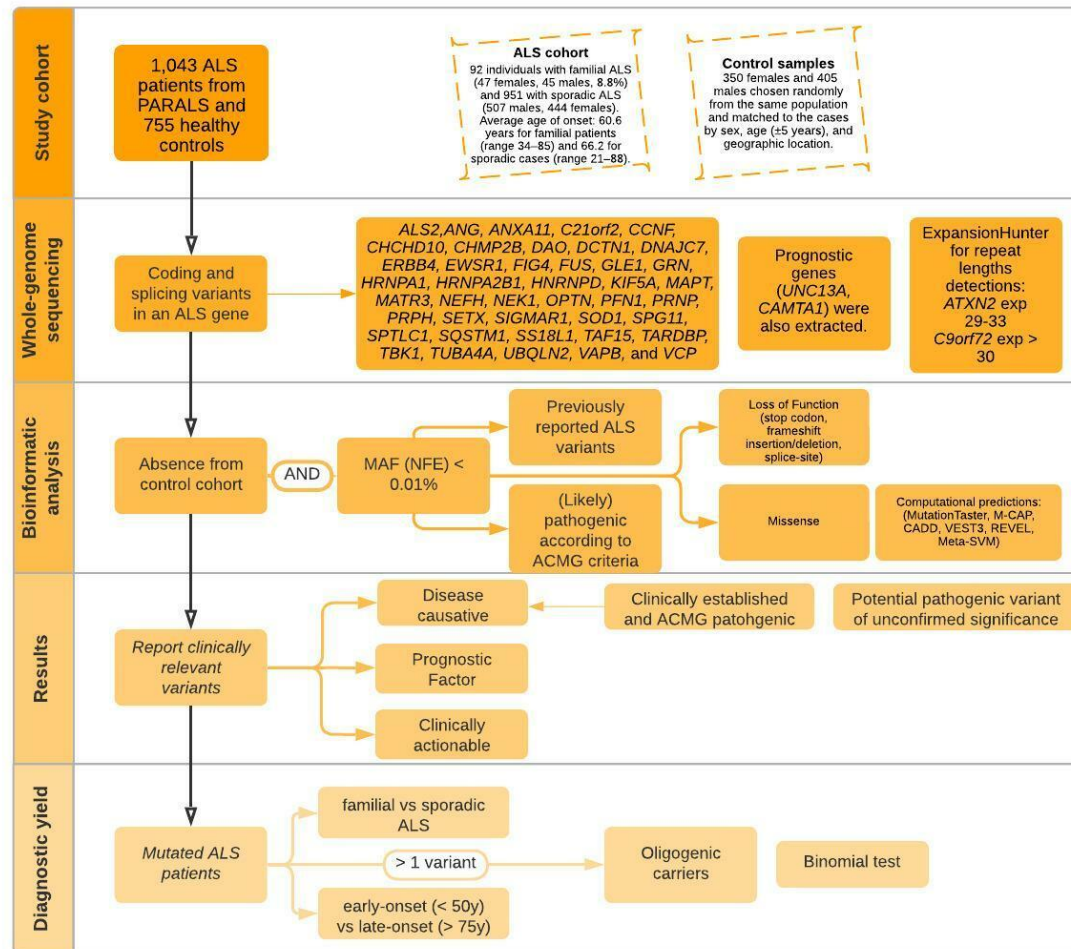
**eFigure 2. Summary of identified variants.**

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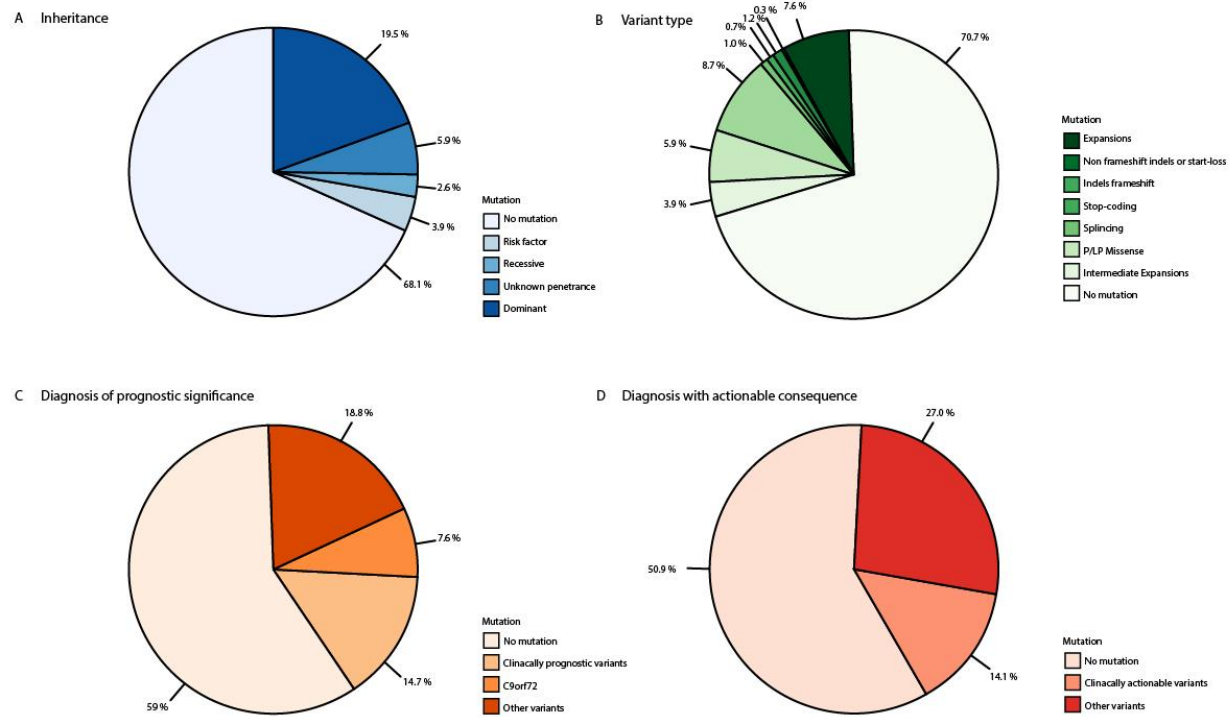
**eTables**

**eTable 1** – Variants detected in our cohort previously reported in ALS cases.

| Gene | Transcript | Nucleotide change | Amino acid change | Exon | Function |
|------|-----------|-------------------|-------------------|------|----------|
| *ALS2* | NM_020919 | c.4867C>T | p.L1623F | exon33 | missense |
| *ALS2* | NM_020919 | c.3836+1G>A | . | exon24 | splicing GT donor |
| *ALS2* | NM_020919 | c.3797C>G | p.P1266R | exon24 | missense |
| *ALS2* | NM_020919 | c.3462G>T | p.Q1154H | exon21 | missense |
| *ALS2* | NM_020919 | c.3394C>A | p.R1132S | exon21 | missense |
| *ALS2* | NM_020919 | c.3206G>A | p.G1069E | exon19 | missense |
| *ALS2* | NM_020919 | c.2979+1G>A | . | exon17 | splicing GT donor |
| *ALS2* | NM_020919 | c.2479A>T | p.T827S | exon13 | missense |
| *ALS2* | NM_020919 | c.1804C>T | p.R602C | exon8 | missense |
| *ALS2* | NM_020919 | c.1649C>T | p.P550L | exon7 | missense |
| *ALS2* | NM_020919 | c.1628A>G | p.D543G | exon6 | missense |
| *ALS2* | NM_020919 | c.1550C>G | p.A517G | exon6 | missense |
| *ALS2* | NM_020919 | c.1472-1G>C | . | exon6 | splicing AG acceptor |
| *ALS2* | NM_020919 | c.37G>A | p.G13R | exon3 | missense |

| *ANXA11* | NM_001157 | c.922C>T | p.R308X | exon8 | stopgain |
|---|---|---|---|---|---|
| *ANXA11* | NM_001157 | c.102G>A | p.M34I | exon3 | startloss |
| *ANXA11* | NM_001157 | c.744+2C>T | . | exon7 | splicing |
| *C21ORF2* | NM_001271441 | c.418_420del | p.G140del | exon5 | non-frameshift deletion |
| *C21ORF2* | NM_001271441.2 | c.735_736insCGT GGGGAGGGAG CATGGAGCCT CACAGGGCCC | p.P245_V246insR GQGAWSLTGP | exon6 | non-frameshift insertion |
| *C21ORF2* | NM_001271441.2 | c.701_702insGCC TCACAGGGCC CCGTGGGGAG GGAGCATGGA GCCTCACAGG GCC | p.V235Pfs*38 | exon6 | frameshift insertion |
| *CCNF* | NM_001761 | c.139C>T | p.L47F | exon2 | missense |
| *CCNF* | NM_001761 | c.316C>G | p.L106V | exon4 | missense |
| *CCNF* | NM_001761 | c.1043C>T | p.P348L | exon9 | missense |
| *CCNF* | NM_001761 | c.1132G>A | p.V378I | exon11 | missense |
| *CHCHD10* | NM_213720 | c.239C>T | p.P80L | exon2 | missense |
| *CHMP2B* | NM_014043 | c.74A>G | p.Q25R | exon2 | missense |
| *CHMP2B* | NM_014043 | c.85A>G | p.I29V | exon2 | missense |
| *DAO* | NM_001917 | c.34G>A | p.G12R | exon2 | missense |

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| | | | | | |
|---|---|---|---|---|---|
| *DAO* | NM_001917 | c.992G>A | p.G331E | exon11 | missense |
| *DCTN1* | NM_001190836 | c.3575C>T | p.A1192V | exon28 | missense |
| *DCTN1* | NM_001190836 | c.3574G>A | p.A1192T | exon28 | missense |
| *DCTN1* | NM_001190836 | c.2588T>C | p.M863T | exon20 | missense |
| *DCTN1* | NM_001190836 | c.2522A>G | p.Y841C | exon20 | missense |
| *DCTN1* | NM_001190836 | c.2321C>G | p.P774R | exon18 | missense |
| *DCTN1* | NM_001190836 | c.2147C>T | p.T716M | exon17 | missense |
| *DCTN1* | NM_001190836 | c.1484G>C | p.R495P | exon12 | missense |
| *DCTN1* | NM_001190836 | c.964C>T | p.Q322* | exon8 | stopgain |
| *DCTN1* | NM_001190836 | c.884T>C | p.V295A | exon7 | missense |
| *DNAJC7* | NM_003315 | c.611G>A | p.R204Q | exon7 | missense |
| *DNAJC7* | NM_003315 | c.203G>A | p.R68Q | exon3 | missense |
| *EPHA4* | NM_001304537 | c.2060C>G | p.S687C | exon12 | missense |
| *EPHA4* | NM_001304537 | c.1702T>C | p.S568P | exon9 | missense |
| *EPHA4* | NM_001304537 | c.1058T>C | p.L353P | exon4 | missense |
| *ERBB4* | NM_005235 | c.3446G>T | p.G1149V | exon27 | missense |
| *ERBB4* | NM_005235 | c.2525G>A | p.R842Q | exon21 | missense |
| *ERBB4* | NM_005235 | c.1913G>A | p.W638* | exon16 | stopgain |
| *ERBB4* | NM_005235 | c.1912T>C | p.W638R | exon16 | missense |
| *ERBB4* | NM_005235 | c.1898G>C | p.C633S | exon16 | missense |
| *ERBB4* | NM_005235 | c.1772A>G | p.E591G | exon15 | missense |

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| Gene | Transcript | cDNA | Protein | Exon | Type |
|------|-----------|------|---------|------|------|
| *ERBB4* | NM_005235 | c.586C>T | p.R196C | exon5 | missense |
| *ERBB4* | NM_005235 | c.532G>C | p.V178L | exon4 | missense |
| *ERBB4* | NM_005235 | c.308G>A | p.R103H | exon3 | missense |
| *EWSR1* | NM_001163285 | c.1447A>G | p.M483V | exon14 | missense |
| *EWSR1* | NM_001163285 | c.1775_1776insTTTTT | p.Q593Ffs*20 | exon17 | frameshift |
| *FIG4* | NM_014845 | c.30C>G | p.S10R | exon1 | missense |
| *FIG4* | NM_014845 | c.52T>C | p.Y18H | exon1 | missense |
| *FIG4* | NM_014845 | c.2200G>A | p.E734K | exon20 | missense |
| *FIG4* | NM_014845 | c.2467C>T | p.Q823* | exon22 | stopgain |
| *FIG4* | NM_014845 | c.2639T>A | p.I880N | exon23 | missense |
| *FIG4* | NM_014845 | c.2650C>T | p.Q884* | exon23 | stopgain |
| *FUS* | NM_001170634 | c.785A>G | p.N262S | exon7 | missense |
| *FUS* | NM_001170634 | c.1480C>T | p.R494* | exon14 | stopgain |
| *FUS* | NM_001170634 | c.1539G>C | p.R513S | exon15 | missense |
| *FUS* | NM_001170634 | c.1552C>G | p.Q518E | exon15 | missense |
| *GLE1* | NM_001003722 | c.1771C>T | p.Q591* | exon12 | stopgain |
| *GRN* | NM_002087 | c.763C>A | p.L255M | exon8 | missense |
| *GRN* | NM_002087 | c.1562G>A | p.C521Y | exon12 | missense |
| *GRN* | NM_002087 | c.1595C>A | p.T532N | exon12 | missense |
| *HNRNPA1* | NM_031157 | c.380A>G | p.Q127R | exon4 | missense |

| Gene | Transcript | cDNA | Protein | Exon | Type |
|---|---|---|---|---|---|
| *HNRNPA1* | NM_031157 | c.666C>G | p.F222L | exon6 | missense |
| *HNRNPA1* | NM_031157 | c.824G>T | p.G275V | exon8 | missense |
| *HNRNPA1* | NM_031157 | c.876C>G | p.N292K | exon8 | missense |
| *HNRNPA1* | NM_031157 | c.883G>A | p.G295R | exon8 | missense |
| *KIF5A* | NM_004984 | c.340C>T | p.R114* | exon4 | stopgain |
| *KIF5A* | NM_004984 | c.1463T>G | p.L488R | exon14 | missense |
| *KIF5A* | NM_004984 | c.2152C>T | p.R718W | exon19 | missense |
| *KIF5A* | NM_004984 | c.2263G>A | p.E755K | exon20 | missense |
| *KIF5A* | NM_004984 | c.2757del | p.K920Nfs*128 | exon25 | frameshift |
| *MAPT* | NM_016834 | c.149T>C | p.I50T | exon3 | missense |
| *MAPT* | NM_016834 | c.736G>A | p.G246S | exon7 | missense |
| *MAPT* | NM_016834 | c.880T>C | p.S294P | exon9 | missense |
| *MAPT* | NM_016834 | c.913G>A | p.V305I | exon9 | missense |
| *MATR3* | NM_018834 | c.368A>G | p.D123G | exon2 | missense |
| *MATR3* | NM_018834 | c.2148+2T>+GGAC | . | exon14 GT donor | splicing |
| *MATR3* | NM_018834 | c.2371+2T>+ATA | . | exon14 GT donor | splicing |
| *NEFH* | NM_021076 | c.1036C>T | p.R346C | exon2 | missense |
| *NEFH* | NM_021076 | c.1723C>T | p.P575S | exon4 | missense |
| *NEFH* | NM_021076 | c.1783C>T | p.P595S | exon4 | missense |
| *NEFH* | NM_021076 | c.2461A>G | p.K821E | exon4 | missense |

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|------|-----------|------|---------|------|------|
| *NEK1* | NM_001199399 | c.3502C>T | p.H1168Y | exon32 | missense |
| *NEK1* | NM_001199399 | c.3451A>C | p.I1151L | exon32 | missense |
| *NEK1* | NM_001199399 | c.3419_3422del | p.I1140Rfs*17 | exon31 | frameshift |
| *NEK1* | NM_001199399 | c.3373G>A | p.E1125K | exon31 | missense |
| *NEK1* | NM_001199399 | c.3214delA | p.T1072Lfs*20 | exon30 | frameshift |
| *NEK1* | NM_001199399 | c.2816C>G | p.S939* | exon28 | stopgain |
| *NEK1* | NM_001199399 | c.2698G>T | p.D900Y | exon28 | missense |
| *NEK1* | NM_001199399 | c.2523_2526del | p.N841Kfs*53 | exon26 | frameshift |
| *NEK1* | NM_001199399 | c.1226G>A | p.W409* | exon15 | stopgain |
| *NEK1* | NM_001199399 | c.1129_1132del | p.Q377Rfs*7 | exon13 | frameshift |
| *NEK1* | NM_001199399 | c.781C>T | p.R261C | exon10 | missense |
| *NEK1* | NM_001199399 | c.577C>T | p.L193F | exon9 | missense |
| *NEK1* | NM_001199399 | c.464+1G>AG+ | . | exon6 GT donor | splicing |
| *NEK1* | NM_001199399 | c.449C>G | p.A150G | exon7 | missense |
| *NEK1* | NM_001199399 | c.380G>A | p.R127Q | exon6 | missense |
| *OPTN* | NM_001008212 | c.247C>T | p.R83C | exon4 | missense |
| *OPTN* | NM_001008212 | c.265C>T | p.Q89* | exon4 | stopgain |
| *OPTN* | NM_001008212 | c.332T>G | p.L111R | exon4 | missense |
| *OPTN* | NM_001008212 | c.403G>T | p.E135* | exon5 | stopgain |
| *OPTN* | NM_001008212 | c.644G>A | p.R215K | exon7 | missense |

| Gene | Transcript | cDNA | Protein | Exon | Type |
|------|-----------|------|---------|------|------|
| *OPTN* | NM_001008212 | c.917_921del | p.T307Sfs*3 | exon9 | frameshift |
| *OPTN* | NM_001008212 | c.1499T>C | p.L500P | exon13 | missense |
| *OPTN* | NM_001008212 | c.1634G>A | p.R545Q | exon15 | missense |
| *OPTN* | NM_001008212 | c.1639C>T | p.Q547* | exon15 | stopgain |
| *OPTN* | NM_001008212 | c.1719G>A | p.M573I | exon15 | missense |
| *PFN1* | NM_005022 | c.67G>A | p.V23M | exon1 | missense |
| *PRPH* | NM_006262 | c.487G>T | p.D163Y | exon1 | missense |
| *PRPH* | NM_006262 | c.611T>A | p.V204E | exon3 | missense |
| *PRPH* | NM_006262 | c.996+1G>A | . | exon6 GT | splicing donor |
| *PRPH* | NM_006262 | c.1024G>A | p.E342K | exon6 | missense |
| *PRPH* | NM_006262.4 | c.1222_1223insGCAG | p.S408Cfs*19 | exon7 | frameshift |
| *PRPH* | NM_006262 | c.1409A>C | p.Y470S | exon9 | missense |
| *SETX* | NM_015046 | c.7240C>T | p.R2414* | exon25 | stopgain |
| *SETX* | NM_015046 | c.6848_6851del | p.T2283Kfs*32 | exon22 | frameshift |
| *SETX* | NM_015046 | c.6685A>G | p.M2229V | exon21 | missense |
| *SETX* | NM_015046 | c.1427A>G | p.H476R | exon10 | missense |
| *SETX* | NM_015046 | c.1427A>C | p.H476P | exon10 | missense |
| *SETX* | NM_015046 | c.1178T>C | p.L393P | exon10 | missense |
| *SETX* | NM_015046 | c.638C>T | p.S213F | exon6 | missense |
| *SETX* | NM_015046 | c.62A>T | p.Y21F | exon3 | missense |

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|---|---|---|---|---|---|
| *SETX* | NM_015046 | c.4A>G | p.S2G | exon3 | missense |
| *SOD1* | NM_000454 | c.16G>A | p.V6M | exon1 | missense |
| *SOD1* | NM_000454 | c.59A>G | p.N20S | exon1 | missense |
| *SOD1* | NM_000454 | c.115C>G | p.L39V | exon2 | missense |
| *SOD1* | NM_000454 | c.197A>G | p.N66S | exon3 | missense |
| *SOD1* | NM_000454 | c.217G>A | p.G73S | exon3 | missense |
| *SOD1* | NM_000454 | c.271G>A | p.D91N | exon4 | missense |
| *SOD1* | NM_000454 | c.281G>A | p.G94D | exon4 | missense |
| *SOD1* | NM_000454 | c.357+1G>+T | . | exon5 GT | splicing donor |
| *SOD1* | NM_000454 | c.409A>T | p.K137* | exon5 | stopgain |
| *SOD1* | NM_000454 | c.435G>C | p.L145F | exon5 | missense |
| *SOD1* | NM_000454 | c.435G>T | p.L145F | exon5 | missense |
| *SOD1* | NM_000454 | c.442G>A | p.G148S | exon5 | missense |
| *SPAST* | NM_001363875 | c.455T>C | p.I152T | exon2 | missense |
| *SPAST* | NM_001363875 | c.806C>T | p.S269F | exon5 | missense |
| *SPAST* | NM_001363875 | c.1526A>G | p.D509G | exon14 | missense |
| *SPG11* | NM_025137 | c.6877C>T | p.R2293W | exon38 | missense |
| *SPG11* | NM_025137 | c.6857G>C | p.R2286P | exon38 | missense |
| *SPG11* | NM_025137 | c.6094T>C | p.C2032R | exon32 | missense |
| *SPG11* | NM_025137 | c.6010T>G | p.L2004V | exon32 | missense |

| Gene | Transcript | cDNA | Protein | Exon | Type |
|------|-----------|------|---------|------|------|
| *SPG11* | NM_025137 | c.5414G>A | p.R1805H | exon30 | missense |
| *SPG11* | NM_025137 | c.3095C>T | p.P1032L | exon17 | missense |
| *SPG11* | NM_025137 | c.148C>T | p.Q50* | exon1 | stopgain |
| *SQSTM1* | NM_003900 | c.447C>G | p.D149E | exon3 | missense |
| *SQSTM1* | NM_003900 | c.775G>A | p.V259M | exon6 | missense |
| *SQSTM1* | NM_003900 | c.775G>C | p.V259L | exon6 | missense |
| *SQSTM1* | NM_003900 | c.1043C>T | p.P348L | exon7 | missense |
| *SQSTM1* | NM_003900 | c.1084G>A | p.E362K | exon7 | missense |
| *SQSTM1* | NM_003900 | c.1142C>T | p.A381V | exon7 | missense |
| *SS18L1* | NM_001301778 | c.253A>C | p.M85L | exon7 | missense |
| *TAF15* | NM_139215 | c.116G>A | p.G39E | exon4 | missense |
| *TAF15* | NM_139215 | c.329A>G | p.Y110C | exon6 | missense |
| *TAF15* | NM_139215 | c.1088+1G>A | . | exon14 | splicing GT donor |
| *TARDBP* | NM_007375 | c.800A>G | p.N267S | exon6 | missense |
| *TARDBP* | NM_007375 | c.1144G>A | p.A382T | exon6 | missense |
| *TARDBP* | NM_007375 | c.1169A>G | p.N390S | exon6 | missense |
| *TARDBP* | NM_007375 | c.1178C>T | p.S393L | exon6 | missense |
| *TBK1* | NM_013254 | c.254T>C | p.I85T | exon4 | missense |
| *TBK1* | NM_013254 | c.454G>C | p.V152L | exon5 | missense |
| *TBK1* | NM_013254 | c.521A>G | p.Y174C | exon5 | missense |

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| Gene | Transcript | cDNA | Protein | Exon | Effect |
|------|-----------|------|---------|------|--------|
| *TBK1* | NM_013254 | c.992+1G>A | . | exon9 | splicing GT donor |
| *TBK1* | NM_013254 | c.1343_1346del | p.I450Kfs*15 | exon12 | frameshift |
| *TIA1* | NM_022173 | c.1154C>T | p.T385I | exon13 | missense |
| *TIA1* | NM_022173 | c.746A>C | p.K249T | exon10 | missense |
| *TIA1* | NM_022173 | c.95C>A | p.P32H | exon2 | missense |
| *TIA1* | NM_022173 | c.94C>A | p.P32T | exon2 | missense |
| *TUBA4A* | NM_001278552 | c.1312G>A | p.D438N | exon4 | missense |
| *TUBA4A* | NM_001278552 | c.1184_1190del | p.G395Afs*67 | exon4 | frameshift deletion |
| *TUBA4A* | NM_001278552 | c.1045A>T | p.T349S | exon4 | missense |
| *TUBA4A* | NM_001278552 | c.148delG | p.A50Qfs*89 | exon2 | frameshift deletion |
| *UBQLN2* | NM_013444 | c.401C>T | p.T134I | exon1 | missense |
| *UBQLN2* | NM_013444 | c.1172A>G | p.Y391C | exon1 | missense |
| *UBQLN2* | NM_013444 | c.1505G>A | p.G502D | exon1 | missense |
| *VAPB* | NM_004738 | c.332C>T | p.P111L | exon4 | missense |
| *VCP* | NM_001354927 | c.2086C>T | p.R696C | exon16 | missense |
| *VCP* | NM_001354927 | c.2002A>G | p.R668G | exon15 | missense |
| *VCP* | NM_001354927 | c.265T>C | p.Y89H | exon4 | missense |

**eTable 2A. Oligogenic combinations in our cohort**

| Mutation 1 | Mutation 2 |
|---|---|
| *C9orf72* GGGGCC exp | *CHCHD10*:c.239C>T:p.P80L |
| *C9orf72* GGGGCC exp | *KIF5A*:c.T1463G:p.L488R |
| *C9orf72* GGGGCC exp | *C21orf2*:c.700_701ins:p.V246 Wfs*35 |
| C9orf72 GGGGCC exp | *NEK1*:c.G3373A:p.E1125K |
| *C9orf72* GGGGCC exp | *OPTN*:c.G644A:p.R215K |
| *SOD1*:c.59A>G:p.N20S | *FUS*:c.1542G>C:p.R514S |
| *SOD1*:c.115C>G:p.L39V | *MATR3*:c.2148+2->GGAC |
| *SOD1*:c.271G>A:p.D91N | *CHCHD10*:c.239C>T:p.P80L |
| *SOD1*:c.435G>C:p.L145F | *OPTN*:c.403G>T:p.E135* |
| *SOD1*:c.435G>C:p.L145F | *ERBB4*:c.308G>A:p.R103H |
| *TARDBP*:c.1144G>A:p.A382T | *UBQLN2*:c.1612G>C:p.V538L |
| *DCTN1*:c.C673T:p.Q225* | *MATR3*:c.2148+2->GGAC |
| *ANXA11*:c.C922T:p.R308X | *ERBB4*:c.A1772G:p.E591G |

**eTable 2B. Analysis of oligogenic frequency and effect of multiple variants on age at disease onset in our cohort**

| | Oligogenic cases | Monogenic cases | Binomial p-value | ANOVA p-value |
|---|---|---|---|---|
| **Cases (% cohort)** | 13 (1.3%) | 266 (25.6%) | 0.98 | - |
| **Age at onset (SD)** | 59.9 (11.9) | 63.9 (11.2) | - | 0.1310 |

## Supplementary References

1.      Brooks BR, Miller RG, Swash M, Munsat TL. El Escorial revisited: Revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders*. 2000;1(5). doi:10.1080/146608200300079536

2.      Renton AE, Majounie E, Waite A, et al. A Hexanucleotide Repeat Expansion in C9ORF72 Is the Cause of Chromosome 9p21-Linked ALS-FTD. *Neuron*. 2011;72(2). doi:10.1016/j.neuron.2011.09.010

3.      Van Damme P, Veldink JH, van Blitterswijk M, et al. Expanded ATXN2 CAG repeat size in ALS identifies genetic overlap between ALS and SCA2. *Neurology*. 2011;76(24):2066-2072. doi:10.1212/WNL.0b013e31821f445b

4.      Dolzhenko E, van Vugt JJFA, Shaw RJ, et al. Detection of long repeat expansions from PCR-free whole-genome sequence data. *Genome Research*. 2017;27(11). doi:10.1101/gr.225672.117

5.      Grassano M, Calvo A, Moglia C, et al. Mutational analysis of known ALS genes in an Italian population-based cohort. *Neurology*. Published online November 2020. doi:10.1212/WNL.0000000000011209

6.      Dewan R, Chia R, Ding J, et al. Pathogenic Huntingtin Repeat Expansions in Patients with Frontotemporal Dementia and Amyotrophic Lateral Sclerosis. *Neuron*. 2021;109(3):448-460.e4. doi:10.1016/j.neuron.2020.11.005

7.      Anderson D, Lassmann T. A phenotype centric benchmark of variant prioritisation tools. *npj Genom Med*. 2018;3(1):5. doi:10.1038/s41525-018-0044-9

8.      Morgan S, Shatunov A, Sproviero W, et al. A comprehensive analysis of rare genetic variation in amyotrophic lateral sclerosis in the UK. *Brain*. 2017;140(6):1611-1618. doi:10.1093/brain/awx082