ARTICLE

Mutational Analysis of Known ALS Genes in an Italian Population-Based Cohort

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Abstract

Objective

To assess the burden of rare genetic variants and to estimate the contribution of known amyotrophic lateral sclerosis (ALS) genes in an Italian population-based cohort, we performed whole genome sequencing in 959 patients with ALS and 677 matched healthy controls.

Methods

We performed genome sequencing in a population-based cohort (Piemonte and Valle d'Aosta Registry for ALS [PARALS]). A panel of 40 ALS genes was analyzed to identify potential disease-causing genetic variants and to evaluate the gene-wide burden of rare variants among our population.

Results

A total of 959 patients with ALS were compared with 677 healthy controls from the same geographical area. Gene-wide association tests demonstrated a strong association with *SOD1*, whose rare variants are the second most common cause of disease after *C9orf72* expansion. A lower signal was observed for *TARDBP*, proving that its effect on our cohort is driven by a few known causal variants. We detected rare variants in other known ALS genes that did not surpass statistical significance in gene-wise tests, thus highlighting that their contribution to disease risk in our cohort is limited.

Conclusions

We identified potential disease-causing variants in 11.9% of our patients. We identified the genes most frequently involved in our cohort and confirmed the contribution of rare variants in disease risk. Our results provide further insight into the pathologic mechanism of the disease and demonstrate the importance of genome-wide sequencing as a diagnostic tool.

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Glossary

ALS = amyotrophic lateral sclerosis; **FDR** = false discovery rate; **GATK** = Genome Analysis Toolkit; **MAF** = minor allele frequency; **OR** = odds ratio; **PARALS** = Piedmont and Valle d'Aosta Registry for ALS; **SKAT** = Sequence-Based Kernel Association Test.

Amyotrophic lateral sclerosis (ALS; OMIM 105400) is a neurologic disorder characterized by progressive degeneration of upper and lower motor neurons resulting in progressive paralysis and, ultimately, death from respiratory failure. Approximately 10% of patients with ALS report a family history, whereas the remainder of cases occur randomly.[1] Since the discovery of pathogenic mutations in *SOD1* in 1993,[2] researchers have made great strides in delineating the genetic basis underlying ALS. More than 40 genes have been reported in association with the disease, and the genetic etiology of approximately two-thirds of familial cases is known.[1,3]

With few exceptions, targeted mutational screening has been performed on samples collected from patients attending ALS clinics. Such clinic-based cohorts represent a self-selected subset of the ALS population, making it challenging to determine accurate mutational rates. In contrast, the Piedmont and Valle d'Aosta Registry for ALS (PARALS) was established in 1995 to study the epidemiologic characteristics of the disease in Northern Italy.[4] This comprehensive registry offers an opportunity to explore the frequency of mutations in a population-based cohort that has been in operation for over 20 years and has near-complete case ascertainment.

We analyzed the frequency and burden of mutations in 40 known ALS genes within a population-based cohort of nearly 1,000 Italian patients who underwent whole genome sequencing. Our efforts provide insight into the precise frequency of mutations in ALS genes in Northern Italy.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

Informed written consent was obtained from all participants, and the ethics committee of Azienda Ospedaliero Universitaria Città della Salute e della Scienza of Turin approved the study.

Study Participants

We included 959 patients who had been diagnosed with ALS and who had been enrolled in PARALS. PARALS is a prospective epidemiologic register that covers a population of 4.5 million from January 1, 1995.[5] Patients are included in PARALS if they meet the diagnosis of definite, probable, or probable laboratory-supported ALS according to the revised El Escorial criteria.[6] The 959 patients represent 72.9% of incident cases in the 2007–2015 period. Controls were chosen randomly from the same population and matched to the cases by sex, age (±5 years), and geographic location. Medical history was obtained for all controls and those with

Table 1 Clinical and Demographic Characteristics

	Values
Patients	959
Sex	
Men	505 (52.66)
Women	454 (47.34)
Clinical syndrome	
ALS	959
Additional feature	
FTD-ALS	124 (12.93)
Site of onset	
Spinal	647 (67.47)
Bulbar	312 (32.53)
Family history of ALS	
Familial	107 (11.16)
Sporadic	852 (88.84)
Average age at onset, y	
All patients	65.95 (21–88)
Familial patients	61.86 (27–86)
Sporadic patients	66.46 (21–88)
Men	65.88 (34–88)
Women	66.03 (21–88)
Controls, n	677
Sex	
Men	343 (50.66)
Women	334 (49.34)
Average age at onset, y	
All controls	63.96 (24–97)
Men	64.05 (34–97)
Women	63.87 (24–93)

Abbreviations: ALS = amyotrophic lateral sclerosis; FTD = frontotemporal dementia.
Values are n, n (%), or mean (range).

Table 2 Amyotrophic Lateral Sclerosis–Associated Genes Analyzed in our Cohort

Gene	Locus	Inheritance
ALS2	2q33	AR
ANG	14q11	RF
ANXA11	10q22	AD
C9orf72	9p21	AD
CAMTA1	1p36	RF
CCNF	16p13	AD
CHCHD10	22q113	AD
CHMP2B	3p11	AD
DAO	12q24.11	AD
DCTN1	2p13	AD, RF
ELP3	8p21	Undefined
ERBB4	2q34	AD
FIG4	6q21	AD, AR
FUS	16p11	AD, AR
HNRNPA1	12q13	AD
KIF5A	12q13.3	AD
MAPT	17q21.31	RF
MATR3	5q31.2	AD
NEFH	22q12	AD, RF
NEK1	4q33	AD, AR
OPTN	10p13	AD, AR
PFN1	17p13	AD
PON1	7q21.3	AD
PON2	7q21.3	AR
PRPH	12q13.12	AR
PGRN	17q21.31	AD
SETX	9q34	AD
SIGMAR1	9p13.3	AR
SOD1	21q22	AD, AR
SPAST	2p22.3	AR
SPG11	15q14	AR
SQSTM1	5q35	AD
TAF15	17q12	Undefined
TARDBP	1p36	AD, AR
TBK1	12q14.2	AD
TUBA4A	2q35	AD
UBQLN2	Xp11	X-linked AD
UNC13A	19p13.11	Modifier
VAPB	20q13	AD
VCP	9p13	AD, de novo

Abbreviations: AD = autosomal dominant; AR = autosomal recessive; RF = risk factor.

history of neurologic disease were excluded. Table 1 lists the clinical and demographic details of this cohort.

Mutation Screening

DNA was extracted from blood according to standard protocols. All participants were screened for the C9orf72 intronic expansion using a conventional repeat-primed PCR, as previously described.[7] Repeat lengths of ≥30 units with the characteristic sawtooth pattern were considered to be pathogenic. Whole-genome sequencing was performed at The American Genome Center located at the Uniformed Services University on the Walter Reed National Military Medical Center campus in Bethesda, Maryland. Libraries were prepared using TruSeq DNA PCR-Free High Throughput Library Prep Kit (Illumina) as per the manufacturer's instructions, before sequencing on an Illumina HiSeqX10 sequencer using paired-end 150 base pair reads. Sequence reads were processed according to Genome Analysis Toolkit's (GATK) best practices (software.broadinstitute.org/gatk/best-practices/). Variant quality control was performed using the GATK variant quality score method with default filters. Genome Reference Consortium Human Build 38 was used as the reference. We extracted variant information from the data for 40 genes previously implicated in the pathogenesis of ALS[3,8] (table 2).

Variant Annotation

We annotated all genotyped variants using KGGseq version 1.0 (grass.cgs.hku.hk/limx/kggseq/). We selected coding, nonsynonymous, and loss-of-function *single nucleotide polymorphisms* for analysis. Synonymous variants were used as a negative control.

Variant Filtering

Variants were filtered based on their minor allele frequencies (MAFs) in non-Finnish Europeans derived from the *gnomAD* (gnomad.broadinstitute.org/) database. Only variants with MAF <1% were retained.

Single Variant Association Testing

Firth regression was used for single variant association testing as it performs well in the study of unbalanced rare variants.[9] Odds ratio (OR) estimates of the burden were derived by logistic regression using the Single Score test.

Gene Burden Analysis

To perform gene-based analysis, we further filtered rare variants based on their MAF in our cohort. As standard in gene-burden analysis, the upper ceiling for the MAF threshold was set at 5% and 1%.[10] For gene burden testing, we applied different aggregation tests and set the *false discovery rate (FDR) p* value threshold at 0.05 and the Bonferroni-adjusted *p* value threshold at 1.25×10^{-2}. *p* Values for Sequence-Based Kernel Association Test (SKAT), Combined-Multivariate Collapsing Burden Test, and Madson-Browning Burden Test were obtained using a permutation approach. Because none of these tests offers a clear advantage in terms of performance, we evaluated the concordance across the different tests to assess the importance of each gene. The analysis was repeated after removing the most influential variant from the dataset for significant genes.

All analyses were implemented in RVTEST (github.com/zhanxw/rvtests). The first 5 principal components, as calculated by flashPCA (github.com/gabraham/flashpca), and sex were included as covariates. FDR and Bonferroni-corrected significance threshold were calculated in R v.3.6.0 (r-project.org/).

Estimation of the Population Prevalence of Causative Genetic Variants

In addition to gene-based testing, we investigated the genetic structure of our ALS population by assessing the prevalence of causative mutations. We therefore applied further filtering criteria to select a high-confidence set of pathogenic variants associated with ALS. We report as patients with disease-causing mutation all the patients with ALS who carried a rare variant (MAF in European Non-Finnish population lower than 0.01%) who meet the criteria for clinical significance as recommended by the American College of Medical Genetics and the Association for Molecular Pathology for variant interpretation in Mendelian disorders[11] and absent from the control population in our cohort. This highly conservative design is likely to minimize the risk of a false-positive genetic diagnosis.

Figure 1 provides a schematic visualization of the analytical workflow used in this study.

Data Availability

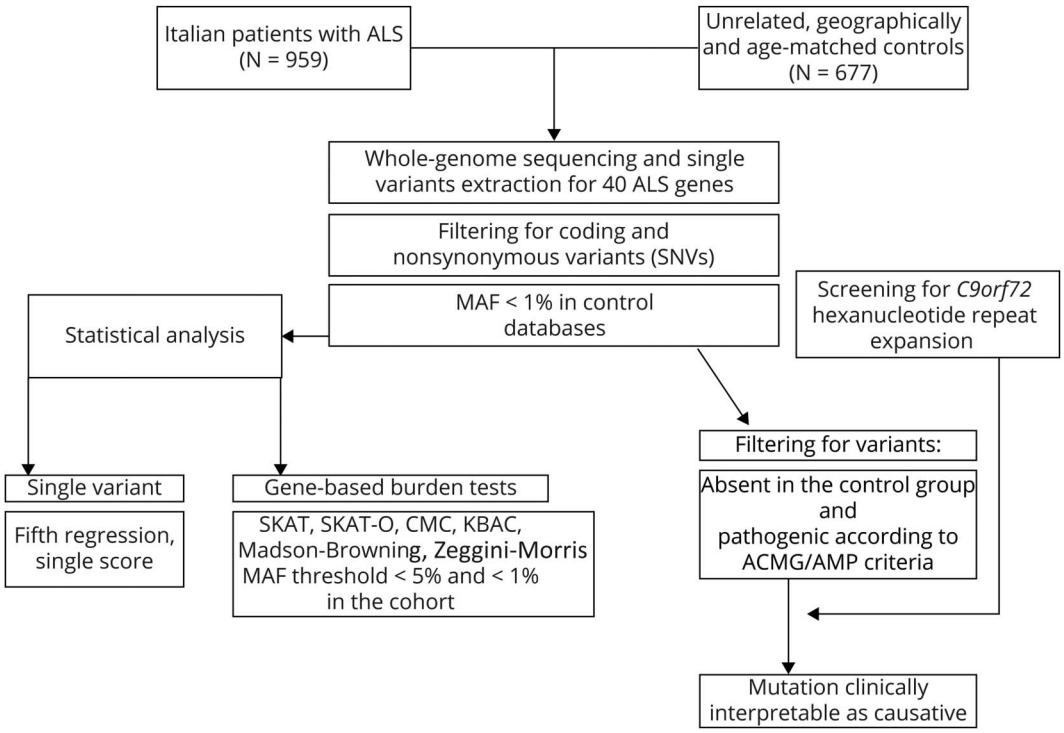
Data are available from the authors upon reasonable request.

Results

Frequency of Mutations in ALS Genes

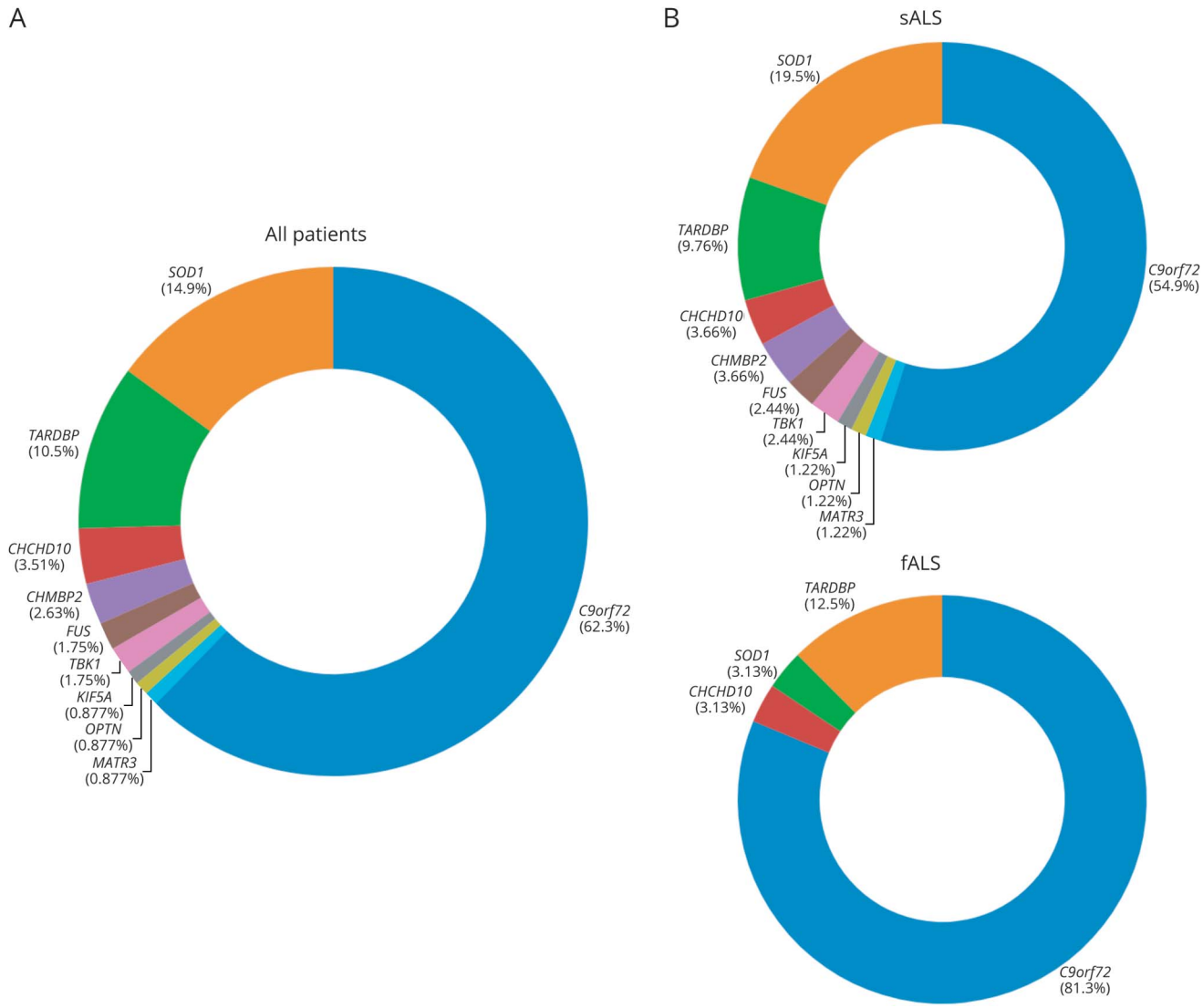
We analyzed the sequences of 40 ALS-related genes in a population-based cohort of 959 Italian patients with ALS and 677 unaffected controls. Of the 959 patients examined, 71 carried the *C9orf72* pathogenic repeat expansions, representing 7.4% of the entire group and 42.1% of the familial cases. An additional 34 patients carried causative mutations, representing a further 3.5% of the overall cohort and 13.1% of

Figure 1 Experimental Workflow



ACMG/AMP = American College of Medical Genetics and Genomics/Association for Molecular Pathology; ALS = amyotrophic lateral sclerosis; CMC = Combined-Multivariate Collapsing Burden Test; KBAC = Kernel-Based Adaptive Clustering Model; MAF = minor allele frequency; SKAT = Sequence-Based Kernel Association Test.

Figure 2 Frequency of Major Amyotrophic Lateral Sclerosis (ALS) Gene Mutations in our Cohort



(A) Relative contributions of ALS genes to the total estimate of patients harboring a causative variant. (B) Distribution of pathogenic variants among patients with sporadic ALS (sALS) and patients with ALS with a family history of the disease (fALS).

the familial cases. Figure 2 shows the mutational frequency of the genes identified in our cohort and table 3 describes the clinical features of mutation carriers.

Single Variant Analysis

After adjustment for multiple testing, only a single variant was associated with the disease. This was a c.2009T>A variant resulting in a valine to glutamic acid change at residue 670 of the neurofilament heavy gene (*NEFH*) (p value = 3.7×10^{-5}, FDR-adjusted p value = 0.047). This variant (rs190692435) decreased the risk of ALS (OR 0.28, 95% confidence interval 0.13–0.55). Variants that are known to cause ALS did not surpass the significance threshold in our cohort, likely reflecting the fact that our sample size was insufficiently powered to detect them (table 4).

Gene Burden Analysis Results

NEFH was significantly associated with the risk of ALS under the SKAT-O model at a minor allele threshold of 5% (p value = 7.7×10^{-5}, FDR p value = 0.0035, figure 3). The gene-level test remained significant after removing the top variants in *NEFH* from the analysis.

The only other significant gene was *SOD1* (p value under Madson-Browning analysis = 8.4×10^{-4}, FDR p value = 0.036, figure 3). Based on Sanger sequence data, we had previously reported that variant to be the most prevalent in our cohort after the *C9orf72* expansion.[4]

Repeating the gene burden analysis at a more stringent minor allele threshold of 1% found that *SOD1* remained significantly associated with the risk of ALS (p value under the KBAC model =

Table 3 Pathogenic Mutations and Corresponding Clinical Features

Gene	Function	Exon	Nucleotide change	AA change	Carriers	Sex	Age at onset, y	*C9orf72* exp	Family history
TARDBP	Missense	6	c.G1144A	p.A382T	8	M	67	<30	
						M	60	<30	Yes
						F	67	<30	
						F	70	<30	
						M	74	<30	Yes
						M	79	<30	
						F	60	<30	Yes
						F	54	<30	
TARDBP	Missense	6	c.A800G	p.N267S	1	M	67	<30	
TARDBP	Missense	6	c.A1169G	p.N390S	1	M	70	<30	
TARDBP	Missense	6	c.C1178T	p.S393L	2	M	70	<30	
						M	74	<30	
KIF5A	Missense	20	c.G2263A	p.E755K	1	M	63	<30	
FUS	Stopgain	14	c.C1480T	p.R494X	2	M	66	<30	
						M	29	<30	
SOD1	Missense	1	c.A59G	p.N20S	2	M	57	<30	
						M	48	<30	
SOD1	Missense	1	c.G16A	p.V6M	1	F	79	<30	
SOD1	Missense	3	c.A197G	p.N66S	2	F	65	<30	
						F	49	<30	
SOD1	Stopgain	5	c.A409T	p.K137X	1	M	45	<30	
SOD1	Missense	5	c.G442A	p.G148S	1	F	68	<30	
SOD1	Missense	2	c.C115G	p.L39V	1	M	47	<30	Yes
SOD1	Missense	3	c.G217A	p.G73S	1	F	48	<30	
SOD1	Missense	4	c.G271A	p.D91N	1	F	71	<30	
SOD1	Missense	4	c.G281A	p.G94D	3	M	58	<30	
						F	38	<30	
						M	69	<30	
SOD1		5	c.G435C	p.L145F	4	M	45	<30	
						M	—	<30	
						M	67	<30	
						F	60	<30	
CHCHD10	Missense	2	c.C239T	p.P80L	4	F	71	<30	
						F	84	<30	
						M	56	>30	Yes
						M	78	<30	
CHMP2B	Missense	2	c.A85G	p.I29V	3	M	70	<30	
						M	37	<30	

Continued

Table 3 Pathogenic Mutations and Corresponding Clinical Features *(continued)*

Gene	Function	Exon	Nucleotide change	AA change	Carriers	Sex	Age at onset, y	*C9orf72* exp	Family history
						F	75	<30	
OPTN	Missense	3	c.C247T	p.R83C	1	M	70	<30	Yes
TBK1	Missense	8	c.C992T	p.T331I	1	M	50	<30	
TBK1	Missense	14	c.G1603A	p.A535T	1	M	64	<30	
MATR3	Missense	15	c.C2120T	p.S707L	1	M	84	<30	

Abbreviation: AA = amino acid change.
We observed 18 variants in *SOD1*: based on the results from gene-based tests, we interpreted all of them to be pathogenic. This confirms *SOD1* as the second most common mutated gene in our cohort, representing 1.88% of our cases. Eight patients with amyotrophic lateral sclerosis (ALS) were heterozygotic for known pathogenic TARDBP c.G1144A:p.A382T mutation. We observed 3 other TARDBP variants that had been previously reported in ALS cases.[4] Overall, TARDBP was mutated in 12 patients (1.25%) in our cohort. It should be noted that 2 patients with ALS in our cohort had putative oligogenic disease (1 patient carrying the *C9orf72 GGGGCC* expansion along with the *CHCHD10 p.P80L* and 1 patient carrying *SOD1 p.D91N* along with *CHCHD10 p.P80L*). According to our study design, we defined as oligogenic only patients carrying 2 or more variants with unambiguous evidence supporting their pathogenicity. Given these restrictive criteria, the observed frequency of patients carrying 2 or more established highly penetrant mutations in our cohort is 1.9%.

7.0×10^{-4}, FDR p value = 0.032). *TARDBP* was the second most enriched gene, although it did not reach statistical significance (p value under the KBAC model = 1.9×10^{-3}, FDR p value = 0.084). The common founder mutation *TARDBP p.A382T*[12] mostly drove the association signal for *TARDBP* (see table 4 for details).

As a negative control, we verified that supposedly neutral variation did not differ in frequency between case and control groups at MAF <5% (lowest FDR p value under SKAT-O model = 0.259) or MAF <1% (lowest FDR p value under SKAT-O model = 0.077). This null study is meant to reinforce the validity of the results obtained, excluding possible artifactual inflation or deflation of test statistics.

Discussion

We sequenced the genomes of 959 patients diagnosed with ALS and 677 neurologically healthy individuals obtained from an Italian population-based registry. We then estimated the frequency of mutations of major ALS genes in this population-based epidemiologic series. This study was an opportunity to understand the contribution of variants and genes to the genetic and phenotypic spectrum of ALS. We found that a significant portion of our cohort (n = 105 cases, 11.0%) carried mutations that are known to be pathogenic. This frequency of patients harboring a genetic mutation is consistent with previous epidemiologic studies in the same area.[13] It is reasonable to assume that the application of less stringent criteria for pathogenic mutations would raise the prevalence of patients with ALS carrying at least one genetic variant, albeit at the cost of increasing false-positive results.

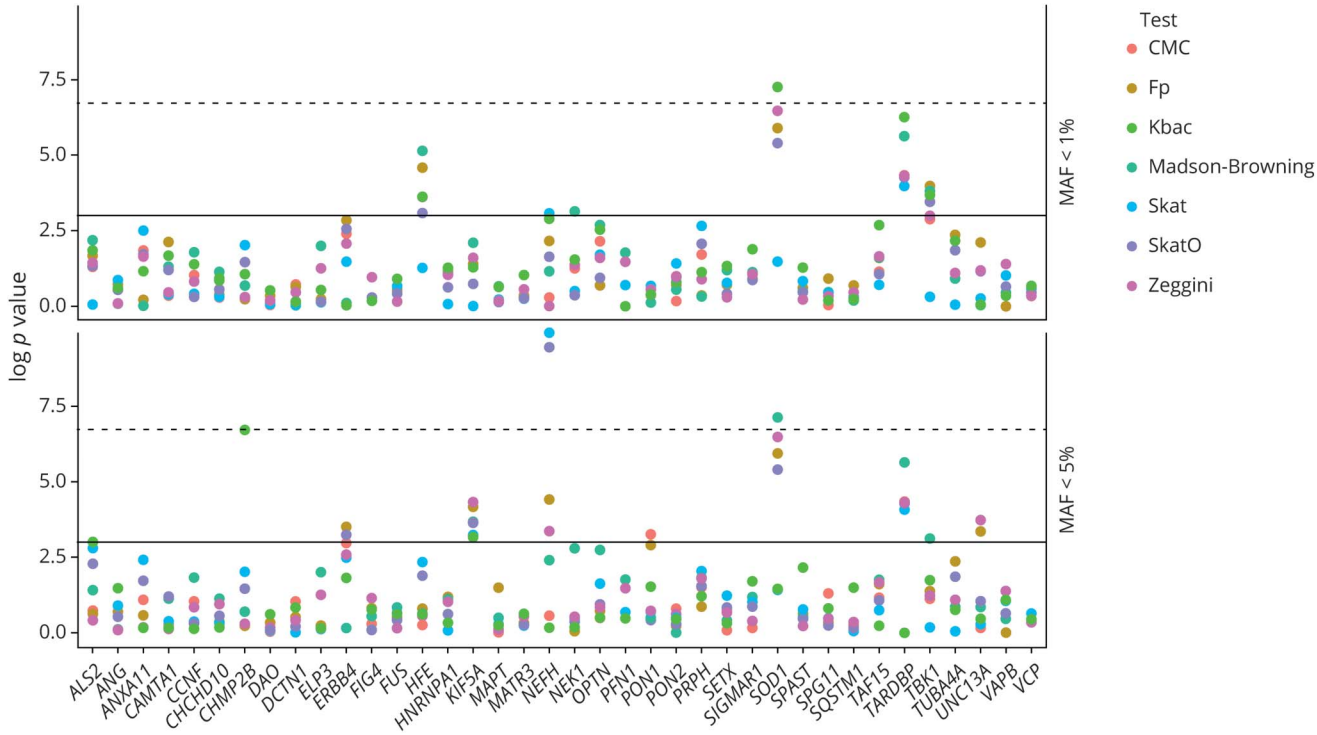
Our analysis demonstrates the utility of genome sequencing to assess the role of multiple genes in disease. Undertaking this type of broad analysis with traditional Sanger sequencing would be costly and time-consuming. Even if we applied restrictive criteria in the definition of causal genetic variants, we demonstrated that genome sequencing

Table 4 Results From Single-Variant Tests

Gene	Exon	Nucleotide change	AA	AF	OR	p Value$_S$	FDR	p Value$_F$
NEFH[a]	4[a]	T2009A[a]	V670E[a]	0.0138[a]	0.28[a]	0.000037[a]	0.0489[a]	0.000106[a]
NEFH	4	C2015A	A672E	0.0156	0.31	0.000054	0.0713	0.000127
NEFH	4	1952_1975del	E658_K665 del	0.0171	0.37	0.000393	0.5152	0.000588
TARDBP	6	G1144A	A382T	0.0025	5.54	0.017639	1	0.057657
SOD1	5	G435C	L145F	0.0012	5.52	0.093306	1	0.243403

Abbreviations: AA = amino acid change; AF = allele frequency in our cohort; FDR = false discovery ratio; OR = odds ratio from Firth regression; p value$_F$ = p value from Firth regression (see also Supplementary Methods); p value$_S$ = p value from Single Score test.
The variant *NEFH* c.2009T>A:p.V670E (rs190692435) is a low-frequency supposedly neutral variation (minor allele frequency in non-Finnish European population 0.6%). Two other variants in *NEFH* (the nonsynonymous substitution *NEFH* c. 2015C>A:p.A672E and the non-frameshift deletion *NEFH* c.1952_1975del>p.E658_K665del) approached statistical significance. Both variants are located in exon 4 and showed a potential protective effect on amyotrophic lateral sclerosis (ALS) (OR 0.31 and 0.37, respectively). No other significant associations with ALS were observed for the analyzed variants after appropriate correction for multiple testing.
[a] Values withstand correction for Bonferroni-adjusted significance threshold or FDR.

Figure 3 Results of Rare Variant Association Testing



Results of rare variant association testing with minor allele frequency (MAF) <5% (A) and <1% (B). Solid line: *p* value 0.05. Dashed line: Bonferroni-adjusted *p* value 0.001. All the log *p* values obtained by the gene-wise association tests applied in our study are reported. CMC = Combined-Multivariate Collapsing Burden Test[17]; Fp = burden test with rare variants upweighted using frequency controls[20]; Kbac = kernel-based adaptive clustering model[16]; Madsen-Browning = Madsen-Browning Burden Test with rare variants upweighted using inverse frequency controls[19]; Skat = Sequence-Based Kernel Association Test[10]; SkatO = Optimized Sequence-Based Kernel Association Test[15]; Zeggini = Zeggini-Morris Burden Test with aggregate counts of rare variants.[18]

yields a broader insight into the genetic architecture of ALS than targeted sequencing or exome sequencing. Whole genome sequencing provides a complete catalog of rare variants present in patients and has the potential not only to answer specific diagnostic questions but also to uncover clinically important genetic information.

In gene-based rare variant association tests, *SOD1* was the only gene to achieve significance for all of the frequency thresholds tested. Interestingly, we found low-frequency variants within the *NEFH* gene that lowered the risk of disease. There are few previous reports in the literature concerning protective variants in this gene in ALS. However, neurofilaments are currently regarded as a promising biomarker in ALS, possibly reflecting involvement in neuronal degeneration.[14] It may be interesting to stratify *NEFH* blood and CSF measurements according to genotype in future studies.

The relatively small size of our cohort limited the power to detect a significant association. Furthermore, we did not consider other types of genetic mutations, such as intronic variants and structural variants that are known to be relevant to ALS. Thus, the mutational frequencies presented here are considered to be floor estimates. Nevertheless, our data allow us to conclude that coding mutations in these genes are not a common cause of sporadic disease.

In summary, we performed genome sequencing in a large Italian case–control cohort (n = 959 cases, n = 677 controls) to capture genetic variation across the ALS spectrum in our population. Our study provides evidence and an effective way to evaluate the role of low-frequency and rare variants underpinning the complex nature of ALS.

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Disclosure

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Andrea Calvo, MD, PhD	"Rita Levi Montalcini" Department of Neuroscience, University of Turin, Italy	Major role in the acquisition of data, interpreted the data, revised the manuscript for intellectual content
Cristina Moglia, MD, PhD	"Rita Levi Montalcini" Department of Neuroscience, University of Turin, Italy	Major role in the acquisition of data, interpreted the data, revised the manuscript for intellectual content
Maura Brunetti, BSc	Laboratory of Genetics, Department of Clinical Pathology, Azienda Ospedaliero Universitaria Città della Salute e della Scienza di Torino, Turin, Italy	Major role in the acquisition of data, revised the manuscript for intellectual content
Marco Barberis, BSc	Laboratory of Genetics, Department of Clinical Pathology, Azienda Ospedaliero Universitaria Città della Salute e della Scienza di Torino, Turin, Italy	Major role in the acquisition of data, revised the manuscript for intellectual content
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Umberto Manera, MD	"Rita Levi Montalcini" Department of Neuroscience, University of Turin, Italy	Major role in the acquisition of data, revised the manuscript for intellectual content
Rosario Vasta, MD	"Rita Levi Montalcini" Department of Neuroscience, University of Turin, Italy	Major role in the acquisition of data, revised the manuscript for intellectual content
Lucia Corrado, PhD	Department of Health Sciences Interdisciplinary Research Center of Autoimmune Diseases, "Amedeo Avogadro" University of Eastern Piedmont, Novara, Italy	Major role in the acquisition of data, interpreted the data, revised the manuscript for intellectual content
Sandra D'Alfonso, PhD	Department of Health Sciences Interdisciplinary Research Center of Autoimmune Diseases, "Amedeo Avogadro" University of Eastern Piedmont, Novara, Italy	Major role in the acquisition of data, interpreted the data, revised the manuscript for intellectual content
Letizia Mazzini, MD	ALS Center, Department of Neurology, Azienda Ospedaliera Universitaria Maggiore della Carità, Novara, Italy	Major role in the acquisition of data, interpreted the data, revised the manuscript for intellectual content
Sonja W. Scholz, MD, PhD	Neurodegenerative Diseases Research Unit, Laboratory of Neurogenetics, National Institute of Neurologic Disorders and Stroke, NIH, Bethesda; Department of Neurology, Johns Hopkins University Medical Center, Baltimore, MD	Analyzed and interpreted the data, drafted the manuscript for intellectual content
Clifton Dalgard, PhD	Department of Anatomy, Physiology & Genetics, Uniformed Services University of the Health Sciences; The American Genome Center, Collaborative Health Initiative Research Program, Uniformed Services University of the Health Sciences, Bethesda, MD	Analyzed the data, revised the manuscript for intellectual content
Jinhui Ding, PhD	Biocomputational Group, Laboratory of Neurogenetics, National Institute on Aging, NIH, Porter Neuroscience Research Center, Bethesda, MD	Analyzed the data, revised the manuscript for intellectual content

Appendix *(continued)*

Name	Location	Contribution
Raphael J. Gibbs, BSc	Biocomputational Group, Laboratory of Neurogenetics, National Institute on Aging, NIH, Porter Neuroscience Research Center, Bethesda, MD	Analyzed the data, revised the manuscript for intellectual content
Ruth Chia, PhD	Neuromuscular Diseases Research Section, Laboratory of Neurogenetics, National Institute on Aging, NIH, Porter Neuroscience Research Center, Bethesda, MD	Design and conceptualized study, analyzed and interpreted the data, revised the manuscript for intellectual content
Bryan J. Traynor, MD, PhD	Neuromuscular Diseases Research Section, Laboratory of Neurogenetics, National Institute on Aging, NIH, Porter Neuroscience Research Center, Bethesda; Department of Neurology, Johns Hopkins University Medical Center, Baltimore, MD	Design and conceptualized study, analyzed and interpreted the data, drafted the manuscript for intellectual content
Adriano Chiò, MD, FAAN	"Rita Levi Montalcini" Department of Neuroscience, University of Turin; Institute of Cognitive Sciences and Technologies, National Council of Research, Rome, Italy	Design and conceptualized study, analyzed and interpreted the data, drafted the manuscript for intellectual content

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