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ORIGINAL ARTICLE

Evaluating the contribution of the gene TARDBP in Italian patients with amyotrophic lateral sclerosis

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Abstract

Background and objectives: Genetic variants in the gene *TARDBP*, encoding TDP-43 protein, are associated with amyotrophic lateral sclerosis (ALS) in familial (fALS) and sporadic (sALS) cases. Objectives of this study were to assess the contribution of *TARDBP* in a large cohort of Italian ALS patients, to determine the *TARDBP*-associated clinical features and to look for genotype–phenotype correlation and penetrance of the mutations.

Methods: A total of 1992 Italian ALS patients (193 fALS and 1799 sALS) were enrolled in this study. Sanger sequencing of *TARDBP* gene was performed in patients and, when available, in patients' relatives.

Results: In total, 13 different rare variants were identified in 43 index cases (10 fALS and 33 sALS) with a cumulative mutational frequency of 2.2% (5.2% of fALS, 1.8% of sALS). The most prevalent variant was the p.A382T followed by the p.G294V. Cognitive impairment was detected in almost 30% of patients. While some variants, including the p.G294V and the p.G376D, were associated with restricted phenotypes, the p.A382T showed a marked clinical heterogeneity regarding age of onset, survival and association with cognitive impairment. Investigations in parents, when possible, showed that the variants were inherited from healthy carriers and never occurred de novo.

Conclusions: In our cohort, *TARDBP* variants have a relevant frequency in Italian ALS patients and they are significantly associated with cognitive impairment. Clinical presentation is heterogeneous. Consistent genotype–phenotype correlations are limited to some mutations. A marked phenotypic variability characterizes the p.A382T variant, suggesting a multifactorial/oligogenic pathogenic mechanism.

KEYWORDS
amyotrophic lateral sclerosis, clinical heterogeneity, TARDBP

Serena Lattante and Mario Sabatelli contributed equally to this work.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the concomitant involvement of upper motor neurons (UMNs) in the cerebral cortex and lower motor neurons (LMNs) in the brainstem and the spinal cord. The disease has an aggressive course, with a median survival of about 3 years from onset. A family history of motor neuron disease is recorded in about 10% of cases (fALS), following an autosomal dominant pattern of inheritance, while the majority of cases (90%) have a sporadic presentation (sALS) [1]. ALS undergoes high heterogeneity, both clinically and genetically. Clinically, ALS overlaps with other neurological disorders. In particular, 15% of ALS patients present with frontotemporal dementia (FTD), caused by the degeneration of the frontal and temporal lobes of the brain. Genetically, more than 100 genes have been associated with ALS. However, only a few of them, including *SOD1*, *C9orf72*, *TARDBP* and *FUS*, act as "major ALS genes" with overlapping prevalence among different populations. Genetic variants in these major ALS genes cause about 60% of fALS and 15% of sALS [2].

A unique pathological signature characterizes almost all ALS cases, consisting in the presence of aggregates of the transactive response DNA binding protein (TDP-43) in the cytoplasm of neuronal and glial cells. The protein TDP-43 is encoded by the gene *TARDBP* (OMIM: 605078), one of the "major" ALS-genes, with mutations identified in both sALS and fALS cases [3, 4]. Multiple cohorts have been screened so far, showing very heterogeneous frequencies. A mutational hotspot has been identified in exon 6, encoding for the C-terminal region of the protein, where RNA binding sites reside. Pathogenic variants in *TARDBP* usually cause isolated ALS but they have been rarely identified in patients with ALS/FTD, with pure FTD [5, 6] and parkinsonism [7, 8]. In order to investigate the contribution of *TARDBP* to ALS pathogenesis in Italy, to better define the *TARDBP*-associated clinical features and to look for genotype–phenotype correlations and penetrance of individual gene variants we analysed a large cohort of Italian patients.

METHODS

Patients

A cohort of 1992 unrelated Italian patients, admitted to ALS Referral Center at Policlinico Universitario Agostino Gemelli IRCCS in Rome from 2013 to 2022, was enrolled to participate in this study. The study conformed with the World Medical Association Declaration of Helsinki and was approved by the Ethics Committee of Università Cattolica del Sacro Cuore (prot. A.133/C.E./2013). It was undertaken with the understanding and written informed consent of each involved subject. ALS diagnosis was made by expert neurologists considering El Escorial diagnostic criteria. Family history for ALS and for cognitive impairment were accurately investigated. Patients with family history for ALS or FTD were considered definite, probable or possible fALS according to previously proposed family history criteria [9]. In total, 193 patients (9.7%) were classified as fALS and 1765 (90.3%) as sALS. For each family, only one index case was considered. Patients were clinically classified into four different phenotypes: classic, upper motor neuron dominant (UMN-D), flail arm and pure LMN. An Italian version of the Edinburgh Cognitive and Behavioural Amyotrophic Lateral Sclerosis Screen (ECAS) was administered to a group of patients to assess cognitive performance. For cognitive and behavioral impairment, patients were evaluated according to the revised criteria for the diagnosis of frontotemporal dysfunction in ALS [10]: normal cognition, ALS with cognitive impairment (ALSci), ALS with behavioural impairment (ALSbi), ALS with combined cognitive and behavioural impairment (ALScbi) and ALS with frontotemporal dementia (ALS-FTD). The Kaplan–Meier method was used to analyse survival and the log-rank test was used to compare survival curves in different groups, considering as significant a p value <0.05. The survival time was calculated from disease onset to last follow-up or death or tracheostomy.

Methods

Peripheral blood samples were collected from patients after written informed consent and genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega). Coding exons and flanking intronic regions of *TARDBP* (NM_007375.4) were sequenced on an ABI3130XL Genetic Analyzer (Applied Biosystems), using standard protocols for Sanger sequencing, and analysed using DNA Sequencing Analysis Software v.5.1. Variants were classified, according to the ACMG criteria, using Varsome browser [11]. Parental DNA was collected when available to test the inheritance of the identified variants. Eight microsatellite markers in 1p36.22 (D1S450, D1S244, D1S2736, D1S1151, D1S2667, D1S434, D1S489, D1S2697), surrounding the *TARDBP* gene, were used for haplotype analysis. Electrophoresis was performed on an ABI3130XL Genetic Analyzer (Applied Biosystems) and fragments were analysed using GeneMapper v5.0. Genotype phasing was performed using the software Beagle 5.4 (http://faculty.washington.edu/browning/beagle/beagle.html).

RESULTS

Genetic analysis of *TARDBP* revealed 13 different rare variants, all in a heterozygous state, in 43 index cases: 10 fALS and 33 sALS (Table 1). According to ACMG recommendations, 8 variants were classified as "pathogenic", 3 as "likely pathogenic" and 2 as "of uncertain significance". All variants were extremely rare in control populations, with an allelic frequency from 0 to 0.00007964 reported on gnomAD. The majority of variants (11/13) were previously identified in ALS patients (Table 1).

Clinical characteristics of our patients carrying *TARDBP* variants are detailed in Table 2. The majority of mutated patients (67.4%) showed a spinal onset (29 in total, 17 upper limb and 12 lower limb)

TABLE 1 List of genetic variants identified in *TARDBP*.

Patients (n)	Nucleotidic change	Aminoacidic change	ACMG criteria	VarSome	Reference	gnomAD (exomes total allele frequency)
1	c.-12–1 G>C		PM2	Uncertain significance	–	0
1	c.484 A>C	p.M162L	PM2, PP2, BP4	Uncertain significance	–	0
3	c.800 A>G	p.N267S	PP5, PM1, PP2, BP4	Pathogenic	18	0.00007566
7	c.881G>T	p.G294V	PP5, PM1, PM5, PM2, PP2	Pathogenic	18	0.00001194
1	c.881G>C	p.G294A	PM1, PS3, PM5, PM2, PP2	Pathogenic	4	0
5	c.883G>A	p.G295S	PM1, PS1, PM2, PM5, PP5, PP2	Pathogenic	18	0.000007963
1	c.909 A>C	p.Q303H	PM1, PM2, PP2	Likely pathogenic	12	0.000007964
1	c.995G>A	p.S332N	PM1, PM2, PM5, PP2, PP5	Pathogenic	18	0
1	c.1127G>A	p.G376D	PM1, PM2, PM5, PP2	Likely pathogenic	30	0
19	c. 1144G>A	p.A382T	PP5, PM1, PP2	Pathogenic	3	0.00003041
1	c.1147 A>G	p.I383V	PP5, PM1, PM2, PP2, BP4	Pathogenic	40	0.00001908
1	c.1153T>G	p.W385G	PM1, PM2, PP2, PP3, PP5	Pathogenic	24	0
1	c.1170 A>G	p.N390S	PM1, PM5, PP5, PP2	Likely pathogenic	3	0.00002276

Abbreviations: ACMG, American College of Medical Genetics and Genomics; BP, supporting evidence of benign impact; PM, moderate evidence of pathogenicity; PP, supporting evidence of pathogenicity; PS, strong evidence of pathogenicity.

Note: For each variant, number of carriers, nucleotidic change, aminoacidic change, ACMG and VarSome classification and frequency reported in gnomAD have been reported.

and the remaining 14 (32.6%) had a bulbar onset. Most patients ($n = 28$) showed the classic phenotype (65.2%), while 23.2% ($n = 10$) had the UMN-D form and 9.3% ($n = 4$) the flail arm phenotype. LMN form was very rare, being detected in only 1 patient (2.3%). Patients with *TARDBP* variants had an average age at onset of 54.1 ± 13.64 (range 17–76) years with a sex ratio M:F = 2.07:1 and a median survival time of 61 months (Figure 1a).

Twelve identified variants were of missense type, all located in exon 6. A unique missense variant, the p.M162L, lies in exon 4. It has never been reported in ALS patients nor in controls, thus it was considered as a "variant of uncertain significance". It was identified in a patient with UMN-D phenotype whose maternal cousin, not tested for the genetic variants, was affected by ALS. In our patient, the variant was inherited from the mother, who died aged 84 years after a cerebral ischaemia with no signs of motor neuron disease. Interestingly, the patient had concomitant multiple sclerosis, which started with sensory symptoms 15 years before bulbar ALS clinical signs. One variant (c.-12-1G>C) was located in the 5' untranslated region (5'UTR) of the gene. We consider that it can affect the splicing, as predicted by in silico analysis (NN Splice acceptor = −0.997, Gene Splicer acceptor = −13.646). The variant was inherited from the healthy mother, with no neurological issues by the age of 78 years. All remaining variants were previously described in ALS patients and are classified as "pathogenic" or "likely pathogenic".

In our cohort, the most frequent variant was the p.A382T, detected in 19 patients: 4 fALS (3 probable, 1 possible) and 15 sALS. The mean age at onset was 56.4 ± 15.4 (range 17–75) years and the median survival time was 54 months (Figure 1b). One patient with onset at 36 years underwent invasive pressure positive ventilation (IPPV) through tracheostomy after 66 months and was still alive 23 years later, without showing ocular movement impairment. In our patients, the p.A382T variant was associated more frequently with a spinal onset (73.7%) and with a classical phenotype (68.4%). Two patients had overt FTD; interestingly, one of them developed progressive non-fluent aphasia (PNFA) 4 years before bulbar motor signs. The majority of carriers (73.7%) were of Sardinian origin.

The p.G294V variant was identified in 7 patients (2 fALS and 5 sALS) with prevalent bulbar onset (71.4%) and classic phenotype (71.4%), a mean age at onset of 59.4 ± 6.9 years and a median survival time of 28 months (Figure 1b). Patient 505 inherited the variant from her father, who died aged 90 years without motor signs but with memory deficits.

The p.G294A variant, involving the same codon but a different nucleotide, was identified in one sALS patient with a spinal onset and a classic phenotype.

The p.G295S variant was identified in 5 patients, all sporadic and with classic phenotype and average age at onset of 55.8 ± 5.9 years. Two female patients with bulbar onset showed concomitant cognitive impairment at neuropsychological evaluation, diagnosed as

TABLE 2 Clinical characteristics of our patients carrying *TARDBP* variants.

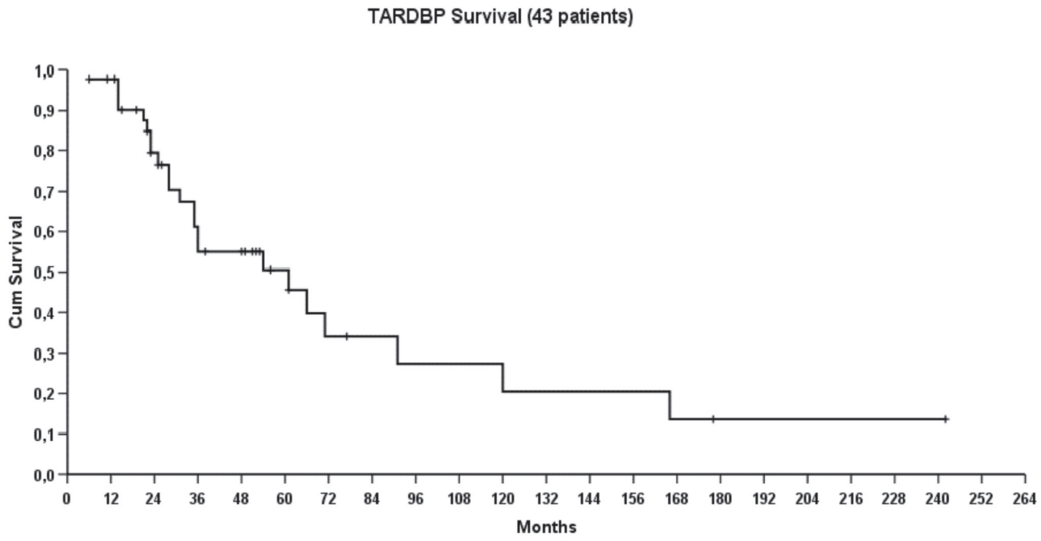
TARDBP variant	Patient ID	Sex	Age of onset (year)	Disease duration (months)	Outcome	Site of onset	Phenotype	SALS/FALS	Familiarity for cognitive impairment	Cognitive/behavioural impairment
c.-12-1G>C	2187	M	48	15	Alive	Spinal (UL)	Flail arm	SALS	No	No
p.M162L	1470	M	52	23	Deceased	Bulbar	UMN-D	FALS (possible)	No	N/A
p.N267S	468	M	66	91	Deceased	Spinal (UL)	Flail arm	SALS	No	No
	610	M	27	178	Alive	Spinal (LL)	Classic	SALS	No	N/A
	2245	M	67	19	Alive	Spinal (UL)	Classic	SALS	Yes (dementia)	No
p.G294A	2134	F	57	22	Alive	Spinal (LL)	Classic	SALS	Yes (dementia)	No
p.G294V	505	F	62	25	Deceased	Bulbar	UMN-D	SALS	Yes (memory disorder)	No
	586	M	58	11	Lost at follow-up	Bulbar	Classic	SALS	No	N/A
	765	M	56	71	Deceased	Spinal (LL)	LMN	SALS	No	No
	802	F	71	14	Deceased	Bulbar	Classic	FALS (definite)	No	No
	1083	M	61	6	Tracheostomy (alive after 58 m)	Bulbar	Classic	FALS (probable)	No	N/A
	2045	M	48	28	Tracheostomy (alive after 6 m)	Bulbar	Classic	SALS	No	No
	2181	F	60	26	Alive	Spinal (LL)	Classic	SALS	No	Yes (ALSbi)
p.G295S	989	M	60	77	Lost at follow-up	Spinal (LL)	Classic	SALS	Yes (dementia and PD)	No
	1639	M	55	52	Lost at follow-up	Spinal (UL)	Classic	SALS	No	N/A
	1907	F	46	38	Alive	Bulbar	Classic	SALS	No	Yes (ALSci)
	1943	F	57	56	Alive	Bulbar	Classic	SALS	No	Yes (ALSci)
	2082	M	61	48	Alive	Spinal (UL)	Classic	SALS	No	No
p.Q303H	367	F	52	166	Deceased	Spinal (LL)	UMN-D	SALS	No	No
p.S332N	51P	F	54	35	Deceased	Bulbar	UMN-D	FALS (definite)	Yes (dementia)	Yes (FTD-BV)
p.G376D	889	M	36	14	Tracheostomy (deceased after 71 m)	Neck muscles	Flail arm	FALS (definite)	No	No
p.A382T	13P	M	76	120	Deceased	Bulbar	UMN-D	SALS	Yes (dementia)	Yes (ALS-FTD)
	18	M	36	66	Tracheostomy (alive after 276 m)	Spinal (LL)	UMN-D	SALS	No	No
	288	F	63	35	Tracheostomy (alive after 30 m)	Spinal (LL)	UMN-D	SALS	Yes (dementia)	Yes (ALSbi)
	303	F	43	51	Lost at follow-up	Spinal (UL)	UMN-D	SALS	Yes (dementia)	N/A

TABLE 2 (Continued)

TARDBP variant	Patient ID	Sex	Age of onset (year)	Disease duration (months)	Outcome	Site of onset	Phenotype	SALS/FALS	Familiarity for cognitive impairment	Cognitive/behavioural impairment
	528	M	75	36	Deceased	Spinal (LL)	Classic	SALS	No	Yes (ALSbi)
	548	F	58	242	Alive	Spinal (LL)	Classic	SALS	No	No
	574	M	60	36	Deceased	Bulbar	UMN-D	SALS	Yes (dementia)	Yes (FTD-PNFA)
	935	M	57	28	Deceased	Spinal (LL)	Classic	SALS	No	No
	1325	M	17	54	Tracheostomy (alive after 32 m)	Spinal (UL)	Classic	SALS	No	No
	1445	M	36	21	Deceased	Bulbar	Classic	FALS (probable)	No	N/A
	1466	M	65	14	Deceased	Spinal (UL)	Classic	FALS (probable)	No	No
	1476	M	52	13	Lost at follow-up	Spinal (UL)	Classic	SALS	No	N/A
	1483	F	75	61	Deceased	Bulbar	Classic	SALS	No	Yes (ALSbi)
	1792	M	50	6	Lost at follow-up	Spinal (UL)	Flail arm	FALS (possible)	No	N/A
	1835	F	58	61	Alive	Spinal (UL)	Classic	SALS	No	No
	1984	M	53	22	Deceased	Bulbar	Classic	SALS	Yes (delirium)	No
	2186	F	71	53	Alive	Spinal (UL)	Classic	SALS	No	No
	2243	M	57	25	Alive	Spinal (UL)	Classic	SALS	No	Yes (ALSbi)
	2322	M	70	49	Alive	Spinal (UL)	Classic	FALS (probable)	No	No
p.I383V	9	M	37	31	Tracheostomy (deceased after 28 m)	Spinal (LL)	Classic	SALS	No	No
p.W385G	2017	M	24	23	Alive	Spinal (UL)	Classic	FALS (definite)	No	No
p.N390S	17	M	40	23	Tracheostomy (deceased after 1 m)	Spinal (UL)	UMN-D	SALS	No	N/A

Abbreviations: ALS, amyotrophic lateral sclerosis; ALSbi, ALS with behavioural impairment; ALScbi, ALS with combined cognitive and behavioural impairment; ALSci, ALS with cognitive impairment; BV, behavioral variant; F, female; FALS, familial ALS; FTD, frontotemporal dementia; LL, lower limb; LMN, lower motor neuron; m, month; M, male; N/A, not available; PD, Parkinson's disease; PNFA, progressive non-fluent aphasia; SALS, sporadic ALS; UL, upper limb; UMN-D, upper motor neuron dominant.

(a)

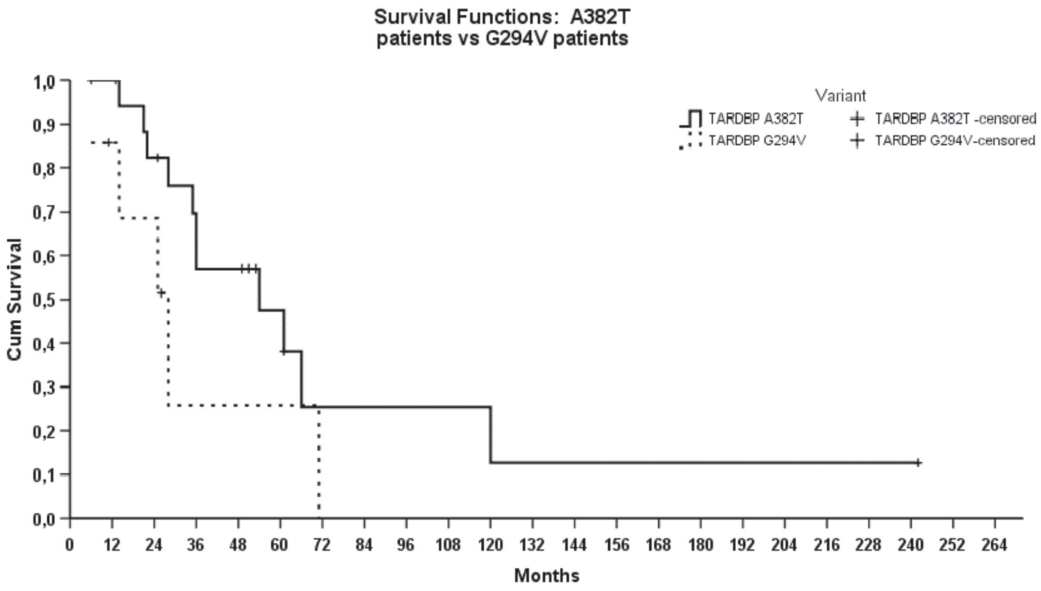


Means and Medians for Survival Time

Mean[a]				Median			
		95% confidence interval				95% confidence interval	
Estimate	Std. Error	Lower Bound	Upper Bound	Estimate	Std. Error	Lower Bound	Upper Bound
83,384	15,839	52,340	114,427	61,000	18,306	25,120	96,880

[a] Estimation is limited to the largest survival time if it is censored.

(b)



Means and Medians for Survival Time

	Mean[a]				Median			
			95% confidence interval			Std.	95% confidence interval	
Variant	Estimate	Std. Error	Lower Bound	Upper Bound	Estimate	Error	Lower Bound	Upper Bound
TARDBP A382T	77,059	21,527	34,867	119,251	54,000	17,692	19,324	88,676
TARDBP G294V	33,000	11,832	9,809	56,191	28,000	6,809	14,654	41,346
Overall	64,776	16,352	32,726	96,827	36,000	12,172	12,144	59,856

[a] Estimation is limited to the largest survival time if it is censored.

FIGURE 1 Kaplan–Meier curves of survival in patients with TARDBP mutations (a) and comparison of patients with the p.A382T and p.G294V variants (b).

ALSci, according to Strong criteria [10]. Patient 1907, classified as sALS, inherited the variant from her father who had a depressive disorder but no motor impairment at age 75 years.

Three sALS patients, all with spinal onset (average age at onset 53.3 ± 22.8 years), carried the p.N267S, and one of them had a family history of cognitive impairment.

The p.W385G was identified in a very young patient, aged 24 years, from a family with a definite fALS, in which three additional cases were diagnosed. He inherited the variant from his healthy mother with no neurological issues at age 47 years. His maternal uncle, carrying the mutation, had depression and behavioural symptoms with no motor neuronal signs at the age of 63 years.

The p.Q303H variant was identified in a patient previously described by our group [12] and no more reported in the literature. It is very rare, as it was never found in control exomes. The patient had a long history of disease and died 166 months after disease onset.

The p.G376D variant was detected in a fALS patient with an early onset (36 years), a flail arm phenotype with a peculiar neck muscle weakness at onset and an aggressive course, with a disease duration of 14 months.

The p.I383V mutation was identified in a sALS patient with an early spinal onset (37 years) and a short disease duration (31 months).

Analysis of microsatellite markers surrounding *TARDBP*, performed in patients carrying the p.G294V and the p.A382T variants, detected a haplotype that spans about 2.7 Mb in 2/7 patients with the p.G294V (505 and 2181) and a large haplotype spanning about 6.8 Mb in 2/19 patients with the p.A382T (1835 and 2186). Shorter haplotypes are identifiable in different patients (Table S1). These results did not allow the identification of a common large haplotype that would have confirmed the hypothesis of a founder effect.

DISCUSSION

The present study deals with the largest investigation of the *TARDBP* gene in Italian ALS patients. By analysing 1992 patients, genetic variants were identified in 43, with a cumulative mutational frequency of 2.2% (5.2% of fALS and 1.8% of sALS). Nearly all missense variants lie in the C-terminal domain, encoded by exon 6. This domain contains nuclear localization (NLS) and export (NES) signals, which allow TDP-43 protein to shuttle between the nucleus and the cytosol. Atypical variants were detected in two patients, consisting of the p.M162L located in exon 4, and the c.-12-1G > C located in the 5'UTR region, respectively. The latter represents a novel 5'UTR variant not present in healthy controls (https://gnomad.broadinstitute.org). Variants in the 5'UTR are very rare in controls and their role is still a matter of debate [13]. Furthermore, the promoter of *TARDBP* has been experimentally characterized: it contains regulatory elements that have been acquired via evolution, being highly conserved in primates [14, 15]. For these reasons, the c.-12-1G > C variant could affect the normal splicing process, as predicted by bioinformatics tools, and could interfere with regulatory elements to modulate the expression of TDP-43.

Furthermore, our patient inherited the variant from the healthy mother who is 78 years old. Two previously reported variants, located in the promoter region, were described as not influencing the promoter activity but with a potential effect on transcript levels [14].

The contribution of *TARDBP* in ALS pathogenesis is different among cohorts of different ancestry, varying from 0.8% to 12.5%. In particular, *TARDBP* variants account for 1.9% of fALS in Australia [16], 8.2% of fALS and 0.4% of sALS in the Netherlands [17] and 7.0% of fALS and 0.8% of sALS in China [18]. Notably, in Italian patients the frequency of *TARDBP* variants is higher, accounting for 4.8%–12.5% of fALS and 2.2%–9.7% of sALS [19, 20]. The presence of a founder effect for the p.A382T mutation in the Italian island of Sardinia, which has a phylogeny distinct from the rest of Italy [21], was hypothesized and could explain such a discrepancy. In Sardinia, this variant is detected in 25%–30% of fALS and in 9%–19.3% of sALS [22, 23]. Therefore, considering all the Italian ALS cohorts together, the p.A382T appears to be the most prevalent variant, accounting for 44.2% of mutated cases, which are mostly of Sardinian origin. All carriers described to date are of Italian or French origin [3, 24]. However, in our cohort it was not possible to identify a large common haplotype shared by mutated patients, at least by using microsatellite marker analysis.

In our cohort, clinical manifestations varied greatly among patients, as the age of onset ranged from 17 to 76 years and disease duration from 1 to 20 years. Six patients had cognitive and/or behavioural impairment: 1 had overt FTD, 1 had PNFA and 4 patients had ALSbi.

The second most frequent variant was p.G294V, identified in 16.2% of patients. Almost all cases reported in the literature are of Italian or Chinese origin with the exception of two Moroccan patients, one of whom was homozygote for the variant [18, 25, 26]. Although a reduced penetrance has been shown for this variant in pedigrees of carriers [26], functional studies supported its pathological impact [27]. Clinical characteristics of patients carrying the same genetic variants identified in our cohort are summarized in Table S2.

In our patients this variant was associated with later onset, shorter disease duration (Figure 1b) and more frequent bulbar onset with respect to other mutations, confirming previously reported findings in Chinese patients in which the p.G294V variant was the most frequent *TARDBP* mutation [28]. In particular, the high prevalence of bulbar onset appears to be a peculiar feature, being observed in 71% of our series and in 66% of those described in the literature [18, 27, 29]. In one of our patients a diagnosis of ALSbi was made.

The p.G295S and the p.A382T variants have been previously described only in European patients from Italy and France [3, 19, 24, 30–32], who suffered from ALS associated with behavioural abnormalities. Notably, the p.G295S variant was found only in sALS patients in our cohort, suggesting low penetrance. Also in literature, carriers of the variant with pure ALS were all sporadic in families [19, 33] while familiarity for the disease was recorded in patients with FTD [24, 31]. Sardinian patients with the p.G295S variant were mostly sporadic (73%) but a number of fALS cases (27%) [23] were

also observed. Both the p.G294V and p.G295S variants are located in the conserved glycine-rich domain of TDP-43, where the majority of pathogenic variants lie [34].

The p.G376D variant was first described in an Italian fALS patient [30] and then reported only in two additional families, one in Switzerland (but in patients of Italian origin [35]) and one in Japan [36]. Our patient belongs to a large previously described family with ALS [29, 30], where the mutation has high penetrance. Nine additional family members, affected by ALS and carrying the variant, were evaluated in our Centre, confirming that this mutation is associated with a peculiar phenotype characterized by early onset, predominant upper limb impairment and aggressive course (Table 3). With the exception of 1 patient (889:E, who is the father of siblings 889:C and 889:D), who had an older age at onset and a milder disease, mean age at onset was 44.5 years and median duration of 11 (range 5–19) months. All patients had a classic or flail arm phenotype and, most frequently, a spinal onset. In 2 patients (889 and 699) the disease started with a neck muscles impairment (Tables 2 and 3). Cognitive impairment was never detected in patients from this family.

The p.I383V mutation was identified in one of our patients who had spinal onset at 37 years and a short disease duration (31 months). Previously described patients with the p.I383V mutation showed a relatively rapid course of the disease, with a median duration of 29.5 months and even less than 1 year in occasional patients [18].

Mutations in *TARDBP* are an established cause of FTD and are frequently associated with language or semantic disorders [6]. However, *TARDBP* mutations are rarely associated with cognitive dysfunction and more frequently with isolated ALS [18, 34]. Clinical evaluation for cognitive impairment using the ECAS test was performed in 33 patients, demonstrating that 10 patients (30%) had variable degree of frontal dysfunction. Interestingly, cognitive symptoms preceded ALS manifestations in 2 patients. One sporadic patient (with the p.A382T variant) had a diagnosis of PNFA 4 years before developing UMN-D ALS with bulbar onset; another one (carrying the p.S332N variant) presented with FTD and showed signs of UMN-D ALS with bulbar onset 2 years later. Our findings confirm that FTD can be the only or the initial clinical manifestation of *TARDBP* mutations [6, 24, 31, 37]. Furthermore, 1 patient with the p.G294V variant had ALSbi and the uncle of patient 2017, harbouring the p.W385G variant, had psychiatric symptoms, without clinical and electromyography signs of motor neuron disease. One limitation of this study is that neuropsychological evaluation was not performed in all mutated patients. Nevertheless, our findings suggest that the contribution of *TARDBP* to the ALS/FTD spectrum is higher than generally assumed.

Interestingly, 5 patients (2 with the p.N267S, 1 with the p.G295S, 1 with the p.Q303H and 1 with the p.A382T variants, respectively) had familiarity for Parkinson disease. In the literature, the p.N267S variant was already described in patients with a family history of dementia and/or parkinsonism [7] as well as in sporadic patients with pure Parkinson's disease [38]. As suggested by in silico studies, this variant can create a novel phosphorylation site, resulting in reduced protein levels [38]. Furthermore, the p.A382T variant was reported in patients with Parkinson's disease and with atypical parkinsonism in familial and sporadic forms [39]. Of relevance, we report for the first time the association of the p.G295S and p.Q303H variants with familiarity for Parkinson's disease.

We were able to perform familial segregation analysis in 4 sALS patients carrying the c.-12-1G > C, the p.G294V, the p.G295S and the p.M162L variants. All these variants were identified in a parent with

TABLE 3 Clinical characteristics of patients carrying the p.G376D variant, belonging to the same family.

Patient ID	Sex	Age at onset (years)	Disease duration (months)	Outcome	Site of onset	Phenotype	Cognitive impairment
298	M	47	5	Deceased	Bulbar (dysphagia)	Classic	No
699	M	31	13	Deceased	Neck muscles	Classic/flail arm	No
1435	M	57	14	Tracheostomy (alive after 60 m)	Spinal (UL, proximal)	Classic/flail arm	No
1566	F	35	11	Tracheostomy (deceased after 41 m)	Spinal (UL, distal)	Classic	No
1567	F	43	10	Tracheostomy (deceased after 38 m)	Spinal (UL, distal)	Classic	No
1758	F	49	19	Tracheostomy (alive after 26 m)	Spinal (UL, distal)	Flail arm	No
889:C	F	56	1	Alive	Spinal (UL, distal)	Classic	No
889:D	F	47	7	Tracheostomy (alive after 97 m) Locked-in syndrome (after 4 years from IPPV)	Bulbar (dysphagia and dysarthria)	Classic	No
889:E	M	70	48	Deceased	Spinal (UL)	Classic	No

Abbreviations: F, female; IPPV, invasive pressure positive ventilation; m, month; M, male; UL, upper limb.

no neurological dysfunction, leading us to consider that sporadic cases were caused by low penetrance variants and not by de novo mutations in these families.

Our findings showed that clinical manifestations of *TARDBP*-mutated ALS are highly variable concerning the age and site of onset, the relative mix of upper and lower motor neuron signs, the disease duration and the association with cognitive or psychiatric symptoms. Some variants, including p.G294V and p.G376D, were associated with restricted phenotypes. Conversely, the p.A382T variant showed a marked clinical heterogeneity with respect to age of onset, survival and association with cognitive signs, suggesting that additional genetic or environmental factors most likely contribute to disease manifestations. Supporting this hypothesis is the detection of the p.A382T variant in 1.3% of normal controls in Sardinia [39] and the evidence that in our cohort the variant was detected in sporadic as well as in probable and possible fALS but never in definite fALS.

In conclusion, 30% of mutated patients in our cohort had frontal dysfunction, thus showing that cognitive and are not so rare and should be investigated in patients carrying *TARDBP* variants. Mutations identified in our cohort of ALS patients of Italian ancestry showed differential penetrance and, in some cases, can be correlated to specific clinical signs. All our observations could help clinicians in determining the prognosis of the disease, providing efficient genetic counselling and better addressing research strategies.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

ETHICS STATEMENT

This study involved human participants and was approved by the Ethics Committee of Università Cattolica del Sacro Cuore (prot. A.133/C.E./2013). All involved patients signed a written informed consent before blood sampling.

FINANCIAL DISCLOSURES

None.

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SUPPORTING INFORMATION

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