ORIGINAL CONTRIBUTION

ONLINE FIRST

Large Proportion of Amyotrophic Lateral Sclerosis Cases in Sardinia Due to a Single Founder Mutation of the *TARDBP* Gene

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Objective: To perform an extensive screening for mutations of amyotrophic lateral sclerosis (ALS)–related genes in a consecutive cohort of Sardinian patients, a genetic isolate phylogenically distinct from other European populations.

Design: Population-based, prospective cohort study.

Patients: A total of 135 Sardinian patients with ALS and 156 healthy control subjects of Sardinian origin who were age- and sex-matched to patients.

Intervention: Patients underwent mutational analysis for *SOD1*, *FUS*, and *TARDBP*.

Results: Mutational screening of the entire cohort found that 39 patients (28.7%) carried the c.1144G>A (p.A382T) missense mutation of the *TARDBP* gene. Of these, 15 had familial ALS (belonging to 10 distinct pedigrees) and 24 had apparently sporadic ALS. None of the 156 age-, sex-, and ethnicity-matched controls carried the pathogenic variant. Genotype data obtained for 5 ALS cases carrying the p.A382T mutation found that they shared a 94–single-nucleotide polymorphism risk haplotype that spanned 663 Kb across the *TARDBP* locus on chromosome 1p36.22. Three patients with ALS who carry the p.A382T mutation developed extrapyramidal symptoms several years after their initial presentation with motor weakness.

Conclusions: The *TARDBP* p.A382T missense mutation accounts for approximately one-third of all ALS cases in this island population. These patients share a large risk haplotype across the *TARDBP* locus, indicating that they have a common ancestor.

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THE IDENTIFICATION OF THE transactivation response DNA-binding protein (TDP-43) as the major constituent of ubiquitin inclusions in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) was a major advance in our understanding of the pathogenesis of these neurodegenerative diseases.[1] Subsequently, it was discovered that mutations in the *TARDBP* gene located on chromosome 1p36 (which encodes the TDP-43 protein) explain approximately 4% of familial ALS cases[2] and a smaller proportion of FTD cases.[3]

Previous studies have shown that genetic studies of population isolates represent a powerful method of finding genes that are causative of ALS.[4] Sardinia, with a population of 1.7 million, is the second largest Mediterranean island, located 120 miles west of the main Italian coastline. Despite numerous invasions over the millennia, genetic, linguistic, and archaeological studies indicate that Sardinians have a phylogeny distinct from other Europeans, including mainland Italians.[4-7] Indeed, Y-chromosome analysis suggests a largely pre-Neolithic settlement of Sardinia with little subsequent gene flow from outside populations despite Phoenician-Carthaginian (535-238 before Christ) and Roman (238 before Christ to 476 anno Domini) dominations of the island.[8] The island population has a uniquely high incidence of several autoimmune diseases such as type 1 diabetes[9] and multiple sclerosis[10] and of monogenic diseases such as Wilson disease.[11]

There are no population-based prospective studies on the incidence of ALS in Sardinia. A recent epidemiological study

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Table 1. Demographic and Clinical Characteristics of the Patients Carrying the *TARDBP* p.A382T Missense Mutation and of the Nonmutated Cases

Characteristic	Patients, No./Total (%) With p.A382T (n=39)	No p.A382T (n=96)	*P* Value
Sex			.29
Male	28	60	
Female	11	36	
Age at onset, mean (SD), y	57.9 (12.2)	59.9 (10.1)	.34
Type of onset	6/33	21/75	.38
Bulbar	6	21	
Spinal	33	75	
FALS	15 (38.5)	17 (17.7)[a]	.01
FTD	12 (30.8)	9 (9.4)	<.001

Abbreviations: FALS, familial amyotrophic lateral sclerosis; FTD, frontotemporal dementia.
[a] Two patients carry the p.A95G *SOD1* missense mutation.

found the frequency of the condition on the island to be 1.88 per 100 000 inhabitants,[12] which is less than that reported elsewhere in Europe.[13,14] However, the catchment area of that study was limited to a single province of Northern Sardinia (representing 19% of the island population), and there are reports from the lay press suggesting that the incidence of this fatal neurodegenerative disease is higher than expected in certain parts of Sardinia.

In this article, we report the results of our mutational screening of the *SOD1*, *TARDBP*, and *FUS* genes in a cohort of 135 Sardinian patients with ALS.

METHODS

SUBJECTS

DNA samples were obtained from a consecutive series of patients diagnosed with definite, probable, probable laboratory-supported, or possible ALS[15] who attended the neurology departments at the University of Cagliari, the capital city of Sardinia situated in the south of the island; the University of Sassari in the north of the island; and the Hospital of Nuoro located in the center of the island. All patients were of Sardinian origin by self-report and were enrolled during a 12-month period between April 2009 and March 2010. Patients underwent neuropsychological testing for the diagnosis of frontotemporal cognitive and behavioral syndromes in ALS according to the consensus criteria.[16] The diagnosis of Parkinson disease was based on the United Kingdom Parkinson disease society brain bank diagnostic criteria.[17] Controls consisted of 156 healthy individuals of Sardinian origin, matched to patients by age and sex. Demographics and clinical features of the cases and controls are shown in eTable 1 (http://www.archneurology.com). The ethical committees of all involved institutions approved the study, and all patients and controls gave written informed consent.

MUTATIONAL SCREENING

All patients underwent mutational analysis for *SOD1*, *FUS*, and *TARDBP*. Specifically, all of the coding exons and 50 base pairs of the flanking intron-exon boundaries of *SOD1*, *FUS*, and *TARDBP* were amplified using polymerase chain reaction (primer sequences available on request), sequenced using the BigDye Terminator v3.1 sequencing kit (Applied Biosystems Inc, Foster City, California), and run on an ABI PRISM 3100-Avant genetic analyzer (Applied Biosystems).

GENOTYPING

Genotyping was performed on a representative sample of familial and sporadic ALS cases carrying the p.A382T mutation using Infinium Human660W SNP chip arrays (Illumina, Inc, San Diego, California), which assay 561 490 single-nucleotide polymorphisms across the genome. These data were used to determine the degree of relatedness of samples (quantified as the pi-hat metric) by applying the identity-by-descent algorithm (–*genome*) within the PLINK toolset.[18]

RESULTS

A total of 135 patients with ALS were consecutively enrolled at the 3 main ALS clinics serving the Sardinian population. These cases were collected during a 1-year time period, and our cohort likely represents almost complete case ascertainment of prevalent cases in the Sardinian population for that time period. Almost a quarter of the cases (n=31; 23.0%) reported a familial history of ALS with autosomal dominant inheritance.

Mutational screening of the entire cohort found that 39 patients (28.7%) carried the known c.1144G>A (p.A382T) missense mutation of the *TARDBP* gene. Of these, 15 had familial ALS (belonging to 10 distinct pedigrees) and 24 had apparently sporadic ALS. Patients who carried the *TARDBP* p.A382T missense mutation originated from all parts of the island. One apparently sporadic case was homozygous for the p.A382T missense mutation. None of the 156 age-, sex-, and ethnicity-matched healthy controls carried the p.A382T mutation.

Of the remaining 96 patients (71.3%) who did not carry the p.A382T mutation, 2 brothers carried a novel c.287C>G (p.A95G) missense mutation of the *SOD1* gene. Based on these 2 cases, the frequency of *SOD1* mutations was approximately 8% of individuals with familial ALS and 1.4% of the whole Sardinian population with ALS. No other mutations of the *SOD1*, *TARDBP*, or *FUS* genes were identified.

Genotype data obtained for 5 ALS cases carrying the p.A382T mutation (3 familial cases from 2 ostensibly unrelated families and 2 sporadic cases) found that they shared a 94–single-nucleotide polymorphism risk haplotype spanning 663 Kb across the *TARDBP* locus on chromosome 1p36.22 (see eTable 2 for risk haplotype). Identity-by-descent analysis based on the same genome-wide genotyping data confirmed that the sporadic cases and the familial cases were cryptically related to each other (mean [SD] pi-hat, 0.21 [0.005], indicating that the apparently sporadic cases carrying the p.A382T variant were roughly second- to third-degree relatives of familial cases carrying the same mutation).

The demographics and clinical characteristics of the patients with and without the p.A382T *TARDBP* mutation are shown in **Table 1**. Frontal lobe dysfunction, flail arm variant of ALS, and upper motor neuron–predominant ALS were more common among Sardinian patients carrying the TARDBP p.A382T mutation than among noncarriers (**Table 2**). Three patients with ALS who carried the p.A382T mutation developed extrapyramidal symptoms several years after their initial presentation with motor weakness. The first patient was a 57-year-old man who presented with proximal leg weakness and subsequently developed a rigid hypokinetic parkinsonian syndrome and frontotemporal dementia. He died 6 years after symptom onset. His older brother, who also carried the p.A382T mutation, developed upper motor neuron–predominant ALS at 70 years of age and was alive 6 years after the onset of ALS without evidence of parkinsonism or FTD. The second patient was a 37-year-old man who initially presented with leg weakness but then progressed to manifest parkinsonism and external ophthalmoplegia 5 years later. He remains alive 12 years after symptom onset. Finally, we identified a 48-year-old man who also presented with upper motor neuron–predominant ALS that was followed 3 years later by the onset of rigid hyperkinetic parkinsonism. Neuropsychological testing confirmed frontal lobe dysfunction consistent with FTD. Of note, the patient had a history of a vocal and motor tics disorder since childhood that had been treated with haloperidol for less than 2 years when aged 18 years. This patient carries a homozygous A382T missense mutation and was alive 4 years after the onset of ALS.

Table 2. Clinical Phenotypes of Patients Carrying the *TARDBP* p.A382T Missense Mutation and of Nonmutated Cases

	Patients, No. (%)		
Phenotype	With p.A382T (n=39)	No p.A382T (n=96)	*P* Value
Classic	15 (38.5)	59 (61.4)	.01
Pyramidal	8 (20.5)	8 (8.3)	.05
Flail leg	2 (5.1)	6 (6.3)	.98
Flail arm	8 (20.5)	2 (2.1)	<.001
Bulbar	6 (15.3)	21 (21.9)	.38

COMMENT

Our mutational screening study of Sardinian patients with ALS found that a single missense mutation in *TARDBP* accounts for approximately one-third of all ALS cases in this island population. Furthermore, these ALS patients shared a large risk haplotype across the *TARDBP* locus, indicating that they had a common ancestor and were part of a larger kindred. This founder effect is comparable with that recently described for the chromosome 9p21 ALS-FTD locus in the Finnish population[4] and highlights the power of performing genetic studies in population isolates with a higher incidence of a disease. In this regard, it is noteworthy that nearly a quarter of the Sardinian ALS cases in our consecutive series of prevalent patients had a known family history of ALS, which is considerably higher than the approximately 5% reported by population-based epidemiological studies in other European populations.[13,14]

The *TARDBP* p.A382T missense mutation has been previously described in 2 French patients with ALS and 11 patients living on mainland Italy.[19-21] Microsatellite analysis suggested that at least a portion of these French and Italian patients carried the same 2.4-Mb haplotype on chromosome 1.[21] The high marker density of the Illumina HumanHap660W genotyping array used in our study allowed this haplotype to be narrowed to a 663-Kb region containing the *TARDBP* gene locus. DNA was not available to test if the mainland Italian or French samples carried the same haplotype, but the geographical closeness of these cases makes it likely that all of them arose from a single mutational event.

Nearly two-thirds of the patients (n=24) carrying the p.A382T mutation were classified as having sporadic ALS. Although it is possible that some of these cases arose from multiple mutational events affecting the same nucleotide, our data strongly support the notion that these cases are part of the same extended pedigree: genome-wide analysis demonstrated that both the familial and sporadic samples shared an average of 21% of their genome with each other, indicating that they had a common ancestor as recently as 3 generations ago. Cryptic relatedness, in which 2 individuals are related to each other without their knowledge, is a well-described issue in ALS and may arise from poor diagnosis in past generations, incomplete knowledge of family history, and even varying manifestations of motor and cognitive dysfunction among mutation carriers within the same family.[22] The relative late age of symptom onset in patients carrying the p.A382T mutation also indicates that this pathogenic variant is only fully penetrant by the eighth decade of life and that mutation carriers in previous generations may have died of other diseases prior to developing motor neuron degeneration. An alternative possibility is that the p.A382T mutation represents a risk factor for ALS in these sporadic cases rather than being the direct cause of disease. Although this possibility cannot be fully excluded, the lack of this known pathogenic mutation in neurologically normal Sardinian control samples (or in other populations), the shared haplotype, and relatedness among the cases carrying the mutation would not support this hypothesis.

Sardinian patients carrying the p.A382T mutation had a heterogeneous spectrum of ALS phenotypes, a pattern that has been observed for mutations in other ALS genes.[23] Despite these observations, the flail arm variant of ALS occurred with greater than expected frequency among mutation carriers. This clinical variant has also been previously described in patients with *TARDBP* mutations,[24] and all 7 French patients with the p.A382T mutation manifested a lower motor neuron predominant form of the disease with onset in the upper limbs.[20]

We observed extrapyramidal symptoms in multiple Sardinian patients with ALS carrying the *TARDBP* p.A382T mutation, representing the first time that parkinsonism has been described in individuals with mutations in this gene.[25] Some caution is required in interpreting these findings. First, one of the patients with extrapyramidal symptoms also manifested external ophthalmoplegia, a finding more commonly associated with mitochondrial disorders. Though supranuclear palsy has already been described in a patient with a different *TARDBP* mutation,[26] the mitochondrial genome of our case was not sequenced, so the existence of a second mitochondrial disease-causing variant in addition to his known pathogenic p.A382T mutation remains possible. Second, it is possible that the neuroleptic medications taken by the third patient for treatment of childhood-onset tic disorder contributed to his extrapyramidal symptoms, though it is noteworthy that these medications were discontinued more than 30 years prior to the onset of parkinsonism. Third, parkinsonism is a well-recognized feature of ALS,[27,28] suggesting that the finding of 3 cases in a cohort of this size may have been a chance occurrence. In addition, the diagnosis of extrapyramidal signs in patients with ALS is often complicated by the presence of upper and lower motor neuron signs inherent to the disease. Finally, autopsy material was not available from any of the patients with extrapyramidal symptoms, so histopathological confirmation of substantia nigra involvement was not possible.

Despite this, we believe that our observations, taken together with the previous report of chorea in a patient with a *TARDBP* mutation,[26] expand the clinical spectrum associated with mutations in this gene to include basal ganglia dysfunction. Given that pathological deposition of TDP-43 in the central nervous system has been described in a variety of neurological disorders,[29] it is perhaps not surprising that *TARDBP* mutations can be associated with a wide range of neurodegeneration processes with corresponding clinical manifestations. The basis of this pathological and phenotypic heterogeneity is not clear, though genetic or environmental factors may influence the precise clinical manifestations of *TARDBP* mutation carriers. These genotype-phenotype observations suggest that screening of Sardinian patients with diverse neurological diseases for the *TARDBP* p.A382T mutation may reveal additional unrecognized clinical consequences of this pathogenic variant.

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