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A genetic association study of two genes linked to neurodegeneration in a Sardinian multiple sclerosis population: The *TARDBP* Ala382Thr mutation and *C9orf72* expansion

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ABSTRACT

Multiple sclerosis (MS) is a chronic disease of the central nervous system characterized by inflammation and accompanied and followed by neurodegeneration. Missense mutations of the TAR DNA Binding Protein gene (TARDBP) located in the chromosome 1p36.22 region, and the hexanucleotide repeat expansions in chromosome 9 open reading frame 72 (C9orf72) are pathogenic in other neurodegenerative diseases such as amyotrophic lateral sclerosis and frontotemporal lobar degeneration. Assuming that TARDBP Ala382Thr mutation and C9orf72 expansion may underlie MS, we evaluated their frequency in a large cohort of MS patients and controls from Sardinia, an island characterized by a very high frequency of MS and an unusual genetic background.
Genomic DNA was extracted from peripheral blood and analyzed for the presence of a TARDBP Ala382Thr mutation and C9orf72 expansion. Difference in the frequency of these mutations between MS patients and controls was calculated using the χ^2 test with a standard 2×2 table. The Ala382Thr mutation in its heterozygous state was found in 27/1833 patients (1.4%) and 20/1475 controls (1.3%), whereas C9orf72 pathogenic repeat expansion was found in 6/1014 MS patients (0.6%) and 2/333 controls (0.6%). Individuals carrying the mutations did not present with other neurodegenerative conditions and any differences were reported between groups.
TARDBP Ala382Thr mutation and C9orf72 expansion do not play a major role in MS pathogenesis in the Sardinian population. Further analyses on larger samples of MS patients from other populations are needed to better define the possible role of these genes in the complex interplay between neuroinflammation and neurodegeneration in MS.

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system characterized by inflammatory demyelination and degenerative processes, resulting in axonal and neuronal damage [1,2]. The neurodegenerative components of MS have been shown to occur very early in the course of the disease in concomitance to inflammatory demyelination, and are only partially related to it [3]. Its etiology is thought to be multifactorial, arising from genetic and environmental interplay, although the mechanisms underlying these associations remain unclear [4,5].

It is noteworthy that the Sardinian population is genetically homogeneous and presents a distribution of alleles at various loci that are different to those seen in narrowing populations [6]. The genetic component of the disease is conferred by a rather large number of small genetic variants, with the main genetic determinant located at the human leukocyte antigen class II DRB1 and DQB1 loci that are implicated in immune response modulation [7]. However, to date, no data exist on the role that the specific genetic aberrations play to induce molecular mechanisms at the basis of neurodegeneration in MS. Instead, a greater number of papers concerning genetic abnormalities that seem to be related to other neurodegenerative diseases are published [8–10].

Some missense mutations of the TAR DNA Binding Protein gene (TARDBP) located in the chromosome 1p36.22 region have been observed in degenerative diseases characterized by TDP-43 immunoreactivity (the protein encoded by TARDBP), which identifies accumulating inclusions in neuronal and oligodendroglial cells. This protein is an ubiquitous and heterogeneous nuclear ribonucleoprotein involved in

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the prevention of the neurodegeneration by gene expression regulation, including RNA splicing and transport [11].

In the Sardinian population, the c.1144G>A (p.A382T) missense mutation of TARDBP was recently reported in approximately 30% of familial and sporadic patients with amyotrophic lateral sclerosis (ALS) [12,13]. In addition, the mutation was found in some individuals affected by frontotemporal dementia, Parkinson's disease, and atypical parkinsonism, leading to the question of whether TARDBP has a role in a widespread range of neurodegenerative diseases [14–16]. It is noteworthy that TDP-43 inclusions have been observed in neurons of two MS patients that presented with comorbidity with ALS and semantic dementia [17,18]. However, to date, no data exist on the role of the TARDBP gene in MS.

Recently, the hexanucleotide repeat expansions in chromosome 9 open reading frame 72 (C9orf72) were identified and are now considered to be the most common genetic abnormality in familial and sporadic ALS and frontotemporal lobar degeneration (FTLD) [19,20].

C9orf72 is a gene encoding for two protein isoforms (C9ORF72a and b). Its function in cellular processes involves the regulation of membrane trafficking and gene expression [21]. The expansion of C9orf72 gene induces the formation of repeat RNA aggregates that are shown to sequester proteins involved in RNA splicing, editing, nuclear export and nucleolar function [22]. The mechanism by which the expanded GGGGCC repeat could induce neurodegeneration is considered to be mediated by three mechanisms independent but not mutually exclusive: the mt C9orf72 toxicity haploinsufficiency, the generation of toxic RNA foci and peptide accumulation in neurons [23,24].

C9orf72-related diseases are characterized by extremely variable clinical phenotypes, including motor and non-motor phenotypes such as dementia and psychosis [25]. In a population of North England where more than 1% of ALS patients are affected by MS, the C9orf72 expansion was identified in 80% of MS-ALS cases and these patients had a more rapidly progressive disease than pure C9orf72-ALS. The hypothesis is that MS-associated neuroinflammation may affect penetrance and progression of the C9orf72 expansion [26].

Considering the broad clinical manifestations related to both C9orf72 mutations and the TARDBP gene, all involving mechanisms of neurodegeneration, we performed a case–control association study of the C9orf72 repeats and of the p.Ala382Thr variant in a large cohort of MS patients and healthy controls (HCs) from Sardinia.

2. Methods

2.1. Patients and controls

MS outpatients, according to the McDonald 2010 criteria [27], and HCs, consisting of family members of MS patients, were consecutively recruited from the Multiple Sclerosis Center of the University of Cagliari. A smaller group of unrelated healthy controls (uHCs) was also recruited. A categorization of MS in familial (another first-degree relative affected) and sporadic (no other first-degree relative affected by MS) cases was also made. Age, sex, and the complete medical, family, and personal history were collected for all subjects.

Blood samples were collected from all subjects, after obtaining informed written consent. All the samples were analyzed for the presence of the TARDBP Ala382Thr mutation and, a part of them, after 2011 was analyzed for C9orf72 expansion. All subjects were neurologically examined at the time of sampling, while at the end of the study only subjects with mutations were re-examined. MS patients with C9orf72 expansion underwent an extensive neuropsychological examination using Rao's battery test [28], with normative values and correction for age, sex, and education available for the Italian population [29]. The Ethics Committee of the University of Cagliari approved the study protocol.

2.2. TDP-43 mutation and C9orf72 expansion analysis

Genomic DNA was extracted from peripheral blood using standard methods (30 MRITA). A 438-bp fragment encompassing exon 6 of TARDBP (NM_007375.3) was PCR amplified, sequenced on an ABI3130xl instrument using BigDye terminator v3.1 sequencing chemistry, and analyzed for the presence of c.1144G>A (p.A382T) with Applied Biosystems Sequence Scanner v1.0 Software (Applied Biosystems Inc., Carlsbad, CA, USA).

C9orf72 genotyping was carried out by Repeat Primed PCR (Anchor PCR) as it was previously described by Renton AE [20]. In addition, we genotyped also the samples by the DeJesus-Hernandez PCR-based method to confirm the number of detected expansions [19].

PCR products were electrophoresed on an ABI 3500xl capillary analyzer and allele scoring was performed using GeneMapper v4.0 software (Applied Biosystems Inc.).

The samples were categorized into those that carry only wild-type alleles (<20 repeats), intermediate-repeat alleles (20–30 repeats), and a pathogenic repeat expansion (>30 repeats).

Difference in TARDBP Ala382Thr mutation and C9orf72 expansion frequency between MS patients and HCs was calculated using the χ^2 test with a standard 2×2 table. $P < 0.05$ was established as a statistically significant differential value.

3. Results

3.1. TDP-43p.Ala382Thr mutation

TDP43 analysis was performed on 1833 MS patients and 1475 HCs. The mean age of the whole sample at the time of sampling was 46.9 years (SD ± 14.9); 41.1 years (SD ± 11.8) for MS, and 54.1 years (SD ± 15.2) for HCs. The MS group comprised 1397 sporadic and 436 familial cases. The follow-up period after sampling was 6.9 years (SD ± 4.3) for MS and 8.4 years (SD ± 5.3) for HCs. The TARDBP p.Ala382Thr mutation in the heterozygous state was detected in 27 of 1833 (1.4%) MS patients (all in sporadic group) and 20 of 1475 (1.3%) HCs. No difference in its frequency between MS patients and HCs was observed ($P = 0.8$) (Table 1).

Distribution of the Ala382Thr mutation is summarized in Table 2, which subdivides the subjects (MS patients and HCs) with respect to the cut-off age of 50 years at the time of sampling.

The TARDBP p.Ala382Thr mutation in heterozygous (G/A) was reported in 21 and 5 subjects aged <50 years of MS and HCs group, respectively. In subjects aged >50 years, it was reported in 6 and 15 subjects of MS and HCs group, respectively.

A smaller group of 64 uHCs was also included (35 female (54%); mean age in years 80.6 ± 8.3; no TARDBP Ala382Thr mutation reported) showing no difference in the frequency of this mutation with respect to HCs (p 0.3).

No evidence of signs or symptoms of neurodegenerative diseases such as ALS, FTLD, or Parkinson's disease was present at the sampling at the time of genotyping or later during the follow-up in both MS patients with mutations and HCs.

Table 1
Demographic feature of the overall subject TDP-43 mutation analyzed in this study.

TDP-43 sample	HCs	uHCs	MS	
3308	1475	64	1833	
Mean age, years	54.1 ± 15.2	80.6 ± 8.3	41.1 ± 11.8	
			Sporadic MS (1397)	*Familial MS* (436)
			40.8 ± 11.2	42.3 ± 13.8
TARDBPAla382Thr mutation	20 (1.3%)	0	27 (1.4%)	

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