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ATNX2 is not a regulatory gene in Italian amyotrophic lateral sclerosis patients with *C9ORF72* GGGGCC expansion

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ABSTRACT

There are indications that both familial amyotrophic lateral sclerosis (ALS) and sporadic ALS phenotype and prognosis are partly regulated by genetic and environmental factors, supporting the theory that ALS is a multifactorial disease. The aim of this article was to assess the role of *ATXN2* intermediate length repeats in a large series of Italian and Sardinian ALS patients and controls carrying a pathogenetic *C9ORF72* GGGGCC hexanucleotide repeat. A total of 1972 ALS cases were identified through the database of the Italian ALS Genetic consortium, a collaborative effort including 18 ALS centers throughout Italy. The study population included: (1) 276 Italian and 57 Sardinian ALS cases who carried the *C9ORF72* expansion; (2) 1340 Italian and 299 Sardinian ALS cases not carrying the *C9ORF72* expansion. A total of healthy 1043 controls were also assessed. Most Italian and Sardinian cases and controls were homozygous for 22/22 or 23/23 repeats or heterozygous for 22/23 repeats of the *ATXN2* gene. *ATXN2* intermediate length repeats alleles ($\geq$28) were detected in 3 (0.6%) Italian ALS cases carrying the *C9ORF72* expansion, in none of the Sardinian ALS cases carrying the expansion, in 60 (4.3%) Italian cases not carrying the expansion, and in 6 (2.0%) Sardinian ALS cases without *C9ORF72* expansion. Intermediate length repeat alleles were found in 12 (1.5%) Italian controls and 1 (0.84%) Sardinian controls. Therefore, ALS patients with *C9ORF72* expansion showed a lower frequency of *ATXN2* polyQ intermediate length repeats than both controls (Italian cases, $p = 0.137$; Sardinian cases, $p = 0.0001$) and ALS patients without *C9ORF72* expansion (Italian cases, $p = 0.005$; Sardinian cases, $p = 0.178$). In our large study on Italian and Sardinian ALS patients with *C9ORF72* GGGGCC hexanucleotide repeat expansion, compared to age-, gender- and ethnic-matched controls, *ATXN2* polyQ intermediate length does not represent a modifier of ALS risk, differently from non-*C9ORF72* mutated patients.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive degenerative disorder of the central nervous system, almost invariably fatal, characterized by a loss of cortical, bulbar, and spinal motor neurons. In 10%–15% of cases it is genetically transmitted (familial ALS, fALS), while in the remaining cases it appears sporadically in the population (sporadic ALS, sALS) (Renton et al., 2014). More than 20 major genes have been related to ALS, the most common in the Caucasian population being *C9ORF72, SOD1, TARDBP*, and *FUS* (Renton et al., 2014). However, there are now indications that both fALS and sALS phenotype and prognosis are partly regulated by genetic and environmental factors, supporting the theory that ALS is a multifactorial and oligogenic disease (Al-Chalabi et al., 2014; van Blitterswijk et al., 2012).

ATNX2 intermediate length repeats have been identified as a risk factor for ALS (Neuenschwander et al., 2014) and their presence are additionally associated with reduced survival in ALS patients (Chiò et al., 2015). More recently, it has been reported that *ATXN2* is also a risk factor for ALS patients carrying the GGGGCC hexanucleotide repeat in the first intron of the *C9ORF72* gene (van Blitterswijk et al., 2014a). This gene accounts for 40% of fALS and 7% sALS in European and American series (Majounie et al., 2012). Phenotypes associated with this repeat expansion include ALS and/or frontotemporal dementia (FTD), psychotic symptoms (hallucinations and delusions), and extrapyramidal signs. The wide and heterogeneous symptomatology related to *C9ORF72* has yet not been fully explained (Rohrer et al., 2015).

The aim of this article was to assess the role of *ATXN2* intermediate length repeats in a large series of Italian and Sardinian ALS patients and controls carrying a pathogenetic *C9ORF72* GGGGCC hexanucleotide repeat.

2. Methods

2.1. Patients

A total of 1972 ALS cases were identified through the database of the Italian ALS Genetic consortium, a collaborative effort including 18 ALS centers throughout Italy. The study population included: (1) 276 Italian and 57 Sardinian ALS cases who carried the *C9ORF72* expansion and (2) 1340 Italian and 299 Sardinian ALS not carrying the *C9ORF72* expansion.

2.2. Controls

The 1043 controls were included in the analysis. This included: (1) 686 regionally-matched, unrelated Italian subjects, reported in previous articles (Conforti et al., 2012; Corrado et al., 2011). These individuals were predominantly blood donors; (2) 243 regionally-matched, unrelated Sardinian subjects; and (3) 114 matched subjects identified through the patients' general practitioners (population-based controls) (Chiò et al., 2015).

2.3. Genetic analysis

Genomic DNA was isolated from peripheral blood lymphocytes using a standard protocol. The *ATXN2* CAG repeat in exon 1 (NM_002973.3) was amplified using a fluorescent primer and sized by capillary electrophoresis on an ABI 3130 genetic analyzer (Applied Biosystem, Foster City, CA) (Cancel et al., 1997). As reported in recent guidelines for molecular genetic testing of spinocerebellar ataxias, capillary electrophoresis is the preferred method to size alleles as it allows resolution of alleles that are one triplet apart (Sequeiros et al., 2010). As a quality control, 20 samples have been genotyped in the 6 laboratories that performed the molecular genetic testing for the present study. The results showed a consistent allele assignment for all the samples.

To compare our findings to those of van Blitterswijk et al. (2014a), we used a threshold of 28 repeats (or greater) as the definition of intermediate size repeats. However, data using a threshold of 27 repeats (the most common used cut off for *ATXN2* intermediate length repeats in the literature) are reported as Supplementary Table.

All ALS cases were also tested for *SOD1* (all exons), *TARDBP* (exon 6), *FUS* (exons 14 and 15), and *C9ORF72* using the methodology described elsewhere (Chiò et al., 2012a).