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ATXN1 intermediate-length polyglutamine expansions are associated with amyotrophic lateral sclerosis

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ABSTRACT

To clarify the possible involvement of intermediate *ATXN1* alleles as risk factors for amyotrophic lateral sclerosis (ALS), we tested *ATXN1* in a cohort of 1146 Italian ALS patients, previously screened for variants in other ALS genes, and in 529 controls. We detected *ATXN1* alleles with ≥33 polyglutamine repeats in 105 of 1146 patients (9.16%) and 29 of 529 controls (5.48%) ($p = 0.003$). The frequency of *ATXN1* alleles with ≥33 polyglutamine repeats was particularly high in the group of ALS patients carrying the *C9orf72* expansion (12/59, 20.3%). We confirmed this result in an independent cohort of *C9orf72* Italian patients (10/80 cases, 12.5%), thus finding a cumulative frequency of *ATXN1* expansion of 15.82% in *C9orf72* carriers ($p = 2.40E-05$). Our results strongly support the hypothesis that *ATXN1* could act as a disease risk gene in ALS, mostly in *C9orf72* expansion carriers. Further studies are needed to confirm our results and to define the mechanism by which *ATXN1* might contribute to neuronal degeneration leading to ALS.

1. Introduction

The genetic architecture of amyotrophic lateral sclerosis (ALS) is complex as the disease is associated to a multitude of causative genes. A limited number of genes, including *C9orf72*, *SOD1*, *TARDBP*, *FUS*, and *TBK1*, are responsible for a significant percentage of both familial and sporadic ALS cases. On the other hand, several genes are detected in a small number of cases or even in isolated ALS families (Sabatelli et al., 2016).

Furthermore, there is evidence that some variants may have small effect size and can act as predisposing factors or modifiers of the disease phenotype (Lattante et al., 2015; Renton et al., 2014; Sproviero et al., 2017; van Blitterswijk et al., 2014). An established risk factor for ALS is *ATXN2*, which normally contains a tract of 22 or 23 CAG repeats, encoding for a polyglutamine (polyQ) stretch. Intermediate-length (29–33 CAG) repeats are significantly associated with increased risk for ALS, while expansions greater than 34 cause spinocerebellar ataxia type 2 (SCA2) (Elden et al., 2010).

SCA1 is a late-onset fatal progressive neurodegenerative disease caused by the expansion of a polyQ tract within the *ATXN1* gene. Normal alleles contain from 6 to 42 CAG repeats, whereas in SCA1 patients, disease alleles range from 39 to 82 units (Orr et al., 1993). *ATXN1* has been analyzed in ALS patients in only 2 studies, with conflicting results (Conforti et al., 2012; Lee et al., 2011).

To elucidate the role of *ATXN1* in ALS in the present study, *ATXN1* polyQ expansion was investigated in a cohort of 1146 Italian ALS patients, including 106 patients with variants in well-established ALS-related genes, as well as in a cohort of 529 healthy controls to compare results.

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2. Materials and methods

2.1. Patients

A total of 1146 DNA samples, extracted from the blood of consecutive ALS patients, were collected at ALS Center of the NEMO Clinical Center-Gemelli Hospital in Rome. All the patients and control individuals signed a written informed consent, and the study was approved by the local Ethical Committee. All patients were diagnosed as having definite or probable ALS according to the El Escorial criteria. Almost all our patients were from the center or the south of Italy. The cohort included 112 index patients with familial ALS (9.7%) and 1034 sporadic ALS (90.3%), and it consisted of 655 males and 491 females, with a mean age at the onset of 61.5 years. A group of 529 geographically and age-matched unrelated Italian individuals without history of neurodegenerative disease were used as controls.

An independent cohort of 80 ALS patients carrying the *C9orf72* expansion was collected at San Raffaele Scientific Institute and NEMO Clinical Center in Milan and was used to further confirm preliminary results.

2.2. PolyQ repeat size determination

The polyQ repeat size in *ATXN1* gene (OMIM: 601556) was determined using a fluorescent polymerase chain reaction and performing a capillary electrophoresis on an ABI3130 sequencer, as previously described (Conforti et al., 2012). Data were analyzed using GeneMapper 4.0 software (Applied Biosystems). Control subjects with different repeat sizes of homozygous alleles were checked by direct sequencing and used as calibrators.

All patients were previously screened for variants in *SOD1* (OMIM: 147450), *TARDBP* (OMIM: 605078), and *FUS* (OMIM: 137070) genes and for expansions in *C9orf72* (OMIM: 614260), as previously described (Lattante et al., 2012).

2.3. Statistical analysis

The χ^2 and Fisher exact tests were used to evaluate genetic association between polyQ repeats in *ATXN1* gene and different groups of ALS patients. All *p*-values have been computed using the R software and adjusted using Benjamini-Hochberg method (R Core Team, 2017). A *p*-value below 0.05 was considered significant.

3. Results

ATXN1 trinucleotide CAG repeats were analyzed in 1146 ALS patients and in 529 neurologically normal controls from Italy (Fig. 1). Complete results of statistical analysis conducted applying χ^2 and Fisher exact tests with Benjamini-Hochberg correction for multiple comparisons were reported in Table 1, whereas *p*-values

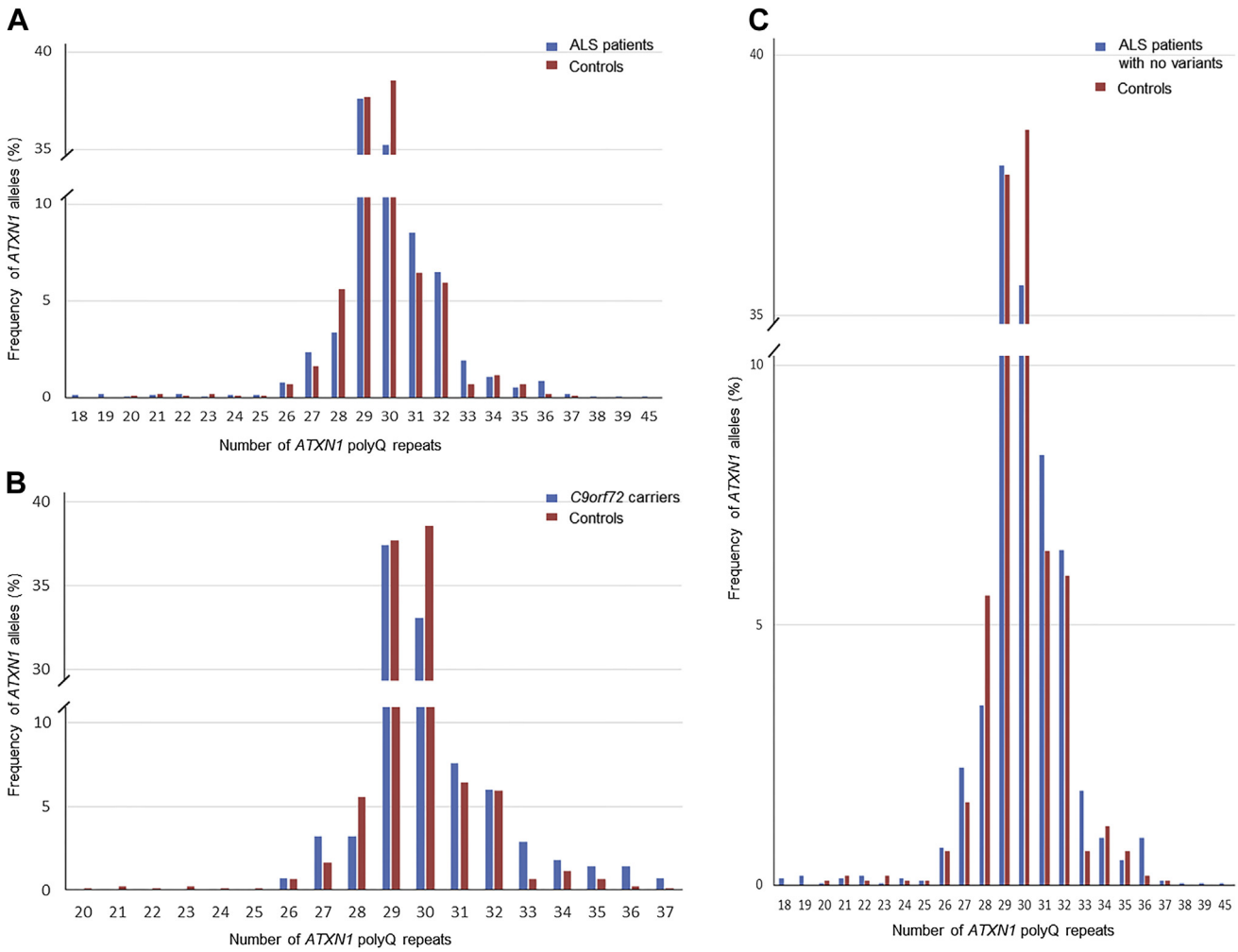

Fig. 1. Distribution of *ATXN1* polyQ repeats. Frequencies of *ATXN1* polyQ repeats are reported comparing results of ALS patients and controls (A), *C9orf72* carriers and controls (B) and ALS patients with no variants and controls (C). Abbreviations: ALS, amyotrophic lateral sclerosis; polyQ, polyglutamine.