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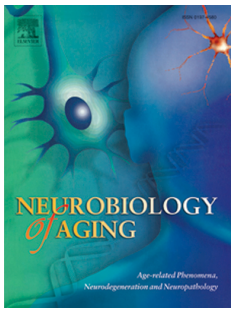

ATXN1 intermediate-length polyQ expansions are associated with Amyotrophic Lateral Sclerosis

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PII: S0197-4580(17)30382-2

DOI: 10.1016/j.neurobiolaging.2017.11.011

Reference: NBA 10090

To appear in: *Neurobiology of Aging*

Received Date: 13 July 2017

Revised Date: 30 October 2017

Accepted Date: 19 November 2017

Please cite this article as: Lattante, S., Pomponi, M.G., Conte, A., Marangi, G., Bisogni, G., Patanella, A.K., Meleo, E., Lunetta, C., Riva, N., Mosca, L., Carrera, P., Bee, M., Zollino, M., Sabatelli, M., *ATXN1* intermediate-length polyQ expansions are associated with Amyotrophic Lateral Sclerosis, *Neurobiology of Aging* (2017), doi: 10.1016/j.neurobiolaging.2017.11.011.

ATXN1 intermediate-length polyQ expansions are associated with Amyotrophic Lateral Sclerosis

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Title character count: 92- Number of references: 19- Number of tables: 2- Number of figures: 1

Word count abstract: 153- Word count paper: 1758

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Abstract

To clarify the possible involvement of intermediate *ATXN1* alleles as risk factors for 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 polyQ repeats in 105/1146 patients (9.16%) and 29/529 controls (5.48%) (*p*= 0.003). ~~We found that polyQ repeats were strongly enriched in the group of ALS patients with the *C9orf72* expansion (12/59, 20.3%) and in an independent cohort of *C9orf72* patients (10/80 cases, 12.5%) with a cumulative frequency of *ATXN1* expansion of 15.82% in *C9orf72* carriers (*p*=2.40E-05). When ALS patients without *C9orf72*, *SOD1*, *TARDBP* and *FUS* variants were considered, the proportion of patients with ≥33 repeats was still significantly increased compared to controls.~~ The frequency of *ATXN1* alleles with ≥33 polyQ 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.

Key words: Amyotrophic lateral sclerosis, Spinocerebellar ataxia, Frontotemporal dementia, *ATXN1, C9orf72*

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 (Renton et al., 2014; van Blitterswijk et al., 2014; Lattante et al., 2015; Sproviero et al., 2017). 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).

Spinocerebellar ataxia-1 (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, while in SCA1 patients disease alleles range from 39 to 82 units (Orr et al., 1993). *ATXN1* has been analyzed in ALS patients in only two studies, with conflicting results (Lee et al., 2011; Conforti et al., 2012).

To elucidate the role of *ATXN1* in ALS, in the present study *ATXN1* polyQ expansion were 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.

2. MATERIALS AND METHODS

2.1 Patients. A total of 1146 DNA samples, extracted from 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 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 PCR 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) ~~and *ATXN2* (OMIM: 601517)~~, as previously described (Lattante et al., 2012).

2.3 Statistical analysis. Chi squared 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 analysed in 1146 ALS patients as well as in 529 neurologically normal controls from Italy (Figure 1). Complete results of statistical analysis conducted applying Chi-squared test and Fisher exact tests with Benjamini-Hochberg correction for multiple comparisons were reported in Table 1, while *p*-values obtained applying Chi-squared test were reported in the manuscript. To double-check the results obtained via the Chi-squared and

Fisher exact tests, the Monte Carlo tests proposed by Sham and Curtis (1995) were also applied. Table 2 shows the *p*-values of the T1 test (T2, T3 and T4 give almost identical outcomes), which confirm the results of the Fisher exact test.

In the control group, alleles with 29 and 30 repeats were the most represented. Alleles with 31 and 32 repeats had a similar frequency of about 6%, while alleles with ≥33 repeats were rare, each allele presenting with a frequency lower than 1%. The longest repeat was 37, found in one individual. The cumulative frequency of rare alleles with ≥33 polyQ repeats was 2.74%.

We considered 33 as the cut-off to discriminate between normal and intermediate repeats after performing the receiver operating characteristic (ROC) analysis and considering data distribution in our population as well as previously published data about the Italian population (Conforti et al., 2012).

In the total group of ALS patients, 9.16% (105/1146) had an allele with ≥33 polyQ repeats, compared to 5.48% of controls (29/529) (p= 0.003). The proportion of patients with ≥33 polyQ repeat was 9.8% (11/112) in the fALS group and 9.1% (94/1034) in the sALS group, with no significant differences between the two groups.

In our cohort, 59 patients (5.14%) carried the *C9orf72* hexanucleotide expansion, 25 carried variants in *SOD1* (2.18%), 18 in *TARDBP* (1.57%), 4 in *FUS* (0.34%) while 1040 had no variants in such genes. We analyzed *ATXN1* data separately in these genetic subgroups (Table 1 and 2) and we found that 12/59 *C9orf72* cases (20.3%) had at least one allele with a polyQ repeat length ≥33, a proportion which is strongly higher compared to controls (p=6.88E-05). The number of carriers of *ATXN1* ≥33 polyQ repeat length in *SOD1* or *TARDBP* patients was not different from controls. No allele with CAG repeats ≥33 was detected in patients with *FUS* variants. In the 1040 ALS patients with no variants in the genes analyzed, 8.65% (90 patients) had alleles with ≥33 repeats, a percentage significantly higher than controls (p=0.032).

To confirm the results found in *C9orf72* carriers, we examined an independent cohort of 80 *C9orf72*-ALS patients collected in two different ALS Centres located in Milan. Alleles with ≥33

repeats were detected in 10/80 patients (12.5%; *p*=0.031). Cumulating all *C9orf72* patients tested, *ATXN1* expansion was present in 15.82% of cases (22/139; p=2.40E-05).

~~No significant difference was observed between *C9orf72* expansion carriers with and without CAG repeats ≥33, considering the mean age of onset (57 years vs 57.5), the prevalence of FTD (30% vs 25%) and the survival (33 vs 34 months).~~

We evaluated the clinical characteristics in the *C9orf72* cohort in order to assess differences between patients carrying ≥33 *ATXN-1* repeats and not. We considered in the two groups the mean age of onset (57 years vs 57.5), the prevalence of FTD (30% vs 25%) and the survival (33 vs 34 months) and we did not find any significant difference.

~~We also examined *ATXN2* in the same cohorts of patients and controls. Part of the results were previously described (Chiò et al., 2016). We found ≥29 CAG repeats alleles in 3.6% of patients (41/1146) and 1.1% of control individuals (6/529). Intermediate length alleles were absent in patients with *SOD1* and *FUS* variants while they were detected in 3/139 (2.15%) patients with the *C9orf72* expansion, 1/18 *TARDBP* patients (5.5%). In the 1040 patients without *SOD1*, *C9orf72* and *TARDBP* variants, 3.6% had ≥ 29 CAG repeats (38/1040). Five patients in our cohort (0.4%) had both *ATXN1* and *ATXN2* intermediate CAG expansion, a proportion that was not significantly higher than that expected by chance (0.33%).~~

4. DISCUSSION

In the present study we analyzed the frequency of *ATXN1* expanded alleles in a cohort of 1146 consecutive Italian ALS patients, including 106 patients carrying variants in the four major ALS genes. Specifically, 59 had an expansion in *C9orf72*, 25 a variant in *SOD1*, 18 in *TARDBP* and 4 in *FUS*.

Patients with *ATXN1* intermediate length ≥33 repeats were significantly more frequent in the whole group of ALS patients (9.16%) than in controls (vs 5.48%) (*p*=0.003). Analysis of different genetic

ALS subgroups showed that polyQ repeats were strongly associated with ALS patients bearing the pathological expansion in *C9orf72* gene. On the contrary, this association was not detected for patients with *SOD1* and *TARDBP* variants. When ALS patients without *C9orf72, SOD1, TARDBP* and *FUS* variants were considered, the proportion of patients with ≥33 repeats was still significantly increased in ALS patients as compared to controls. The strong association between *C9orf72* expansion and intermediate *ATXN1* polyQ expansion was confirmed in an independent cohort of 80 *C9orf72* ALS patients from different Italian ALS Centres. In the whole group of 139 *C9orf72* patients tested, *ATXN1* expansion was present in 15.82% of cases (22/139) (p=2.40E-05). Importantly, we found a statistically significant difference between ALS patients with *C9orf72* expansion and ALS cases without variants in other genes. This is a novel result, as the two previous studies did not analyze groups with different genetic backgrounds. In *C9orf72* patients the presence of *ATXN1* intermediate expansion did not correlate with phenotype as it had no influence on age of onset or survival. These results are similar to those observed for *ATXN2*, which does predict disease risk of ALS but does not correlate with the phenotype (Sproviero et al., 2017).

Two previous studies on *ATXN1* in ALS gave conflicting results. One study examined 526 ALS patients from USA and found no significant association between *ATXN1* polyQ length and ALS. Another study analysing 418 Italian ALS patients, mostly sporadic (405 in total), showed that *ATXN1* polyQ intermediate expansions were associated with an increased risk of developing ALS. A possible explanation for this discrepancy is that the genetic variants contributing to ALS can differ among populations and geographic regions.

Other polyQ proteins, including SCA3 (*ATXN3*), SCA6 (*CACNA1A* or *ATXN6*), SCA7 (*ATXN7*), SCA17 (*TBP*), dentatorubral-pallidoluysian atrophy (*ATN1*) and Huntington disease (*HTT*), have been studied in sporadic ALS patients but no significant association have been observed (Lee et al., 2011) .

The genetic architecture of ALS is complex, as the list of identified ALS-related genes includes numerous genes and their variants may have large, small or intermediate size effect (Al-Chalabi et

al., 2017). Consistent observations in the literature suggest that multiple variants in ALS-related genes might cooperate in disease onset or phenotype. In particular, *C9orf72* expansion has been described in association with variants in *SOD1*, *FUS*, *TARDBP*, *OPTN*, *ANG*, *UBQLN2*, *VAPB* (for review see van Blitterswijk et al., 2012). The mechanism by which *ATXN1* might contribute to neuronal degeneration in *C9orf72* ALS is unclear. There is growing evidence that C9orf72 protein exists in a complex with SMCR8 and WDR41 and that this complex acts as a GDP/GTP exchange factor involved in the autophagy/lysosome pathway (Sellier et al., 2016; Sullivan et al., 2016). The expanded polyQ *ATXN1* has been shown to interfere with the clearance of misfolded cytosolic proteins via the Ubiquitin Proteosome System (Park et al., 2013; Cortes & La Spada, 2015) and via autophagy (Vig et al., 2009). Thus, dysfunction of proteostasis might represent a common pathway shared by C9orf72 and ATXN1.

Our results are in keeping with those observed in an Italian ALS cohort (Conforti et al., 2012) suggesting that *ATXN1* is a genetic risk factor for ALS, at least in the Italian population. The hypothesis that *ATXN1* intermediate length plays a role in specific subgroups of patients, including *C9orf72* related ALS, needs to be verified in larger cohorts of patients with different geographic origins.

Acknowledgments

The authors are grateful to the patients for taking part in this study. This work was supported by ICOMM Onlus.

Disclosure statement

The authors declare no conflict of interest.

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Legends

Figure 1. Distribution of ATXN1 polyQ repeats. Frequencies of *ATXN1* polyQ repeats are reported comparing results of ALS patients and controls (1A), *C9orf72* carriers and controls (1B) and ALS patients with no variants and controls (1C).

Table 1. Numbers and percentages of patients carrying ≥33 ATXN1 repeats and *p*- values obtained using chi squared and two-tailed Fisher exact tests and adjusted using Benjamini-Hochberg method. All different genetic subgroups analyzed have been reported. Abbreviations: fALS: familial amyotrophic lateral sclerosis, sALS: sporadic amyotrophic lateral sclerosis

Table 2. Numbers and percentages of patients carrying ≥33 *ATXN1* repeats and *p*-values obtained using the T1 test proposed by Sham and Curtis (1995) and implemented in Dave Curtis' CLUMP2 program. They are finally adjusted for multiple comparisons using Benjamini-Hochberg method. The number of Monte Carlo replications used for computing the T1 *p*-value is equal to 10000.

Verification

All authors disclose to not have any actual or potential conflicts of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the work submitted that could inappropriately influence their work.

They have reviewed the contents of the manuscript being submitted, approved its contents and validated the accuracy of the data.

The manuscript has not been previously published, submitted elsewhere and will not be submitted elsewhere while under consideration at Neurobiology of Aging.

All patients involved in the study signed an informed consent.

Highlights

ATXN1 intermediate-length polyQ expansions are associated with Amyotrophic Lateral Sclerosis

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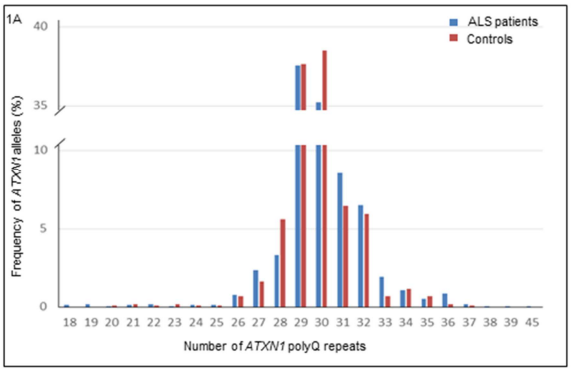
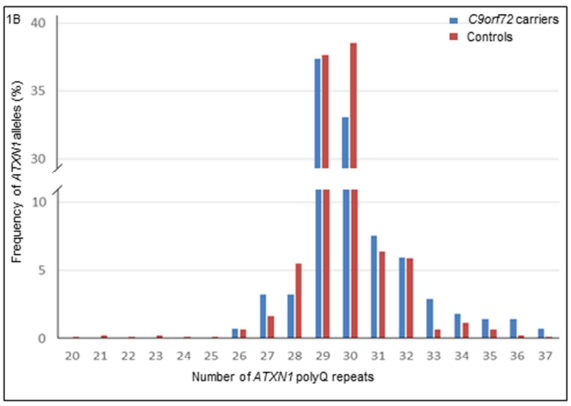
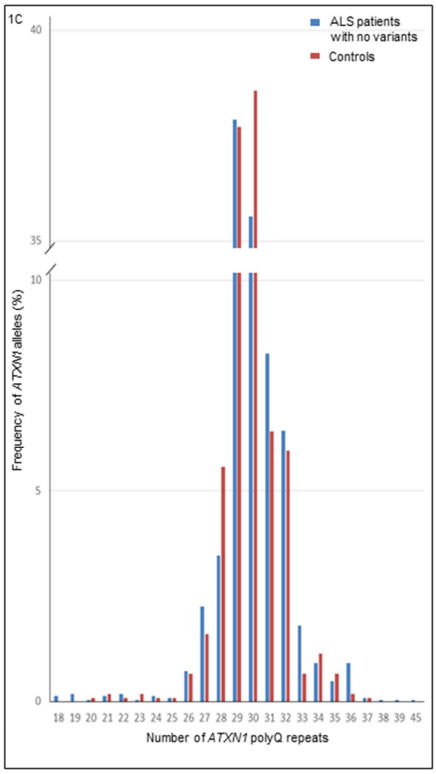
- The ~~possible~~ involvement of *ATXN1* as risk factor for ALS has been ~~proposed~~ described.
- *ATXN1* alleles with ≥33 polyQ repeats are more frequent in Italian ALS patients than in controls.
- *ATXN1* repeats are strongly enriched in ALS patients with the *C9orf72* expansion.







Patients	**Total n**	**≥33 *ATXN1* repeats, n (%)**	***Chi squared test***		***Fisher exact test***	
			***p*-values**	**Benjamini-Hochberg**	***p*-values**	**Benjamini-Hochberg**
All patients	1146	105 (9.16)	0.003024	0.009071	0.002115	0.006346
fALS	112	11 (9.8)	0.131108	0.168568	0.088494	0.113778
sALS	1034	94 (9.1)	0.016039	0.036088	0.012842	0.028893
C9orf72 carriers	59	12 (20.3)	6.88E-05	0.000309	0.000277	0.001245
SOD1 variant carriers	25	2 (8)	0.928276	1	0.64386	0.724342
TARDBP variant carriers	18	1 (5.5)	1	1	1	1
FUS variant carriers	4	0	-	-	-	-
ALS with no variants	1040	90 (8.65)	0.03215	0.048226	0.026311	0.039467
Independent C9orf72 cohort	80	10 (12.5)	0.031985	0.048226	0.025522	0.039467
Total of C9orf72 carriers	139	22 (15.82)	2.40E-05	0.000216	6.00E-05	0.00054
Controls	529	29 (5.48)				

Table 1. Numbers and percentages of patients carrying ≥33 *ATXN1* repeats and *p*-values obtained using Chi squared and two-tailed Fisher exact tests and adjusted using Benjamini-Hochberg method. All different genetic subgroups analyzed have been reported. Abbreviations: fALS: familial amyotrophic lateral sclerosis, sALS: sporadic amyotrophic lateral sclerosis.

Patients	**Total n**	**≥33 *ATXN1* repeats, n (%)**	***T1 test***	
			***p*-values**	**Benjamini-Hochberg**
All patients	1146	105 (9.16)	0.0019	0.0057
fALS	112	11 (9.8)	0.087191	0.112103
sALS	1034	94 (9.1)	0.011899	0.026773
C9orf72 carriers	59	12 (20.3)	0.0004	0.0018
SOD1 variant carriers	25	2 (8)	0.649035	0.730164
TARDBP variant carriers	18	1 (5.5)	1	1
FUS variant carriers	4	0	-	-
ALS with no variants	1040	90 (8.65)	0.028097	0.042146
Independent C9orf72 cohort	80	10 (12.5)	0.025697	0.042146
Total of C9orf72 carriers	139	22 (15.82)	0.0002	0.0018
Controls	529	29 (5.48)		

Table 2. Numbers and percentages of patients carrying ≥33 *ATXN1* repeats and *p*-values obtained using the T1 test proposed by Sham and Curtis (1995) and implemented in Dave Curtis' CLUMP2 program. They are finally adjusted for multiple comparisons using Benjamini-Hochberg method. The number of Monte Carlo replications used for computing the T1 *p*-value is equal to 10000.