BRAIN
REPORT

ATXN2 intermediate expansions in amyotrophic lateral sclerosis

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Intermediate CAG (polyQ) expansions in the gene ataxin-2 (ATXN2) are now recognized as a risk factor for amyotrophic lateral sclerosis. The threshold for increased risk is not yet firmly established, with reports ranging from 27 to 31 repeats. We investigated the presence of ATXN2 polyQ expansions in 9268 DNA samples collected from people with amyotrophic lateral sclerosis, amyotrophic lateral sclerosis with frontotemporal dementia, frontotemporal dementia alone, Lewy body dementia and age matched controls.

This analysis confirmed ATXN2 intermediate polyQ expansions of ≥31 as a risk factor for amyotrophic lateral sclerosis with an odds ratio of 6.31. Expansions were an even greater risk for amyotrophic lateral sclerosis with frontotemporal dementia (odds ratio 27.59) and a somewhat lesser risk for frontotemporal dementia alone (odds ratio 3.14). There was no increased risk for Lewy body dementia. In a subset of 1362 patients with amyotrophic lateral sclerosis with complete clinical data, we could not confirm previous reports of earlier onset of amyotrophic lateral sclerosis or shorter survival in 25 patients with expansions.

These new data confirm ≥31 polyQ repeats in ATXN2 increase the risk for amyotrophic lateral sclerosis, and also for the first time show an even greater risk for amyotrophic lateral sclerosis with frontotemporal dementia. The lack of a more aggressive phenotype in amyotrophic lateral sclerosis patients with expansions has implications for ongoing gene-silencing trials for amyotrophic lateral sclerosis.

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Introduction

Ataxin-2 (ATXN2) is an RNA binding protein and regulator of stress granule assembly that is increasingly implicated in the pathogenesis of neurodegenerative diseases. Polyglutamine (CAG) expansions of >34 repeats within ATXN2 are causative of the disease spinocerebellar ataxia type 2 (SCA2).[1] Intermediate expansions that do not reach the threshold for SCA2 are now recognized as a risk factor for amyotrophic lateral sclerosis (ALS). ATXN2 is shown to be a modifier of TDP43 toxicity in yeast, flies and mouse models of ALS.[2–4] Indeed, in TDP-43 transgenic mice, an animal model of ALS, lifespan was significantly extended either by crossing these animals with ATXN2 knockout mice, or by treatment with antisense oligonucleotides directed at silencing the ATXN2 gene.[3] The importance and clinical implications of these findings lead to the current ongoing trial of ATXN2 antisense oligonucleotides for the treatment of people with ALS (Clinical Trial NCT04494256).

The initial publication, which compared 915 ALS patients to 980 controls, identified polyglutamine (polyQ) expansions of 27–33 repeats to be enriched in the ALS population compared to controls.[4] Subsequently, several groups have replicated the increased risk of intermediate repeat length expansions, although the appropriate threshold cut-off has varied from ≥29 to ≥31.[5–10] Further, it has been reported that patients with intermediate expansions may experience a more aggressive ALS phenotype, including earlier age of onset[4] and shorter survival.[5,6] Last, no studies have directly investigated the association of ATXN2 intermediate expansions with ALS combined with frontotemporal dementia (ALSFTD) or with Lewy body dementia (LBD). We chose LBD as a comparison because it is a late life neurodegenerative disorder with clinical features and pathology distinct from FTD and where a large genomic dataset was available.

To address these issues, we investigated the prevalence of ATXN2 intermediate expansions in DNA collected from 9268 people, comparing age-appropriate controls to cohorts with diagnoses of ALS, ALSFTD, FTD without ALS and LBD. Further, we attempted to establish an association between ATXN2 intermediate repeat expansions and disease phenotypes, including age of onset and survival.

Materials and methods

The sources of DNA for sequencing are as described previously,[11,12] from Wellderly,[13] and from the Baltimore longitudinal study of ageing (https://www.blsa.nih.gov).[14] All samples were subject to whole-genome sequencing and the clinical phenotype of cases were provided by the source clinicians. As described previously,[11,12] samples were subject to several quality control assessments, including call rate and heterozygosity/homozygosity rates. Further, samples were analysed to assess population stratification via principal component analysis, and divergent samples were removed from the analysis (Supplementary Fig. 1). Genomes were analysed with Expansion Hunter, v.3.0[15] to identify copy numbers in the genes for ATXN2 and C9orf72. A subset of the samples carrying ATXN2 expansions longer than 34, the established repeat threshold underlying SCA2, was re-examined manually for proper graphical realignment. This led to the exclusion of five genomes that did not align correctly at this locus. C9orf72 expansions were identified in 90 people with ≤30 ATXN2 repeats and one individual with an intermediate ATXN2 polyQ expansion. A subset of 1362 patients cared for by J.D.G. (Emory cohort) was used to investigate the influence of ATXN2 intermediate expansions on ALS phenotypes.

Within this cohort, 350 patients who did not undergo whole-genome sequencing were genotyped for ATXN2 repeat length and the C9orf72 expansion using standard and repeat-primed PCR.[16] These ATXN2 data are included in the Emory clinical phenotyping (see next).

Statistical analysis

The binary case-control status was regressed against the presence or absence of ATXN2 repeats ≥31. Analysis was performed using R (v.3.5.2) using the glm() function applying the binary and logit transformation to the model. Estimates from the model for the contribution of ATXN2 repeats ≥31 were converted to odds ratio by exponentiating the estimates. The lower and upper confidence intervals (CIs) were calculated as follows: exp(estimate +/1.96× standard error of estimate). In the per repeat length analysis, to accommodate very small numbers of cases and controls in each repeat length, the binary case-control status was regressed using the Firth logistics regression method[17] using the logisdf package (v.1.23). The logistic and Firth regression models were also corrected for sex and principal components 1–10 to account for any subtle cohort and population stratification.

Data availability

The majority of genomes, including repeat expansion data, have been previously published[11,12] and are publicly available on dbGaP (phs001369, $n = 6907$), the AMP-PD portal ($n = 4579$ including overlapping samples) and the New York Genome Center web portal.

Results

A total of 9268 genomes: 2181 ALS, 71 ALSFTD, 1485 FTD, 2610 LBD and 2921 controls, were evaluated for repeat length of ATXN2. Diagnosis was provided by the submitting physician, and age and site of onset were available for a subset of patients. The designation of ALSFTD was based on the combined clinical diagnoses of FTD and motor neuron disease. The control group was without neurodegenerative disease with a mean age of 73.6 years (median 81 years). Table 1 shows the numbers of patients with polyQ repeat numbers ranging from ≤22 to ≥34, with the frequencies for each repeat length shown graphically in Fig. 1. Comparisons across the four groups showed no differences in ATXN2 repeat length between any case group versus controls at a repeat length up to 30 repeats (Supplementary Table 2 and Supplementary Fig. 2). However, an increased frequency of ALS patients was observed at ≥31 repeats, establishing this as the cut-off for increased risk (Table 2 and Fig. 1). Comparing all ALS cases with ATXN2 repeat length ≥31 resulted in an odds ratio of 6.93 (P-value 9.50×10^{-7}, 95% CI = 3.19–15.02; Table 2). Interestingly, comparing ALSFTD patients versus controls with ≥31 repeats displayed an even higher risk (odds ratio = 29.45, P-value 9.50×10^{-8}, 95% CI = 8.5 to 102.02). FTD alone showed a lower risk, although there was still a significant odds ratio (odds ratio = 2.70, P-value 0.046, 95% CI = 1.02 to 7.13). The risk for a related neurodegenerative disease, LBD was not associated with ATXN2 repeat length (odds ratio = 1.19, P-value 0.735, 95% CI = 0.43 to 3.29).

A subset of 1362 ALS patients with complete clinical information (Emory cohort) was examined for associations between ATXN2 repeat length and sub-phenotypes. Within this cohort, 25 patients (1.8%) harboured ≥31 ATXN2 repeats. Comparing ALS patients with and without expansions (≤30 repeats) did not reveal any difference in the mean age of onset of disease (58.7 years in those

Table 1 Numbers of patients with ATXN2 repeats in each diagnosis group

Longest repeat	ALS	ALSFTD	Control	FTD	LBD
≤22	1767	45	2400	1230	2039
23	265	19	367	179	400
24	19	0	22	14	23
25	4	0	14	3	7
26	6	0	5	3	6
27	61	2	70	33	92
28	4	0	8	1	7
29	11	0	16	4	18
30	5	0	11	5	10
31	17	0	4	6	5
32	12	4	2	4	0
33	5	0	1	3	1
≥34	5	1	1	0	2
Totals	2181	71	2921	1485	2610

≤30 versus 60.5 years in those ≥31, $P = 0.46$). Information on survival was available 900 people who were followed to the time of death. No differences in survival ($P = 0.89$) were found comparing patients with ≤30 repeats (mean = 2.86 years, range 1.4–29.8), and those with ≥31 repeats (3.39 years, range 2.4–8.9; Fig. 2A). Similarly, no difference ($P = 0.44$) was observed when comparing the survival including all patients and censoring those still alive: 3.5 years for ≤30 and 3.6 years for ≥31 repeats (Fig. 2B). Evaluating the cohorts separated by site of onset (limb versus bulbar) also showed no differences in age of onset or survival, with the caveat that there were only four bulbar onset patients with expansions ≥31 (Supplementary Table 1). Fifty-two patients in this clinical cohort had ALSFTD. Only one had an expansion of 31 repeats, and all others had repeat sizes of 28 or fewer.

Discussion

Here we provide new data on the influence of ATXN2 intermediate polyQ expansions for the risk of ALS. These data are among the most extensive set of ALS genomes to address this issue, and among the first to examine ATXN2 expansions in large populations with ALS-related disorders (ALSFTD, FTD), and in an unrelated neurodegenerative disorder, LBD. We confirm the increased risk for ALS and establish a threshold cut-off ≥31 repeats. Further, we identified an even greater risk for the combination of ALS and FTD. Interestingly, we also found an increased risk for FTD without ALS, albeit associated with a lower level of risk. This observation, along with the lack of increased risk of polyQ expansions for LBD, provides some evidence for the specificity of ATXN2 expansions for ALS and ALSFTD.

Contrary to previous reports in Italian populations,[5,6] but consistent with previous data from the UK and the Netherlands,[9] we found no association of intermediate ATXN2 polyQ expansions with clinical features suggestive of a more aggressive disease, including younger age of onset and shorter survival. It is unclear why our findings differ; however, these new data may be important for the ongoing clinical trial using antisense technology to knock down ATXN2 protein. Although all sporadic ALS patients are eligible to participate, the later experimental cohorts are recruiting ALS patients only with ATXN2 polyQ expansions (Clinical Trial NCT04494256), on the basis of the supposition that ATXN2 knockdown will be more effective than in ALS patients without expansions. The lack of increased clinical severity in these patients may argue against this hypothesis since there are no data that we are aware of that those with polyQ expansions generate more ATXN2 protein than those without expansions.

Although ALS and FTD are clinically distinct, these disorders are inescapably linked by their common pathological and genetic underpinnings. These commonalities include the neuropathological finding of phosphorylated cytoplasmic TDP inclusions, and the overlap of ALS and FTD in individual patients and within families. Indeed, the most common disease associated mutation in familial ALS, the C9orf72 hexanucleotide expansion, is also the most common mutation found in familial FTD. Although it might be assumed that FTD seen with or without ALS is the 'same' disease, there are clinical[18] and pathological/proteomic[19] indications that they may have distinct underlying biology. Our finding that ATXN2
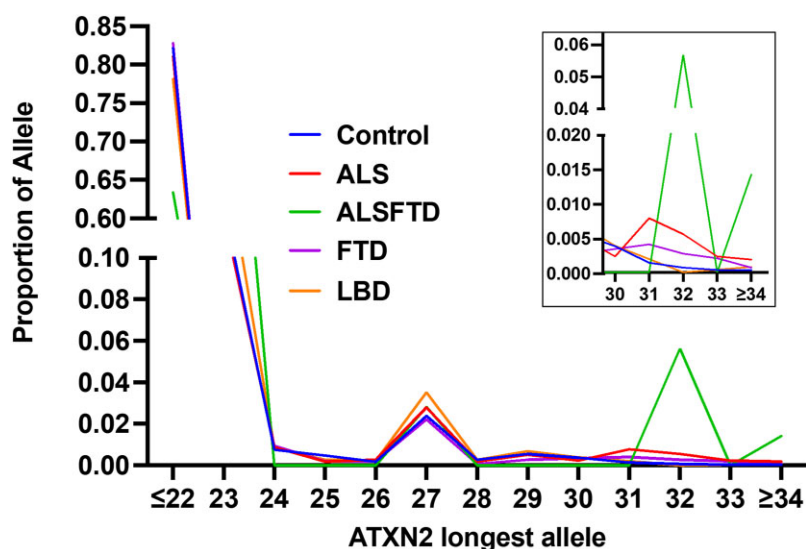

Figure 1 Proportion of genomes with ATXN2 polyQ repeat number of the longest allele. Note that the majority of alleles register 22 and 27 repeats. The *inset* is a magnification of the proportion at ≥31 repeats, showing the increased proportion of people with ALS, FTD and ALSFTD.

Table 2 Comparison of patient groups: ATXN2 repeats ≤30 versus ≥31

Adjustment	Group pair	P-value	Odds ratio	Confidence interval
with covariates	ALS versus controls	9.50×10^{-7}	6.93	3.19 to 15.02
with covariates	ALSFTD versus controls	9.50×10^{-8}	29.45	8.5 to 102.02
with covariates	FTD versus controls	0.046	2.70	1.02 to 7.13
with covariates	LBD versus controls	0.735	1.19	0.43 to 3.29
with covariates	ALS versus FTD	9.18×10^{-3}	2.40	1.24 to 4.64
with covariates	ALSFTD versus FTD	5.54×10^{-5}	9.85	3.24 to 29.97
with covariates	ALSFTD versus ALS	3.91×10^{-3}	4.21	1.59 to 11.18
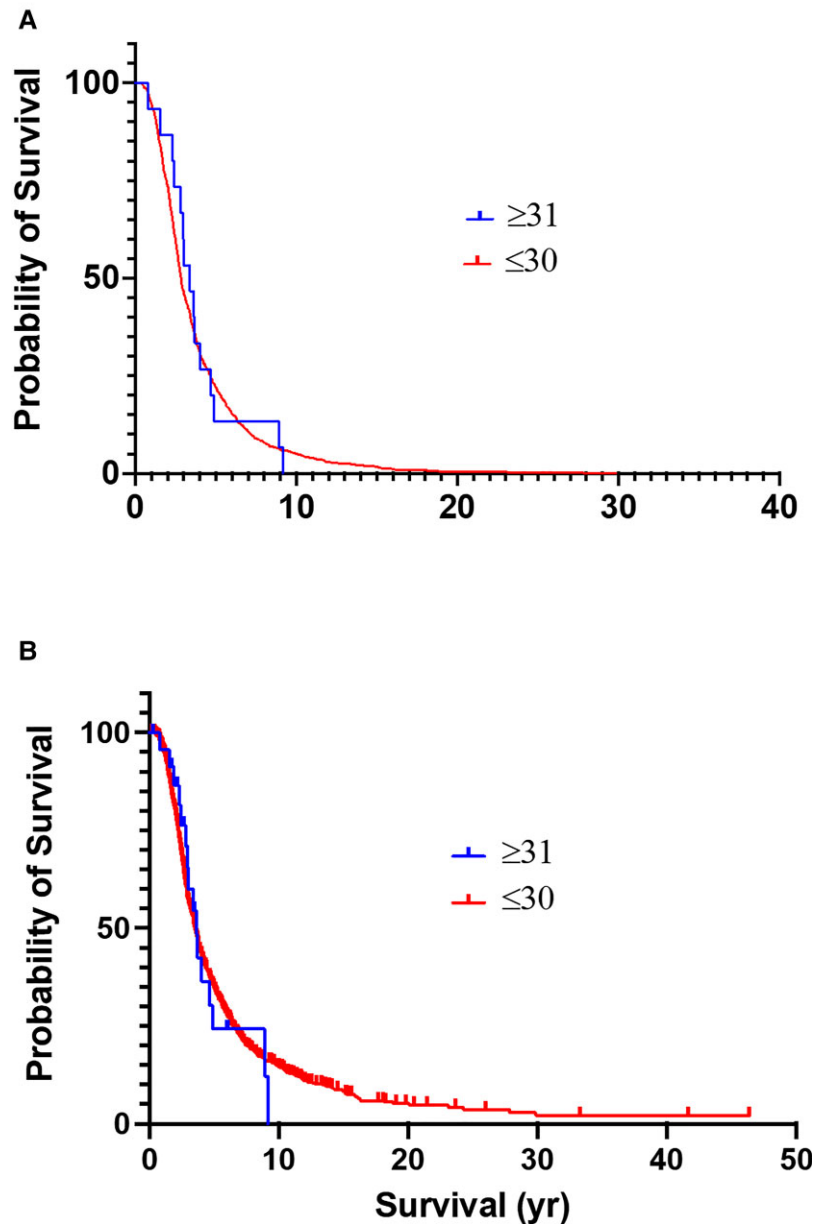
Figure 2 Comparison of survival for ALS patients with <30 or ≥31 ATXN2 polyQ repeats. (**A**) All deceased patients. (**B**) All patients with censoring of those still alive.

intermediate polyQ expansions impart a higher risk for ALSFTD than ALS alone, but a much lower increased risk for FTD, provides additional data supporting the possibility that FTD with and without ALS may represent distinct disease/phenotypes that share overlapping pathways contributing to pathogenesis. We note that a previous analysis of a smaller cohort of 368 FTD patients[20] and another cohort consisting of a combination of 479 clinically diagnosed FTD and 162 pathologically diagnosed FTLD-TDP[21] did not identify

increased risk associated with intermediate ATXN2 repeat expansions.

The mechanisms underlying the relationship between ATX2 and ALS pathogenesis are unknown. ATX2 is a ubiquitously expressed protein with various cellular functions.[22] Perhaps most germane to ALS is that ATXN2 is an RNA binding protein that regulates post-transcriptional control of expression, with direct effects on translation of subsets of RNA.[23] It is not surprising, therefore, that ATXN2 interacts with other ALS genes and their protein products, including C9orf72, TDP43 and FUS.[24] During stress, ATX2 is localized to stress granules where it is involved in stress granule assembly.[25] These functions have been implicated in experimental ALS models, although it is unclear whether intermediate expansions interfere with these pathways.

Conclusion

The discovery of ATXN2 intermediate polyQ expansions in ALS is undoubtedly an important, yet unresolved, clue into the pathobiology ALS. The ongoing therapeutic trial targeting ATXN2 in ALS is a direct and positive outcome of this discovery. The lack of phenotypic and survival differences between ALS patients with and without expansions supports the inclusion of both groups in this trial. These new data demonstrating an even higher risk for ALSFTD in those with intermediate expansions adds to the genetic and pathological connections between these two neurodegenerative disorders.

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Competing interests

B.J.T. holds patents on the clinical testing and therapeutic intervention for the hexanucleotide repeat expansion of C9orf72 (patent numbers EP2751284A1, CA2846307A and 20180187262) and has received research grants from The Myasthenia Gravis Foundation, the Robert Packard Center for ALS Research, the ALS Association (ALSA), the Italian Football Federation (FIGC), the Center for Disease Control and Prevention (CDC), the United States Department of Veterans Affairs, the Muscular Dystrophy Association, Merck and Microsoft Research. B.J.T. receives funding through the Intramural Research Program at the National Institutes of Health. B.J.T. sits on the scientific advisory committee of the American Neurological Association, is an associate editor of *Brain*, and sits on the editorial boards of the *Journal of Neurology*, *Neurosurgery*, and *Psychiatry*, *Neurobiology of Aging* and *eClinical Medicine*. J.D.G. receives research support from the ALS Association, the Muscular Dystrophy Association, the National Institute of Ageing (P30AG066511), the National Institutes of Neurological Disorders and Stroke (P01NS08974), Biogen, Mitsubishi Tanabe, The Healy Center. J.E.L. is a member of the scientific advisory board for Cerevel Therapeutics, a consultant for ACI Clinical LLC sponsored by Biogen, Inc. or Ionis Pharmaceuticals, Inc. J.E.L. is also a consultant for Perkins Coie LLP and may provide expert testimony. J.E.L. was supported by funding from NIH/NINDS (R01NS073873 and R56NS073873).

Supplementary material

Supplementary material is available at *Brain* online.

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