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Ataxin-2 repeat-length variation and neurodegeneration

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Expanded glutamine repeats of the ataxin-2 (ATXN2) protein cause spinocerebellar ataxia type 2 (SCA2), a rare neurodegenerative disorder. More recent studies have suggested that expanded *ATXN2* repeats are a genetic risk factor for amyotrophic lateral sclerosis (ALS) via an RNA-dependent interaction with TDP-43. Given the phenotypic diversity observed in SCA2 patients, we set out to determine the polymorphic nature of the *ATXN2* repeat length across a spectrum of neurodegenerative disorders. In this study, we genotyped the *ATXN2* repeat in 3919 neurodegenerative disease patients and 4877 healthy controls and performed logistic regression analysis to determine the association of repeat length with the risk of disease. We confirmed the presence of a significantly higher number of expanded *ATXN2* repeat carriers in ALS patients compared with healthy controls (OR = 5.57; *P* = 0.001; repeat length >30 units). Furthermore, we observed significant association of expanded *ATXN2* repeats with the development of progressive supranuclear palsy (OR = 5.83; *P* = 0.004; repeat length >30 units). Although expanded repeat carriers were also identified in frontotemporal lobar degeneration, Alzheimer's and Parkinson's disease patients, these were not significantly more frequent than in controls. Of note, our study identified a number of healthy control individuals who harbor expanded repeat alleles (31–33 units), which suggests caution should be taken when attributing specific disease phenotypes to these repeat lengths. In conclusion, our findings confirm the role of *ATXN2* as an important risk factor for ALS and support the hypothesis that expanded *ATXN2* repeats may predispose to other neurodegenerative diseases, including progressive supranuclear palsy.

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INTRODUCTION

The ataxin-2 gene (*ATXN2*) first came to prominence when a number of groups demonstrated that expansion of a glutamine tract (CAG/CAA; polyQ) within the protein resulted in the spinocerebellar ataxia type-2 (SCA2) phenotype (1–3). Subsequent studies determined that a repeat length of >34 units invariably caused disease (4). A few cases with repeat lengths between 31 and 34 units were also reported (5–7), while all normal controls showed repeat lengths ≤30 units (8). The SCA2 phenotype can be heterogeneous, with symptoms that overlap with other ataxias and parkinsonian disorders (7). SCA2 patients have presented with ataxia, Parkinson's disease (PD), progressive supranuclear palsy (PSP), multiple system atrophy (MSA), cortical basal syndrome (CBS) and motor neuron disease (MND) (9–12); furthermore, up to 30% of SCA2 patients develop dementia at some point during their disease (8).

Recently, the yeast orthologue of the ataxin-2 protein (*Pbp1*) was identified as a modifier of TAR DNA-binding protein 43 (TDP-43) toxicity via an unbiased screening in yeast (13). Confirmation of these studies in multiple model organisms and in human cells revealed that ataxin-2 and TDP-43 are part of an RNA-dependent protein complex (13). In humans, TDP-43 pathology in the form of TDP-43 aggregates in neuronal cytoplasmic and intranuclear inclusions is a pathological hallmark of amyotrophic lateral sclerosis (ALS) and is observed in a subset of patients with frontotemporal lobar degeneration (FTLD) (14). Moreover, the ataxin-2 protein was abnormally localized in ALS spinal cord neurons showing more distinct cytoplasmic accumulations compared with control neurons (13). Finally, TDP-43 pathology was observed in patients with SCA2. Based on these findings, Elden *et al.* (13) studied the role of the *ATXN2* repeat in ALS and observed an increased frequency of expanded-length *ATXN2* alleles in patients with ALS (>26 units). This finding was further supported in a European ALS patient population, where longer repeats (>30 units) showed the strongest association with ALS disease risk (15).

The pleiotropism observed in the clinical presentation of patients with *ATXN2* repeat expansions and the presence of TDP-43 pathology in a myriad of neurodegenerative diseases led us to hypothesize that *ATXN2* repeats may be a risk factor for multiple protein aggregation disorders. In the present study, we compared *ATXN2* repeat lengths in study cohorts of well-characterized patients with ALS, FTLD, Alzheimer's disease (AD), PSP and PD with *ATXN2* repeat lengths in an extensive series of healthy controls.

RESULTS

Genotype and allele distributions were within Hardy–Weinberg equilibrium (HWE) in all cohorts. Table 1 displays the Odds ratios (OR), 95% confidence intervals (CIs) and *P*-values for each disease cohort in comparison to our control series ($n = 4877$). At a repeat length cut-off >26 units, statistically significant association was observed for ALS (OR = 1.58; $P = 0.020$); however, more significant association of *ATXN2* repeat length with ALS was observed at a repeat length cut-off >30 units (OR = 5.57; $P = 0.001$). Using the repeat cut-off >30 units, highly significant association was also observed in the PSP series (OR = 5.83; $P = 0.004$). Expanded repeat carriers were further identified in FTLD, AD and PD patients though not at a significantly higher frequency compared with controls. In fact, in contrast to previous studies, we identified a number of healthy control subjects ($n = 9$) with *ATXN2* repeat lengths >30 units.

In total, we identified 20 patients and nine controls carrying at least one *ATXN2* allele with >30 repeat units (Table 2). *ATXN2* repeat sizes in patients varied from 31 to 36 units while all controls had repeat sizes ≤33 units. The mean age at last examination of the controls with expanded repeats was relatively young (54.0 ± 17.4 years; range 29–82) compared with the mean disease onset age in most patient groups (Table 2). In fact, six out of nine controls with expanded repeats are currently below the mean onset age observed in all patient groups, except PD (Table 2). The longest expansion (36 repeats) was observed in a female who presented with lower-limb onset ALS at the age of 54, rapidly progressing to involve all limbs with a mixture of upper and lower motor neuron signs. The patient died at the age of 55. Neuropathological examination confirmed the diagnosis of ALS.

Direct DNA sequencing of the longest *ATXN2* repeat alleles in each of the neurodegenerative disease groups showed that the CAG repeat was interrupted with CAA in all patients analyzed. This analysis also showed at least two internal repeat structures (either a single CAA interruption or three CAA interruptions), suggesting multiple mechanisms of *ATXN2* repeat expansions at this locus (Table 2).

DISCUSSION

Expanded ataxin-2 polyQ repeats have been shown to result in a cerebellar ataxia phenotype (SCA2) (4,8). It was recently proposed that expanded repeat expansions of the *ATXN2* repeat increase the risk of ALS (13). Herein, we have shown that the risk observed for *ATXN2* repeats is not limited to ALS, but is also observed in another neurodegenerative disease, PSP, a four-repeat (4R) tauopathy. The strongest association was observed with expanded *ATXN2* repeats lengths >30 units in ALS (OR = 5.57, $P = 0.001$) and PSP (OR = 5.83, $P = 0.004$), in accordance with a recent follow-up study performed in European ALS patients which suggested that longer *ATXN2* repeats resulted in a more clear association with disease (15).

No significant association was demonstrated for the other neurodegenerative disorders examined in the present study: AD, PD and FTLD; although a number of expanded repeat carriers were observed in these disease groups. In the case of AD and PD, the lack of association may suggest that the aggregation of the major proteins underlying these disorders (amyloid-β and α-synuclein) is not influenced by an expanded polyQ repeat in ataxin-2. However, for FTLD, where the majority of patients are found to have either TDP-43 or tau pathology at autopsy, we also did not observe an association. Given the clinical and pathological heterogeneity in these disorders, even larger sample series and meta-analytical

Table 1. Association analyses of ATXN2 repeat length in neurodegenerative disorders

Disorder (*n*)	CAG repeat length	Patient, *n* (%)	Control, *n* (%)	OR (95% CI)	Uncorrected *P*-value
ALS (532)	>26	33 (6.2)	197 (4.0)	1.58 (1.08–2.31)	0.020
	>30	8 (1.5)	9 (0.2)	5.57 (1.95–15.88)	0.001
FTD (641)	>26	31 (4.8)	197 (4.0)	1.20 (0.82–1.76)	0.352
	>30	3 (0.5)	9 (0.2)	1.94 (0.51–7.37)	0.332
AD (1530)	>26	56 (3.7)	197 (4.0)	0.96 (0.70–1.33)	0.815
	>30	3 (0.2)	9 (0.2)	2.17 (0.40–11.86)	0.370
PSP (514)	>26	24 (4.7)	197 (4.0)	1.20 (0.78–1.85)	0.399
	>30	4 (0.8)	9 (0.2)	5.83 (1.74–19.52)	0.004
PD (702)	>26	28 (4.0)	197 (4.0)	0.95 (0.63–1.43)	0.793
	>30	2 (0.3)	9 (0.2)	0.93 (0.19–4.51)	0.931

approaches may be required to fully resolve the role of *ATXN2* repeats in other neurodegenerative disorders.

Importantly, in contrast to previous reports, we did identify 9 of 4877 (0.2%) healthy control carriers with repeat lengths >30 units. The presence of ataxin-2 polyQ repeat length expansions within our controls series may reflect an age-related reduced disease penetrance. In fact, several of these controls are currently below the average onset age observed in our patient cohorts and may develop disease at an older age. Conversely, the sporadic nature of these late-onset neurodegenerative disorders suggests that many other factors in addition to *ATXN2* repeat length may contribute to the disease penetrance and phenotypic presentation. Together our findings demonstrate the importance of large control series and promote caution in the designation of pathogenicity due to an expanded repeat in *ATXN2* for phenotypes other than SCA2.

The most frequent *ATXN2* repeat length appears to be a 22 repeat consisting of the $(CAG)_8CAA(CAG)_4CAA(CAG)_8$ trinucleotide sequence (16). It has been observed that SCA2 patients with expansions (>31 units) have a pure CAG repeat and do not harbor the CAA interruptions (2). Although the CAA codons do not alter the amino acid residue, they can result in branched structures at the DNA and RNA level *in vitro* (17). Interestingly, it has been postulated that expanded repeats that contain the CAA codons can produce a more heterogeneous clinical phenotype resembling a myriad of parkinsonian and neurodegenerative disorders, including PD, PSP, MSA and most recently ALS (7,12). In fact, in a recent study, expanded repeat alleles of 40 ALS patients and 9 controls were sequenced and all repeats were found to be interrupted (18). Cloning and sequencing of the expanded repeat alleles in a selection of our patients diagnosed with ALS, FTLD, AD, PSP and PD also showed CAA interruptions. More detailed analysis of the internal repeat structure further demonstrated that expansions had occurred via at least two mechanisms resulting in different internal repeat structures in our carriers. Although we only analyzed a limited number of patients, our findings suggested that there may be no correlation between the internal repeat structure and specific clinical or pathologic phenotypes.

As previously shown by Elden *et al*. (13), expanded ataxin-2 polyQ repeats enhance the interaction of ataxin-2 with TDP-43 and promote TDP-43 mislocalization under situations of stress, which could explain the increased risk for ALS. However, PSP is a 4R-tauopathy and most patients are not found to have TDP-43 pathology at autopsy. Our PSP patient with the longest *ATXN2* repeat was negative for TDP-43 immunostaining (data not shown). Therefore, it will be critically important to determine whether expanded ataxin-2 polyQ repeats could also promote protein aggregation or mislocalization of other neurodegenerative disease associated proteins, including tau.

Future studies are now needed to elucidate the underlying pathomechanism involving ataxin-2 polyQ repeat length expansions. Given the alternate pathologies associated with *ATXN2* repeats observed in our study, we suggest that the ataxin-2 protein may play a role in neurodegenerative diseases other than ALS.

MATERIALS AND METHODS

Subjects

Demographics for the individual study groups are given in Table 3. All patients and controls were of Caucasian ancestry. Our ALS cohort ($n = 532$) consisted of 319 clinically diagnosed patients obtained from the Coriell Institute for Medical Research (including seven patients diagnosed with progressive muscular atrophy) and 102 unrelated patients diagnosed with ALS according to El Escorial criteria from a consecutive clinical case series seen at the Mayo Clinic Jacksonville (MCJ) ALS Center in the period 2008–2010. An additional 111 pathologically confirmed ALS patients were obtained from the MCJ brain bank and the London Motor Neuron Disease (MND) Clinic. The FTLD cohort ($n = 641$) included 479 clinically diagnosed FTLD patients of unknown pathological subtype diagnosed with behavioral variant FTD, semantic dementia or progressive non-fluent aphasia and 162 patients with pathologically confirmed FTLD-TDP. FTLD patients were ascertained from a total of nine Centers between 1995 and 2010 (Table 3). The AD cohort ($n = 1530$) was composed of 626 patients clinically diagnosed according to National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association criteria (19) at Mayo Clinic Rochester (MCR) and 904 patients from the MCJ brain bank diagnosed pathologically according to the National Institute on Aging and Reagan Institute Working Group criteria (20). All PSP patients ($n = 514$) were also derived from the MCJ brain bank and pathologically

Table 2. Clinic, pathologic and genetic characteristics of ATXN2 expanded repeat carriers

Sample ID	Source	Clinical diagnosis	Pathological diagnosis	Sex	Age at onset[a]	Mean age at onset[a] by study group	Age at death	CAG repeat lengths	Composition of expanded repeat
Case 1	London MND Clinic	ALS	ALS	F	54	60.6 ± 13.5	55	22/36	(CAG)27 CAA (CAG)8
Case 2	London MND Clinic	ALS	ALS	M	78		79	22/32	Not analyzed
Case 3	Coriell repository (ND10556)	ALS	n/a	M	54		n/a	22/32	(CAG)8 CAA (CAG)9 CAA (CAG)4 CAA (CAG)8
Case 4	Coriell repository (ND12198)	PMA	n/a	M	66		n/a	28/32	Not analyzed
Case 5	MCJ brain bank	ALS	ALS	F	79		81	22/32	Not analyzed
Case 6	MCJ brain bank	ALS	ALS	F	58		61	22/31	Not analyzed
Case 7	Coriell repository (ND09782)	ALS	n/a	M	58		n/a	22/31	Not analyzed
Case 8	Coriell repository (ND10492)	ALS	n/a	M	38		n/a	27/31	Not analyzed
Case 9	UCSF	FTLD	FTLD-TDP	F	52	63.7 ± 10.1	66	23/32	(CAG)8 CAA (CAG)9 CAA (CAG)4 CAA (CAG)8
Case 10	MCS	FTLD	n/a	F	69		n/a	22/31	Not analyzed
Case 11	MCJ	FTLD	n/a	M	70		n/a	22/31	Not analyzed
Case 12	MCJ brain bank	AD	AD	F	76	74.7 ± 20.0	93	22/34	(CAG)25 CAA (CAG)8
Case 13	MCJ brain bank	AD	AD	F	54		59	26/31	Not analyzed
Case 14	MCR	AD	n/a	F	94		n/a	22/31	Not analyzed
Case 15	MCJ	PD	n/a	M	50	44.0 ± 8.5	n/a	22/32	(CAG)23 CAA (CAG)8
Case 16	MCJ	PD	n/a	F	38		n/a	22/32	Not analyzed
Case 17	MCJ brain bank	FTLD/PSP	PSP	M	62	68.5 ± 6.6	70	22/33	(CAG)23 CAA (CAG)9
Case 18	MCJ brain bank	PSP	PSP	M	76		81	22/31	Not analyzed
Case 19	MCJ brain bank	CBS	PSP	F	72		77	22/31	Not analyzed
Case 20	MCJ brain bank	PSP	PSP	F	64		68	22/31	Not analyzed
Control 1	Coriell repository (ND09211)	Control	n/a	F	38	54.0 ± 17.4	n/a	22/33	Not analyzed
Control 2	Coriell repository (ND04274)	Control	n/a	M	59		n/a	22/32	Not analyzed
Control 3	Coriell repository (ND10475)	Control	n/a	F	48		n/a	22/32	Not analyzed
Control 4	Coriell repository (ND03356)	Control	n/a	M	66		n/a	22/31	Not analyzed
Control 5	Coriell repository (ND09727)	Control	n/a	F	29		n/a	22/31	Not analyzed
Control 6	ISGS	Control	n/a	F	50		n/a	22/31	Not analyzed
Control 7	ISGS	Control	n/a	M	41		n/a	23/31	Not analyzed
Control 8	MCR	Control	n/a	F	82		n/a	22/31	Not analyzed
Control 9	MCR	Control	n/a	F	73		n/a	22/31	Not analyzed

ISGS, Ischemic stroke genetics study; MCJ, Mayo Clinic Jacksonville; MCS, Mayo Clinic Scottsdale; MCR, Mayo Clinic Rochester; UCSF, University of California, San Francisco; MND, motor neuron disease; ALS, amyotrophic lateral sclerosis; PMA, progressive muscular atrophy; FTLD, frontotemporal lobar degeneration; FTLD-TDP, frontotemporal lobar degeneration with TDP-43 pathology; AD, Alzheimer's disease; PSP, progressive supranuclear palsy; CBS, corticobasal syndrome; PD, Parkinson's disease; n/a, not applicable.
[a]For controls, age at most recent examination is shown.

confirmed. PD patients ($n = 702$) were ascertained at MCJ and clinically diagnosed with PD according to published criteria (21). Finally, a cohort of unrelated control individuals free of neurodegenerative diseases ($n = 4877$) was also available for genetic association studies. The Ethics Review Board at Mayo Clinic approved the study.

Genotyping

DNA was extracted from venous blood or frozen brain tissue using standard methods. Genotyping of the *ATXN2* repeat was performed employing fluorescent-labeled primer PCR with capillary electrophoresis on an ABI 3730 Genome Analyzer (primer sequences are available on request) and analyzed with Genemapper software. To ensure standardized sizing of the *ATXN2* repeat, genotyping of all patients and controls was performed at MCJ using a single ABI3730 Genome Analyzer. For all samples with repeat sizes of ≥ 30, PCR amplification and genotyping were repeated for confirmation. Consistency of genotypes with HWE was assessed using χ^2 tests, separately for each series.

Statistical analysis

The association between the *ATXN2* repeat length and each neurodegenerative disease was evaluated using a logistic

Table 3. Demographic data of patients and control cohorts included in the study

Study cohorts	*n*	Age[a] (years)	Females (%)	Sample source (*n* samples)
Controls	4877	71.0 ± 13.1	55.9	Coriell repository (787); Ischemic stroke genetics study (295); Mayo Clinic Jacksonville (1590); Mayo Clinic Rochester (2029); Mayo Clinic Scottsdale (53); University of California, San Francisco (123)
Amyotrophic lateral sclerosis	532	58.1 ± 12.7	40.1	Coriell repository (319)[b]; Mayo Clinic Jacksonville (102); London MND Clinic, Canada (89); Mayo Clinic Jacksonville brain bank (22)
Frontotemporal lobar degeneration	641	66.0 ± 10.2	46.0	Mayo Clinic Jacksonville (189); Mayo Clinic Rochester (136); Mayo Clinic Scottsdale (10); University of California, San Francisco (130); Northwestern University Feinberg School of Medicine (27); Drexel University College of Medicine (28); University of Texas Southwestern Medical Center (9); University of British Columbia, Canada (21); University of Western Ontario, Canada (31); Mayo Clinic Jacksonville brain bank (60)
Alzheimer's disease	1530	80.3 ± 8.4	59.0	Mayo Clinic Rochester (626); Mayo Clinic Jacksonville brain bank (904)
Progressive supranuclear palsy	514	74.9 ± 8.0	47.3	Mayo Clinic Jacksonville brain bank (514)
Parkinson's disease	702	63.4 ± 12.2	36.8	Mayo Clinic Jacksonville (702)

[a]Age is shown as the mean ± standard deviation, describing the age at blood draw for controls and the age at final diagnosis for patients.
[b]ALS patient samples included in the original Elden *et al.* (13) paper were excluded from our study.

regression model adjusted for age (age at final diagnosis for patients and age at blood draw for controls) and gender, where ORs and 95% CIs were estimated. For each disease, association with the length polymorphism was performed after dichotomizing the repeat length as 'short' or 'long', based on previously reported cut-offs, where 'long' is either >26 or >30 units. We examined association under an additive model. *P*-values ≤0.05 were considered statistically significant and analyses were performed using PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) (22).

Cloning and sequencing of expanded repeats

For a selection of patients with different neurodegenerative diseases and an expanded *ATXN2* repeat (>30 units), a 471 bp fragment containing the *ATXN2* repeat region was PCR amplified from genomic DNA. PCR fragments were cloned using a TOPO® TA Cloning® Kit (Invitrogen) and grown on media agar plates. For each patient, 15 individual clones were hand picked and bidirectional DNA sequencing was performed on an ABI 3730 DNA sequencer and analyzed using Sequencher (Applied Biosystems).

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Conflict of Interest statement. None declared.

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