Evaluating the prevalence of polyglutamine repeat expansions in amyotrophic lateral sclerosis

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ABSTRACT

Objective: Given the recent finding of an association between intermediate-length polyglutamine (polyQ) expansions in ataxin 2 and amyotrophic lateral sclerosis (ALS), we sought to determine whether expansions in other polyQ disease genes were associated with ALS.

Methods: We assessed the polyQ lengths of ataxin 1, ataxin 3, ataxin 6, ataxin 7, TBP, atrophin 1, and huntingtin in several hundred patients with sporadic ALS and healthy controls.

Results: Other than ataxin 2, we did not identify a significant association with the other polyQ genes and ALS.

Conclusions: These data indicate that the effects of ataxin 2 polyQ expansions on ALS risk are likely to be rooted in the biology of ataxin 2 or ataxin 2-specific interactions, rather than the presence of an expanded polyQ repeat per se. These findings have important consequences for understanding the role of ataxin 2 in ALS pathogenesis and provide a framework for future mechanistic studies. *Neurology*® 2011;76:2062-2065

GLOSSARY

ALS = amyotrophic lateral sclerosis; **polyQ** = polyglutamine; **SCA** = spinocerebellar ataxia.

We recently identified intermediate-length polyglutamine repeat expansions in ataxin 2 as a risk factor for amyotrophic lateral sclerosis (ALS).¹ Ataxin 2 belongs to a family of at least 9 polyglutamine (polyQ) disease proteins.² The normal ataxin 2 polyQ length is most frequently 22 or 23 Qs, polyQ expansions greater than 34 Qs cause spinocerebellar ataxia type 2 (SCA2),³ and, in our study, intermediate-length (27–33 Qs) repeats were significantly associated with increased risk for ALS.¹

Is the effect of polyQ expansions and ALS specific to ataxin 2 or could other polyQ proteins also contribute to the disease? In the present study, we analyzed the polyQ lengths of 7 additional polyQ disease genes for SCA1 (*ATXN1*), SCA3 (*ATXN3*), SCA6 (*CACNA1A* or *ATXN6*), SCA7 (*ATXN7*), SCA17 (*TBP*), dentatorubral-pallidoluyian atrophy (*ATN1*), and Huntington disease (*HTT*) in patients with sporadic ALS and healthy controls. These include genes whereby the disease in humans, in addition to the typical cerebellar Purkinje neuron degeneration, can also encompass peripheral nerve loss and motor neuron association reminiscent of ALS.^{4–8} Our analysis reveals no significant association between polyQ length and ALS in any of the genes tested beyond ataxin 2. Thus, polyQ expansions in ataxin 2 likely increase risk of ALS by affecting the normal function of ataxin 2, probably through its role in RNA metabolism, rather than from general effects on proteostasis elicited by the polyQ stretch

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Table 1 Analysis of polyQ genes in ALS

PolyQ gene	Patients with ALS tested	Controls tested	PolyQ repeat lengths ^a	Differences in polyQ length in ALS vs control?
SCA1 (ataxin 1)	526	526	21–37 (27, 28)	No
SCA2 (ataxin 2) ^b	915	980	4.7% Q27–Q33 ALS; 1.4% Q27–Q33 control	Yes; $p = 3.6 \times 10^{-5}$
SCA3 (ataxin 3)	488	623	13–33 (13, 21)	No
SCA6 (CACNA1A)	146	167	3–17 (10, 12)	No
SCA7 (ataxin 7)	156	170	1–12 (5)	No
SCA17 (TBP)	116	85	25–41 (35, 36)	No
DRPLA (atrophin 1)	120	106	19–36 (27)	No
Huntington disease (huntingtin)	121	115	7–31 (18)	No
AR (androgen receptor) ^c	146	100	Male: 22–26 (23 ALS; 23, 25 control); female: 21–26 (22, 23 ALS; 25 control)	No

Abbreviations: ALS = amyotrophic lateral sclerosis; DRPLA = dentatorubral-pallidoluysian atrophy; polyQ = polyglutamine; SCA = spinocerebellar ataxia.

^a Range is indicated, with most common length repeat within parentheses.

^b Analysis from reference 1.

^c Reported in reference 4.

per se. These findings provide a conceptual framework for understanding mechanisms by which ataxin 2 contributes to ALS.

METHODS DNA samples. Genomic DNA from human patients with ALS and healthy controls was obtained from the Coriell Institute for Medical Research (Coriell). These genomic DNA samples were from DNA panels from the National Institute of Neurological Disorders and Stroke Human Genetics Resource Center DNA and Cell Line Repository (<http://ccr.coriell.org/ninds>). The submitters that contributed samples are acknowledged in detailed descriptions of each panel: ALS (NDPT025, NDPT026, NDPT030, NDPT100, NDPT103, and NDPT106) and control (NDPT084, NDPT090, NDPT093, NDPT094, NDPT095, NDPT096, NDPT098, and NDPT099). The Coriell non-ALS samples represent unrelated North American Caucasian individuals (ages 36–48 years) who themselves were never diagnosed with a neurologic disorder or had a first-degree relative with one.

Standard protocol approvals, registrations, and patient consents. We received approval for these studies from the Coriell Institute for Medical Research Institutional Review Board. The individual submitters who contributed these DNA samples to Coriell received written informed consent from all patients (or guardians of patients) participating in the study (consent for research).

PolyQ repeat size determination in patients with ALS and controls. For analysis of CAG repeat lengths in polyQ disease genes, a capillary electrophoresis approach was used, incorporating the 6FAM fluorophore into the PCR products in the 5' primer. See table e-1 on the *Neurology*[®] Web site at www.neurology.org for primer sequences and PCR conditions.

PCR products were mixed with Liz-500 size standard (Applied Biosystems) and were processed for size determination on an ABI3730 sequencer. The sizes of the repeats were determined with GeneMapper[™] 4.0 software (Applied Biosystems).

Statistical analysis. Two-tailed Fisher exact tests were used to evaluate genetic association between polyQ repeats in each polyQ disease gene and ALS.

RESULTS To evaluate the potential contribution of other polyQ genes to ALS, we defined the trinucleotide repeat length in 7 polyQ genes in patients with ALS and healthy controls (table 1). We selected the genes for SCA1 (*ATXN1*), SCA3 (*ATXN3*), and SCA6 (*CACNA1A*) genes because of previous case reports documenting prominent motor neuron involvement in these diseases, in addition to the typical cerebellar Purkinje neuron degeneration that characterizes the SCAs.^{5–9} We also analyzed the Huntington disease gene, *HTT*, as well as genes for SCA7 (*ATXN7*), SCA17 (*TBP*), and dentatorubral-pallidoluysian atrophy (*ATN1*). For each gene, we used PCR to amplify the trinucleotide repeat region, incorporating the fluorescent dye 6-FAM into the 5' PCR primer. We determined the CAG repeat length by resolving PCR amplicons by capillary electrophoresis, followed by size determination with Genescan analysis, compared to known size standards. The figure shows the distribution of CAG repeats in each of the polyQ genes analyzed in both cases and controls. Other than for ataxin 2, we did not observe significant differences in the polyQ lengths between ALS cases and healthy controls (figure and table 1).

DISCUSSION ALS is a neurodegenerative disease characterized primarily by the degeneration of cortical, brainstem, and spinal motor neurons.¹⁰ The SCAs are a group of neurodegenerative diseases char-

Figure Distribution of polyglutamine (polyQ) repeat lengths in polyQ disease genes in patients with amyotrophic lateral sclerosis (ALS) and healthy controls

(A) Spinocerebellar ataxia (SCA)1 (ataxin 1), (B) SCA3 (ataxin 3), (C) SCA6 (CACNA1), (D) Huntington disease (huntingtin), (E) SCA7 (ataxin 7), (F) SCA17 (TBP), (G) dentatorubral-pallidoluysian atrophy (DRPLA) (atrophin 1), (H) SCA2 (ataxin 2) (data from reference 1 shown for comparison). The distribution of repeat lengths for the genes assessed, except for ataxin 2, was not different in ALS cases and controls.

acterized primarily by cerebellar Purkinje neuron degeneration.¹¹ Interestingly, there are reports of prominent motor neuron degeneration in some SCA cases,^{5–9} and we have recently noted in a patient with ALS symptoms of ataxia, cerebellar speech, ocular dysmetria, and nystagmus, which are unusual for ALS, but are clearly reminiscent of SCA (see clinical anecdote in supplementary information to reference 1). Thus, there can be clinical similarities between these 2 diseases. However, the molecular underpinnings, if any, of this clinicopathologic overlap have remained unclear. We recently reported that intermediate-length polyQ expansions in ataxin 2 are associated with increased risk for ALS.¹ These results suggest that ataxin 2 could be a molecular link between these 2 seemingly disparate diseases.

However, another possibility is that the effects of intermediate-length polyQ expansions on ALS are not limited to ataxin 2, and that such expansions could contribute to disease in a more general way, perhaps by perturbing global cellular proteostasis networks.^{12,13} This would predict that intermediate-length polyQ expansions in other genes could also contribute to ALS.

The data presented here, together with our ataxin 2 data¹ (figure, table 1), which showed a significant association between ataxin 2 polyQ lengths and ALS, and those on the androgen receptor (reference 4 and included for comparison in table 1), which did not show significant differences between ALS cases and controls, suggest that the effects of polyQ expansions in ALS are likely specific to ataxin 2, though further studies on these genes in additional ALS populations will likely be informative. Thus, the association of repeat expansions in ataxin 2 with risk for ALS probably involves aspects of the normal function of ataxin 2 as a regulator of RNA metabolic pathways, rather than a general effect of polyQ repeats on global protein homeostasis.

The finding of selective repeat expansions in ataxin 2 with ALS risk will have important implications for the design and development of therapeutic interventions aimed at targeting pathogenic ataxin 2/TDP-43 interactions. We recently reported strong genetic and physical interactions between TDP-43 and ataxin 2 in yeast, flies, and mammalian cells, and the altered localization of ataxin 2 in motor neurons of patients with sporadic ALS.¹ Rather than generally inhibiting polyQ or globally boosting proteostasis networks, interventions that are specific to the normal function of ataxin 2 or its pathogenic interaction with TDP-43 will likely be more efficacious. Here we assessed the 9 polyQ disease proteins in ALS. Our results support the hypothesis that intermediate-

length polyQ expansions in ataxin 2 contribute to ALS owing to ataxin 2-specific effects, rather than general misfolding stress associated with slightly longer polyQ repeat lengths across a broader spectrum of polyQ disease genes.

DISCLOSURE

T. Lee, Y.R. Li, Dr. Chesi, M.P. Hart, D. Ramos, N. Jethava, D. Hosangadi, J. Epstein, and B. Hodges report no disclosures. Dr. Bonini serves as Editor for the *Journal of Clinical Investigation* and receives research support from the NIH, the Howard Hughes Medical Institute, the Ellison Medical Foundation, and the Muscular Dystrophy Society. Dr. Gitler receives research support from the NIH and The Pew Charitable Trusts.

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