Neurobiology of Aging 35 (2014) 936.e1–936.e4

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Brief communication

Evaluating noncoding nucleotide repeat expansions in amyotrophic lateral sclerosis

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ARTICLE INFO

Article history:
Received 27 August 2013
Received in revised form 16 September 2013
Accepted 19 September 2013
Available online 23 October 2013

Keywords:
Ataxin
polyQ
Noncoding
Nucleotide repeat expansion
ALS

ABSTRACT

Intermediate-length polyglutamine expansions in ataxin 2 are a risk factor for amyotrophic lateral sclerosis (ALS). The polyglutamine tract is encoded by a trinucleotide repeat in a coding region of the ataxin 2 gene (*ATXN2*). Noncoding nucleotide repeat expansions in several genes are also associated with neurodegenerative and neuromuscular diseases. For example, hexanucleotide repeat expansions located in a noncoding region of *C9ORF72* are the most common cause of ALS. We sought to assess a potential larger role of noncoding nucleotide repeat expansions in ALS. We analyzed the nucleotide repeat lengths of 6 genes (*ATXN8*, *ATXN10*, *PPP2R2B*, *NOP56*, *DMPK*, and *JPH3*) that have previously been associated with neurologic or neuromuscular disorders, in several hundred sporadic patients with ALS and healthy control subjects. We report no association between ALS and repeat length in any of these genes, suggesting that variation in the noncoding repetitive regions in these genes does not contribute to ALS.

1. Introduction

Intermediate-length polyglutamine repeat expansions in ataxin 2 are a risk factor for amyotrophic lateral sclerosis (ALS) (Elden et al., 2010) and longer expansions cause spinocerebellar ataxia type 2 (SCA2) (Fischbeck and Pulst, 2011). Ataxin 2 is a member of a family of polyglutamine (polyQ) disease proteins, which includes huntingtin. The genes encoding polyQ proteins all harbor trinucleotide repeat tracts that, when expanded past a certain threshold, cause neurodegenerative disease (Orr and Zoghbi, 2007). We had previously surveyed other polyQ disease genes in ALS patients and found no significant association between polyQ length and ALS in any of the genes tested beyond ataxin 2 (Lee et al., 2011).

Mutations in the *C9ORF72* gene were recently identified as the most common cause of ALS and frontotemporal dementia (DeJesus-Hernandez et al., 2011; Renton et al., 2011). The pathogenic mechanism in C9ORF72-linked ALS involves the expansion of a noncoding hexanucleotide repeat, GGGGCC, located in an intron of the *C9ORF72* gene, from a few repeats in unaffected individuals to hundreds or even thousands of copies in affected individuals (DeJesus-Hernandez et al., 2011; Renton et al., 2011). The mechanism through which these expansions cause disease is unclear and may involve loss of function of C9ORF72, gain of RNA toxicity from transcribed GGGGCC sequences that accumulate in nuclear and cytoplasmic foci, or even proteotoxicity from the nonconventional translation of GGGGCC into dipeptides in multiple reading frames (Ling et al., 2013). The discovery of *C9ORF72* mutations in ALS indicates that, in principle, noncoding repeat expansions in other genes could also contribute to the disease. Indeed, besides C9ORF72-ALS, there are several other neurodegenerative and neuromuscular diseases that are caused by expansions of repetitive DNA in noncoding regions, including spinocerebellar ataxia type 8, 10, 12, 31, and 36; fragile X–associated tremor/ataxia syndrome; Huntington disease-like 2; and myotonic dystrophy types I and II (Cooper et al., 2009; Li and Bonini, 2010).

In this report, we expand the analysis of nucleotide repeats in ALS beyond those found in coding regions (e.g., polyQ proteins) and assess the potential role of noncoding repeats. We analyzed the disease-linked nucleotide repeat sequences in the following genes: *ATXN8*—spinocerebellar ataxia type 8 (SCA8), *ATXN10*—SCA10, *PPP2R2B*—SCA12, *NOP56*—SCA36, *JPH3*—Huntington disease-like 2 (HDL2), and *DMPK*—myotonic dystrophy type I in patients with sporadic ALS and healthy control subjects. This analysis revealed no significant association between nucleotide repeat length and ALS in any of the genes tested, suggesting that variation in the noncoding repetitive regions in these genes does not contribute to ALS.

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0197-4580/$ – see front matter
http://dx.doi.org/10.1016/j.neurobiolaging.2013.09.024

2. Materials and methods

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, NDPT103, and NDPT106) and control (NDPT084, NDPT090, NDPT093, NDPT094). The Coriell non-ALS control samples represent unrelated North American Caucasian individuals (aged 36–48 years) who themselves were never diagnosed with a neurologic disorder nor had a first-degree relative with one. We used polymerase chain reaction (PCR)-based fragment analysis to determine the repeat lengths of each gene analyzed, using a similar protocol as in (Lee et al., 2011). PCR primers and cycling conditions are available on request. It remains possible that our analysis method (PCR fragment analysis) could have missed exceptionally long repeat expansions that are refractory to PCR amplification, but we think this is unlikely because we did not observe an increase in the frequency of apparently homozygous repeat alleles in ALS cases compared with controls, except for *PPP2R2B*. For that gene, there was a statistically significant increase in the number of ALS samples with homozygous alleles compared with controls ($p = 0.01$ (169 of 358 ALS and 127 of 338 controls)). However, our analysis method for this gene would have detected the presence of any pathogenic expansion (55–78 repeats). We also note that both *ATXN8* and *NOP56* contain another repetitive motif just next to the disease-causing repetitive motif. Like previous studies, our fragment analysis captures the combined number of both repetitive regions (Table 1).

3. Results

To evaluate the potential contribution of noncoding nucleotide repeat genes to ALS, we defined the nucleotide repeat length in 6 noncoding repeat genes in patients with ALS and healthy control subjects (Table 1). We selected the following genes: *ATXN8*—spinocerebellar ataxia type 8 (SCA8), *ATXN10*—SCA10, *PPP2R2B*—SCA12, *NOP56*—SCA36, *JPH3*—HDL2, and *DMPK*—myotonic dystrophy type I. For each gene, we used PCR to amplify the nucleotide repeat region, incorporating the fluorescent dye 6-FAM into the 5′ PCR primer. We determined the repeat length by resolving PCR amplicons by capillary electrophoresis, followed by size determination with fragment analysis, compared with known size standards. Fig. 1A and Table 1 show the genes we analyzed and the normal range of repeat lengths. Fig. 1B–G show the distribution of nucleotide repeats in each of the genes analyzed in both cases and controls. We did not observe significant differences in the repeat lengths between ALS cases and healthy controls (*ATXN8* [>23 repeats or >32 repeats], $p = 0.49$ and $p = 0.08$, respectively; *ATXN10* [>14 repeats], $p = 0.41$; *PPP2R2B* [>10 repeats], $p = 0.11$; *NOP56* [>9 repeats], $p = 0.19$; *JPH3* [>14 repeats], $p = 0.11$; *DMPK* [>5 repeats], $p = 0.61$). Of note, 2 ALS patients had *ATXN8* CAG repeat lengths of 70 and 85 (Fig. 1B), which border the SCA8-causing repeat range, although *ATXN8* expanded repeats show incomplete penetrance (Day et al., 2000).

4. Discussion

The discoveries of nucleotide repeat expansions in *ATXN2* and *C9ORF72* as contributors to ALS (Ling et al., 2013) raise the possibility that repeat expansions in other genes might also contribute to the disease. In this report, we evaluated 6 genes harboring noncoding nucleotide repeats that are expanded in neurologic and neuromuscular diseases and did not find an association with ALS. In a previous report, we assessed 7 polyQ-encoding genes and found no association with ALS (Lee et al., 2011). Other groups have also assessed additional nucleotide repeat expansion genes in ALS. Groen et al. (2012) reported no association of the noncoding CGG-repeat expansions in *FMR1*. Blauw et al. (2012) identified an association between polyalanine-encoding GCG repeats in NIPA1 and ALS. In contrast to our previous findings (Lee et al., 2011), Conforti et al. (2012) reported increased frequency of trinucleotide repeat expansions in the ataxin 1 gene in ALS patients.

Our findings suggest two possibilities. First, the effects of repeat expansions in *ATXN2* and *C9ORF72* that contribute to ALS are specific to those particular genes and thus further understanding the normal functions of these genes will provide insight into their role in disease. Second, our analysis of selected candidate nucleotide repeat genes here and previously (Lee et al., 2011) could certainly have missed other repeat-containing genes. Thus, we suggest that a comprehensive genomewide assessment of nucleotide repeat expansions is warranted. Such an analysis will likely require the development and implementation of novel methods to analyze genome sequencing data because repetitive sequences are typically refractory to standard next-generation sequencing alignment approaches (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Maybe the difficulty in mapping long repeat expansions could be turned into an advantage and used to scour genome sequence data for mapping discrepancies that may in fact be hidden repeat expansions.

Disclosure statement

The authors have no actual or potential conflicts of interest.

Table 1
Noncoding repeat genes analyzed in patients with ALS and control subjects

Gene	Associated disease	Repeat sequence	No. ALS patients analyzed	No healthy controls analyzed	Observed repeat size range (maximum detectable)	Previously reported normal repeat size range[a]	Disease repeat expansion range[a]
ATXN8	SCA8	(CTA)n-(CTG)n	365	350	15–85 (100)	15–50	~71–1400
ATXN10	SCA10	(ATTCT)n	356	355	9–22 (32)	10–29	400–4500
PPP2R2B	SCA12	(CAG)n	358	338	9–25 (126)	7–32	51–78
NOP56	SCA36	(GGCCTG)n-(CGCCTG)n	352	342	6–14 (43)	3–14	~650–2500
JPH3	HDL2	(CTG)n	360	352	5–29 (142)	6–28	>41
DMPK	DM1	(CTG)n	333	329	5–39 (100)	5–38	>50

Key: ALS, amyotrophic lateral sclerosis.
[a] Garcia-Murias et al., 2012; Todd and Paulson 2010.