The evaluation of polyglutamine repeats in autosomal dominant Parkinson's disease

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Abstract

We evaluated the contributions of various polyglutamine (polyQ) disease genes to

Parkinson's disease (PD). We compared the distributions of polyQ repeat lengths in
 40 eight common genes (*ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A*, *ATXN7*, *TBP*, *ATN1*, and
HTT) in 299 unrelated patients with autosomal dominant PD (ADPD) and 329 normal
 controls. We also analyzed the possibility of genetic interactions between *ATXN1* and
ATXN2, *ATXN2* and *ATXN3*, and *ATXN2* and *CACNA1A*. Intermediate-length polyQ
 expansions (>24 Qs) of *ATXN2* were found in seven ADPD patients and no controls
 45 ($7/299 = 2.34\%$ and $0/329 = 0\%$, respectively; $P = 0.0053 < 0.05/8$ after Bonferroni
 correction). These patients showed typical L-DOPA-responsive PD phenotypes.

Conversely, no significant differences in polyQ repeat lengths were found between the
 ADPD patients and the controls for the other seven genes. Our results may support the
 hypothesis that *ATXN2* polyQ expansion is a specific predisposing factor for multiple
 50 neurodegenerative diseases.

Keywords: trinucleotide repeat diseases, Parkinson's disease, polyglutamine,
intermediate length, ataxin-2

1. Introduction

Several genes other than the "PARK" genes are suspected to be responsible for
 55 parkinsonism. Mutations of these genes sometimes confer symptoms that clinically
 mimic idiopathic Parkinson's disease (PD) and present radiological or pathological
 findings characteristic of PD (Klein, et al., 2009). These genes include the

polyglutamine (polyQ) disease genes: *HTT* (Walker, 2007), *ATXN1* (Dubourg, et al., 1995), *ATXN2* (Charles, et al., 2007, Furtado, et al., 2004, Gwinn-Hardy, et al., 2000),
 60 *ATXN3* (Lu, et al., 2004a, Subramony, et al., 2002), *CACNA1A* (Kim, et al., 2010), and
TBP (Kim, et al., 2009). Of these genes, it has been suggested that intermediate-length
 polyQ expansions in *ATXN2* and *TBP* are associated with PD (Charles, et al.,
 2007, Furtado, et al., 2004, Kim, et al., 2009).

In addition, intermediate-length polyQ expansions (24-33 Qs) in *ATXN2* have
 65 recently been suggested as a risk factor for amyotrophic lateral sclerosis (ALS) (Chen,
 et al., 2011, Elden, et al., 2010). This observation has inspired several studies
 investigating how intermediate-length expansions of various polyQ disease genes
 contribute to neurodegenerative diseases other than those with which they were
 originally associated (Gispert, et al., 2012, Lee, et al., 2011b, Ross, et al., 2011).

70 Based on these findings and the suggestion that polyQ diseases may share common
 pathogenic mechanisms (Al-Ramahi, et al., 2007, Bertoni, et al., 2011, Chen and
 Burgoyne, 2012), we hypothesized that polyQ disease genes in general might play a
 role in PD. We focused on autosomal dominant PD (ADPD) because polyQ
 neurodegenerative diseases generally have an AD mode of inheritance, and we
 75 compared the distribution of polyQ repeat lengths in eight common genes between

ADPD patients and normal controls.

2. Methods

We conducted genetic analyses of *ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A*, *ATXN7*, *TBP*, *ATN1*, and *HTT* in a Japanese cohort with ADPD and normal controls. In this study, we
 80 classified the mode of inheritance as autosomal dominant when a family included affected members in two consecutive generations. The diagnosis of PD was confirmed by the participating neurologists based on established criteria (Hughes, et al., 1992).

We recruited the study subjects from the gene bank of our institution. We selected 299 unrelated patients with ADPD (169 females and 130 males; age at onset (AAO) = 57.7
 85 $\pm$ 13.6(SD) years old, range 17-85) from families with unexplained pathogenesis, i.e., those with no known pathogenic mutations in the *SNCA*, *PARK2*, *LRRK2*, and *VPS35* genes. A total of 329 healthy unrelated volunteers with no individual or family history of neurodegenerative disease (203 females and 126 males; age at examination = 57.5 $\pm$ 11.8(SD) years old, range 23-88) were examined as normal controls. Blood samples
 90 were obtained from the patients and controls, all of whom gave informed consent. Our institutional ethics committee approved the genetic study.

DNA was extracted from lymphocytes using standard methods. The polyQ repeat lengths in the polyQ disease genes were detected using capillary electrophoresis with fluorescent 5'-6-FAM-labeled forward primers. The primer sequences and PCR

95 conditions are described in Supplementary Table e1. The PCR products were mixed with the LIZ-500 size standard (Applied Biosystems, Foster City, CA) and processed on an Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems) for size determination. The sizes of the repeats were determined with GeneMapper™ 3.7 software (Applied Biosystems).

100 Statistical analysis was performed using JMP 8 software (SAS Institute, Cary, NC). We evaluated the association between ADPD and the polyQ repeat lengths of each gene using two-tailed Fisher's exact tests, as previously described (Gispert, et al., 2012, Lee, et al., 2011a, Ross, et al., 2011). A P value $< 0.05/8$ after Bonferroni correction was considered significant (8 is for the number of genes investigated in the current study.).

3. Results

3.1. Molecular Genetic Analysis

The range of repeat lengths in *ATXN2* was between 19 and 35. The majority of patients (95.6% of patients with ADPD and 98.6% of the controls) had a repeat length of 22, as reported in previous studies (Lee, et al., 2011a, Pulst, et al., 1996). Of the 253 patients with ADPD, 7 harbored repeat lengths longer than 24, whereas none of the controls did (2.8% and 0%, respectively; $P=0.0053$, Figure 1 and Table 1).

No substantial differences in the repeat lengths in *ATXN1*, *ATXN3*, *CACNA1A*,
ATXN7, *TBP*, *ATN1*, or *HTT* were observed between the ADPD patients and controls
 115 (Table 1 and Supplementary Figure e1).

We supplementarily sequenced entire coding exons and exon/intron boundaries of
 glucocerebrosidase gene (*GBA*) in the 7 probands with intermediate *ATXN2* polyQ
 expansion, because rare *GBA* mutations have been considered to be a risk factor for PD
 (Li, et al., 2013, Mitsui, et al., 2009); no *GBA* mutation was found in these 7 probands.

120 **3.2. Pedigree and clinical information for the seven probands with *ATXN2* polyQ repeat lengths > 24.**

Figure 2 shows the pedigrees of the seven probands with *ATXN2* polyQ repeat lengths
 > 24 and their families. In Family A, AII-2 presented with resting tremor in the
 bilateral lower extremities and left-dominant bradykinesia, which were responsive to
 125 L-DOPA and selegiline. AIII-1, who experienced rigidity and resting tremor
 predominantly in the left extremities, presented with tongue and jaw tremor
 (Supplementary Table e2). All of these signs were relieved by pramipexole. AIII-3 was
 reportedly initially diagnosed with essential tremor because her first sign was bilateral
 postural tremor. She underwent left and right thalamotomy at a one-year interval. She
 130 showed hyperreflexia in the lower extremities, but this symptom was presumably due

to cervical spondylosis, for which surgical decompression was performed. AIV-2 and AIV-3, who inherited an intermediate-length polyQ expansion of 35 Qs, were not affected at the time of this study.

In Family B, BI-2 was affected at an older age than her offspring, although their genotypes were the same, and all had L-DOPA-responsive parkinsonism with laterality (Supplementary Table e2).

In Family C, CII-2 was diagnosed with Parkinson's disease with dementia. Although her parents were consanguineous, her polyQ *ATXN2* lengths were heterozygous (29/22).

All other members of the seven families showed L-DOPA-responsive parkinsonism with laterality and were free of motor neuron signs, cerebellar ataxia, and saccadic eye movement disorder. None was reported to have any significant brain magnetic resonance imaging (MRI) abnormality (Supplementary Table e2).

4. Discussion

We investigated the distributions of the polyQ repeat lengths of eight common polyQ disease genes (*ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A*, *ATXN7*, *TBP*, *ATN1*, and *HTT*) in patients with ADPD. PolyQ repeat lengths > 24 in *ATXN2* were significantly more common in the patients than in the controls. To the best of our knowledge, there have

150 been only two similar studies investigating the distribution of *ATXN2* polyQ repeat
lengths in PD patients and controls to date (Gispert, et al., 2012, Ross, et al., 2011).
Although both previous studies failed to prove any significant difference, one (Gispert,
et al., 2012) showed that PD patients tended to have longer repeat lengths, consistent
with our results. In the other previous study (Ross, et al., 2011), the controls might
155 have included some number of pre-symptomatic patients because the mean age of the
controls was lower than that of the PD patients.

In reference to the recent studies concerning the effect of polyQ repeat length on
neurodegenerative disease, we screened for a threshold of the normal *ATXN2* polyQ
repeat length around a range from 24 to 34 (Charles, et al., 2007, Chen, et al.,
2011, Elden, et al., 2010, Gispert, et al., 2012, Lee, et al., 2011a, Lee, et al., 2011b, Ross,
160 et al., 2011). The distribution of our patients differed significantly from that of controls
only when the cutoff was set to 25. This may be much lower than the threshold for
ATXN2-related PD adopted by previous studies (Charles, et al., 2007), but it is possible
that the cutoff for *ATXN2* polyQ repeat length and its influence on PD may vary from
165 population to population, as is the case for ALS, as indicated in a previous study (Lee,
et al., 2011b). Such variation of the threshold would be consistent with the observation
that previous reports of *ATXN2*-associated PD have mainly been from East Asian

populations (Charles, et al., 2007, Klein, et al., 2009, Lu, et al., 2004b, Sun, et al., 2011, Wang, et al., 2009). Additional factors, such as cis- and trans-acting genetic elements, non-allelic genetic modifiers, and stochastic and environmental factors (Charles, et al., 2007, Pulst, et al., 2005), might have enhanced the toxicity of *ATXN2* intermediate-length polyQ expansion in our population.

We described the details of family members with *ATXN2* intermediate-length expansions (> 24 Qs, Figure 2 and Supplementary Table e2). These patients generally manifested typical PD phenotypes without motor neuron signs, cerebellar ataxia, or saccadic eye movement disorder, as was stated in previous reports (Furtado, et al., 2004, Klein, et al., 2009). A correlation between the association of AAO and polyQ repeat length was not clearly present or absent in our patients with repeat lengths of *ATXN2* > 24, as previously observed (Furtado, et al., 2002, Furtado, et al., 2004, Payami, et al., 2003, Sun, et al., 2011). For example, in Family A, members of the 3rd generation had earlier AAOs than did their mother. However, there was a gap between the AAOs of AIII-1 and AIII-3, even though their genotypes were the same. In addition, AIII-1 and AIII-3 had two allele expansions (35/32 Qs) instead of a single allele expansion, which might have caused their early onsets (Ragothaman, et al., 2004). The 35Q alleles may have been inherited ‘as is’ from AII-1, who reportedly had no neurological

disorder, although it is also possible that an expansion occurred upon transmission.

Thus, AAOs might be affected by features other than polyQ repeat length, such as genetic and epigenetic factors.

In the current study, we did not find any association between the ADPD phenotype

190 and the repeat lengths of polyQ disease genes other than *ATXN2*. This result implies that the contribution of *ATXN2* to ADPD is due to the specific effects of this gene rather than the presence of the polyQ expansion itself, as reported in a previous study of ALS (Lee, et al., 2011a). This result might appear to be inconsistent with recent reports suggesting that the intermediate polyQ expansion of *TBP* is likely to be a risk
195 factor for PD (Kim, et al., 2009, Wu, et al., 2004, Xu, et al., 2010, Yun, et al., 2011).

However, because those reports did not provide significant evidence, and because all of these studies were performed in East Asian patients, further evidence should be accumulated.

As a supplementary analysis, we also applied a multiple logistic regression including

200 the product terms *ATXN1*ATXN2*, *ATXN2*ATXN3*, and *ATXN2*CACNA1A* in order to screen for some interactions among these polyQ disease gene combinations, based on previous studies showing the possibility of interaction among these polyQ genes (Al-Ramahi, et al., 2007, Jardim, et al., 2003, Lessing and Bonini, 2008, Pulst, et al.,

2005). However, no significant difference was detected between the PD patients and

205 controls (with a threshold *P*-value of 0.05, Supplementary Table e3).

In conclusion, an intermediate-length polyQ expansion of *ATXN2* is likely to contribute to the pathogenesis of ADPD, either directly causing the PD phenotype or modifying the effects of unknown genes on the PD phenotype. Our results add to the recent finding that intermediate-length polyQ repeat expansions of *ATXN2* may be a

210 contributing factor in multiple neurodegenerative diseases.

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Figure Legends

Figure 1

The distribution of polyglutamine (polyQ) repeat lengths of *ATXN2* in autosomal

410 **dominant Parkinson's disease patients and normal controls.**

The histogram shows only subjects with ≥ 23 repeats.

Figure 2

The pedigrees of 7 families in which the proband has an *ATXN2* polyQ repeat

415 **length > 24.**

ATXN2 repeat lengths are listed above and to the right of the pedigree symbols of the genotyped individuals.

Table 1.

420 **Fisher's exact tests of polyQ repeat lengths between ADPD patients and controls.**

PolyQ disease gene	PolyQ repeat length	Conventional normal range†	Difference between ADPD patients and controls?
<i>ATXN1</i>	21-36	6-44	No
	19-35		
<i>ATXN2</i>	25-35Qs: 2.3% of ADPD, 0% of control	14-31	Yes, $P=0.0053$ ($<0.05/8$), OR= ∞
<i>ATXN3</i>	13-46	11-44	No
<i>CACNA1A</i>	5-18	4-18	No
<i>ATXN7</i>	1-10	4-19	No
<i>TBP</i>	30-40	25-42	No
<i>ATN1</i>	12-36	6-35	No
<i>HTT</i>	15-35	6-34	No

ADPD: autosomal dominant Parkinson's disease; Q: glutamine.

†The consensus normal ranges of the polyQ repeat lengths associated with the corresponding disease (e.g., *ATXN1* for SCA1). (Hands, et al., 2008, Sequeiros, et al., 2010)

Figure 1

PolyQ length	19	20-21	22	23	24	25	26	27	28	29	30	31-34	35
Patients	1	0	288	3	0	1	1	2	0	1	1	0	1
Controls	0	0	325	2	2	0	0	0	0	0	0	0	0

Figure 2