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 ± 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 ±

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 wasconsidered significant (8 is for the number of genes investigated in the

105 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

110 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

135   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).

140   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).

145   **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.,

160 2011,Elden, et al., 2010,Gispert, et al., 2012,Lee, et al., 2011a,Lee, et al., 2011b,Ross,

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

170   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

175   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,

180   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

185   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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Financial disclosure related to research covered in this article:

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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?
ATXN1	21-36	6-44	No
ATXN2	19-35 25-35Qs: 2.3% of ADPD, 0% of control	14-31	Yes, *P*=0.0053 (<0.05/8), OR=∞
ATXN3	13-46	11-44	No
CACNA1A	5-18	4-18	No
ATXN7	1-10	4-19	No
TBP	30-40	25-42	No
ATN1	12-36	6-35	No
HTT	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)

Supplementary Figure 1

The distribution of polyglutamine (polyQ) repeat lengths of the genes other than *ATXN2* in

autosomal dominant Parkinson's disease patients and normal controls.

Each gene was originally associated with the following diseases:

5   (A) Spinocerebellar ataxia (SCA)1, (B) SCA3, (C) SCA6, (D) SCA7, (E) SCA17, (F) Huntington's

disease, and (G) dentatorubral-pallidoluysian atrophy (DRPLA)

(A) *ATXN1*

(B) *ATXN3*

(C) *CACNA1A*

(D) *ATXN7*

(E) *HTT*

(F) *TBP*

(G) *ATN1*
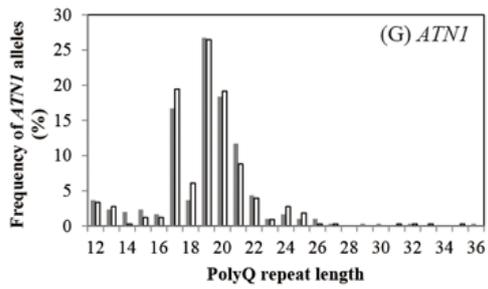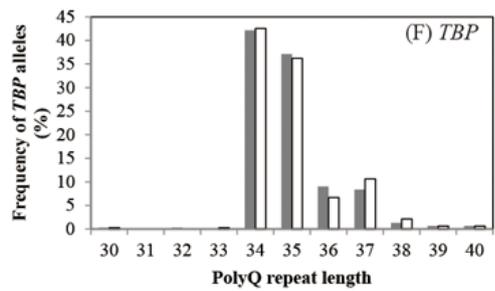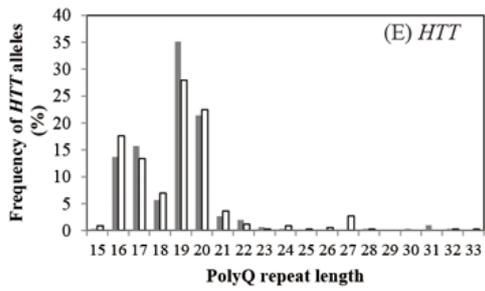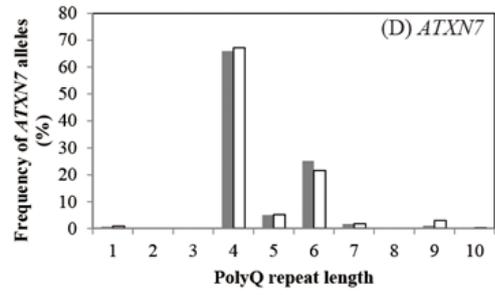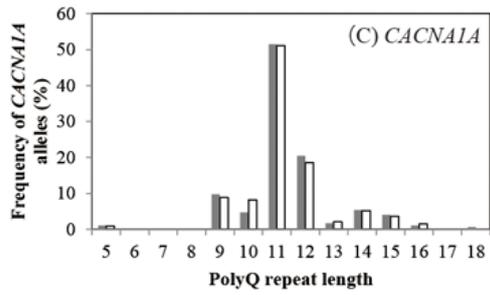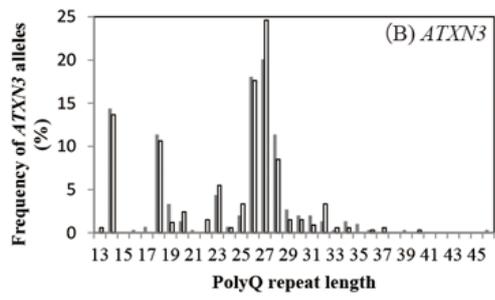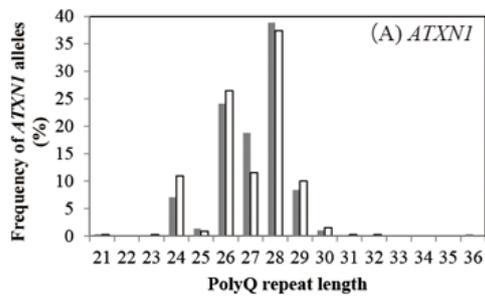
■ Patients

□ Controls

Supplementary Table 1. Primer Sequences and PCR conditions

Gene	Primers	PCR conditions	Notes
ATXN1	/FAM/5' –CAGTCTGAGCCAGACGCCGGGACACAAG– 3' 5' – CGGTGTTCTGCGGAGAACTGGAAATGTGG– 3'	2 min 94ºC, 35 cycles (30 sec 94ºC, 30 sec 62ºC, 1 min 72ºC), and 5 min 72ºC.	AmpliTaq Gold® 360 MasterMix(AppliedBiosystems™)
ATXN2	/FAM/5'–CGGGCTTGCGGACATTG –3' 5'–GCGTGCGAGCCGGTGTAT – 3'	1 min 94ºC, 30 cycles (30 sec 94ºC, 30 sec 60ºC, 2 min 72ºC), and 5 min 72ºC.	TaKaRa LA Taq™, 2×GC Buffer Ⅱ
ATXN3	/FAM/5'–TGGCCTTTCACATGGATGTGAA–3' 5'– CCAGTGACTACTTTGATTCG– 3'	10 min 94ºC, 30 cycles (1 min 95ºC, 1 min 53ºC, 2 min 72ºC), and 10 min 72ºC.	AmpliTaq Gold® 360 MasterMix(AppliedBiosystems™)
CACNA1A	/FAM/5' – CACGTGTCCTATTCCCCTGTGATCC – 3' 5' – TGGGTACCTCCGAGGGCCGCTGGTG – 3'	3 min 94ºC, 30 cycles (30 sec 94ºC, 30 sec 55ºC, 3 min 72ºC), and 5 min 72ºC	AmpliTaq Gold® 360 MasterMix(AppliedBiosystems™), GC-enhancer
ATXN7	/FAM/5' – AAGGAGCGGAAAGAATGTCG– 3' 5' – CAGGAAGTTTGGAAGCCTCA – 3'	2 min 94ºC, 35 cycles (30 sec 94ºC, 30 sec 54ºC, 1 min 72ºC), and 5 min 72ºC.	AmpliTaq Gold® 360 MasterMix(AppliedBiosystems™), GC-enhancer
TBP	/FAM/5' – GACCCCACAGCCTATTCAGA– 3' 5' – GCCTGAGGTTCCCTGTGTT– 3'	2 min 94ºC, 35 cycles (30 sec 94ºC, 30 sec 56ºC, 1 min 72ºC), and 5 min 72ºC.	AmpliTaq Gold® 360 MasterMix(AppliedBiosystems™)
ATN1	/FAM/ 5' – CCCAGTCCACCGCCCACCCACCA– 3' 5' – TGCTCCAGGAGGAGGGGGCCCAGA– 3'	2 min 94ºC, 32 cycles (30 sec 94ºC, 30 sec 65ºC, 1 min 72ºC), and 5 min 72ºC.	AmpliTaq Gold® 360 MasterMix(AppliedBiosystems™)
HTT	/FAM/5' – ATGGCGACCCTGGAAAGCTGATGAA – 3' 5' – GGCGGCTGAGGAAGCTGAGGA – 3'	9 min 95ºC, 30 cycles (20 sec 95ºC, 20 sec 60ºC, 20 sec 72ºC), and 5 min 72ºC.	AmpliTaq Gold® 360 MasterMix(AppliedBiosystems™)

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Supplementary Table 2   Clinical information for the patients with *ATXN2* polyQ repeat lengths > 24

Subjects	AII-2	AIII-1	AIII-2	BI-2	BII-1	BII-2	CII-2	DIII-3	EIII-3	FII-2	GIII-3
Age/Sex	79/F	58/M	56/F	78/F	50/M	48/F	74/F	65/F	68/F	77/F	56/F
Age at onset	70	44	34	76	43	41	58	43	58	64	54
PolyQ repeat lengths	32/22	35/32	35/32	27/22	27/22	27/22	29/22	27/22	25/22	30/22	26/22
Initial sign	Rt L/E Tr	Jaw Tr	Bil U/E Tr	UK	Rt L/E Br	UK	Lt L/E Tr&Br	Rt U/E Tr	UK	Lt U/E&L/E Br	Rt L/E Br
Rigidity	±	+	+	+	+	+	+	+	+	+	+
Bradykinesia	+	+	+	+	+	+	+	+	+	+	+
Resting tremor	+	+	+	+	+	+	+	+	+	+	-
Postural instability	+	-	+	+	+	+	+	+	+	+	+
Asymmetry at onset	+	-	-	+	+	+	+	+	+	+	+
Response to L-DOPA	+	+	+	+	+	+	+	+	+	+	+
Wearing off	-	-	-	-	+	+	-	+	+	+	-
LID	-	-	+	-	+	-	-	+	+	-	-
Dementia	-	-	-	-	-	-	+	-	+	-	-
Hyperreflexia	-	-	+	-	-	-	-	-	-	-	-
Additional info			*1, *2					*3			

Rt: Right, Lt: Left, Bil: Bilateral, L/E: Lower Extremity, U/E: Upper Extremity.

Tr: Tremor, Br: Bradykinesia, LID: L-DOPA-induced dyskinesia, UK: Unknown.

*1 Underwent decompression surgery for cervical spondylosis at the age of 56.

15   *2 Underwent Lt and Rt thalamotomy at the ages of 38 and 39, respectively.

*3 Underwent Lt and Rt thalamotomy at the ages of 46 and 57, respectively.

Supplementary Table 3 Multiple logistic regression with product terms

	Terms	P value (of likelihood ratio tests)	OR (95% CI)
PolyQ repeat length	ATXN1	0.771	1.019 (0.883-1.142)
	ATXN2	0.003*	1.961 (1.173-7.063)
	ATXN3	0.786	0.996 (0.966-1.026)
	CACNA1A	0.811	1.013 (0.913-1.123)
	ATXN7	0.777	0.98 (0.855-1.124)
	TBP	0.590	0.964 (0.841-1.103)
	ATN1	0.911	0.997 (0.945-1.051)
	HTT	0.906	0.996 (0.935-1.061)
Product terms	ATXN1-ATXN2	0.055	
	ATXN2-ATXN3	0.849	
	ATXN2-CACNA1A	0.558	
	Age	0.996	1 (0.987-1.013)
	Sex	0.122	