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IV. 研究成果の刊行物・別刷

Analysis of GWAS-linked loci in Parkinson disease reaffirms PARK16 as a susceptibility locus

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ABSTRACT

Objective: A genome-wide association study (GWAS) in the Japanese population identified 2 new Parkinson disease (PD) susceptibility loci on 1q32 (*PARK16*) (OMIM 613164) and *BST1*. We analyzed single nucleotide polymorphism (SNPs) located at the GWAS-linked loci (*PARK16*, *PARK8*, *PARK1*, and *BST1*) in a Chinese population and also conducted a meta-analysis in Asians by pooling 2 independent replication studies from Japan.

Methods: We conducted an analysis of 13 SNPs associated with PD GWAS-linked loci in 2 case-control cohorts comprised of 1,349 ethnic Chinese subjects.

Results: *PARK16*, *PARK8*, and *PARK1* loci but not *BST1* were found to be associated with PD. *PARK16* SNPs were associated with a decreased risk while *PARK1* and *PARK8* SNPs were associated with an increased risk of PD. A pooled analysis of our Chinese cohorts and 2 Japanese replication cohorts involving 1,366 subjects with PD and 16,669 controls revealed robust association with these 3 loci and also *BST1*. There was a trend toward a stronger protective effect of SNPs at the *PARK16* locus in sporadic PD compared to familial cases and in older compared to younger subjects.

Conclusions: Our study reaffirms the role of GWAS-linked loci in PD in Asian subjects and the strength of association is similar between Chinese and Japanese subjects. Efforts to elucidate the associated gene within *PARK16* locus are warranted. *Neurology*® 2010;75:508-512

GLOSSARY

GWAS = genome-wide association study; **PD** = Parkinson disease; **SNP** = single nucleotide polymorphism.

Parkinson disease (PD) (OMIM168600), a neurodegenerative disorder, is characterized by loss of dopaminergic neurons in the pars compacta of the substantia nigra. In recent years, several causative genes have been associated with PD for both familial and sporadic forms of the disease.¹ However, these mutations probably account for a small percentage of PD cases in most populations. Therefore the search for genetic susceptibility risk factors in the vast majority of PD continues to be of scientific interest. Specific to PD, genetic variants involving pathogenic genes (*LRRK2* [*PARK8*], OMIM 607060, *SYN* [*PARK1*], OMIM 168601) and specific candidate genes have been shown to associate with the disease.²⁻⁹ To date, there have been a few PD genome-wide association studies (GWAS) in the Caucasian population.^{2,10,11} However, their findings have not been consistently replicated.^{12,13} Among the many reasons, sample size and population stratification are some probable limitations. Recently, a GWAS study identified 2 new susceptibility loci on 1q32 (*PARK16*) (OMIM 613164) and *BST1* (bone marrow stromal cell antigen 1) (OMIM 004334) and also associations with known pathogenic genes involved in autosomal dominant forms of parkinsonism (*PARK1* on 4q22 and *PARK8* on 12q12) in the Japanese

Supplemental data at
www.neurology.org

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population.¹⁴ *PARK1* have also been implicated as a genetic risk factor in another GWAS study using samples from subjects of European ancestry.

Moreover, the disease associations at *PARK16* and *PARK8* were replicated in a Caucasian replication study, using individuals of European ancestry.¹⁵ Since Japanese and Chinese are of close Asian ancestry, we conducted a replication study of the GWAS-linked loci (*PARK16*, *PARK8*, *PARK1*, and *BST1*) in a Chinese population and also conducted a meta-analysis in Asian subjects by pooling 2 independent replication studies from Japan.

METHODS Ethnic Han Chinese subjects diagnosed with idiopathic PD by movement disorders neurologists at 2 different centers in Singapore (Singapore General Hospital and National Neuroscience Institute) were included. The diagnosis of PD was based on the UK Parkinson's Disease Society

Brain Bank clinical diagnostic criteria.¹⁶ Sporadic PD was defined as PD without a family history of disease. Controls of similar race, gender, and age from the same region as the patients with PD were also included. For controls, the age was matched ± 5 years to the age at onset of PD cases.

Patient consent. The study received approval from each institutional ethics committee and all the study subjects gave written informed consent for their DNA to be used for genetic research. PD samples that had previously screened positive for pathogenic mutations in [*alpha*]-synuclein, *Parkin*, *DJ-1*, *LRRK2*, and *PINK1* were not included.

Selection of SNPs. Of the 20 SNPs from 4 genes that were reported in the GWAS study,¹⁴ some SNPs are closely correlated ($r^2 > 0.8$). Therefore, we only selected 13 noncorrelated SNPs ($r^2 < 0.8$) (figure) for analysis in this study.

Genetic analysis. Genotyping was carried out with MALDI-TOF mass spectrometry using the Sequenom MassARRAY™ system (San Diego, CA). Multiplex genotyping assays were designed using the Sequenom DESIGNER software (San Diego, CA). PCR (5 ng of genomic DNA) and primer extension reactions were carried out initially according to the Sequenom genotyping assay iPLEX™ protocol. Confirmation of the variants with sequence analysis was carried out for random samples in ABI 3730 automated DNA sequencer (Applied Biosystems).

Statistical analysis. Statistical analyses were performed using R 2.10.1 software. χ^2 and Student *t* tests were used for comparing the categorical and continuous variables. We assessed each variant for departure from Hardy-Weinberg equilibrium. The results from pooled datasets were analyzed together and individual and pooled odds ratios and associated 95% confidence intervals were tabulated. We estimated the per-allele odds ratios using logistic regression and used Wald test to test whether the coefficients are significant. To test for association of allele frequency at each SNP with PD, we used χ^2 test with 1 degree of freedom. A multivariate logistic regression analysis adjusted for age and gender was performed. Stratified analysis by family history and age at onset was also carried out. Meta-analysis was performed to combine our Chinese study with 2 Japanese studies. Heterogeneity among sample sets was accessed using Woolf test. The meta-analysis was conducted using the Mantel-Haenszel method. As this is a replication study involving 4 independent loci, we made a modest correction for multiple comparisons with statistical significance defined at $p < 0.01$.

RESULTS Demographics. We studied a total of 1,349 ethnic Chinese subjects comprised of 2 case-control cohorts including a total of 433 patients with PD and 916 controls from 2 independent centers in Singapore. None of the patients with PD were from consanguineous families and about 3% reported a positive family history. The demographics of the study subjects are summarized in table 1.

Genotyping data. Thirteen SNPs located within the *PARK16*, *PARK8*, *PARK1*, and *BST1* loci, which were associated with PD in the GWAS study,¹⁴ were analyzed. The 2 case-control cohorts were analyzed together to improve the power of analysis. The frequency of all the SNPs in the studied sample followed Hardy-Weinberg equilibrium, with the

Figure Selection of genome-wide association study single nucleotide polymorphisms (SNPs)

Gene	SNP	Position No.	SNPs selected for genotyping	r2
<i>BST1</i>	rs11931532	26	rs11931532_1	>0.8
<i>BST1</i>	rs12545693	37		
<i>BST1</i>	rs4538473	62		>0.8
<i>BST1</i>	rs4698412	59	rs4698412_1	
<i>PARK8</i>	rs1994090	16	rs1994090_1	
<i>PARK8</i>	rs2046932		rs2046932_1	>0.8
<i>PARK8</i>	rs4768212			
<i>PARK8</i>	rs2708453	85		>0.8
<i>PARK8</i>	rs7304279	54	rs7304279_1	
<i>PARK16</i>	rs11240572		rs11240572_1	
<i>PARK16</i>	rs16856139	7	rs16856139_1	
<i>PARK16</i>	rs708730	85		>0.8
<i>PARK16</i>	rs823156	78	rs823156_1	
<i>PARK16</i>				
<i>PARK16</i>			rs823128_1	>0.8
<i>PARK16</i>	rs947211	70	rs947211_1	
<i>PARK1</i>	rs11931074	5	rs11931074_1	>0.8
<i>PARK1</i>	rs3857088	25		
<i>PARK1</i>	rs6532194	130	rs6532194_1	
<i>PARK1</i>	rs894278	76	rs894278_1	

Only 1 SNP from each shaded box was selected for analysis as the 2 SNPs in each shaded box are in close linkage disequilibrium ($r^2 > 0.8$).

Table 1 Demographics of subjects with Parkinson disease and controls (total n = 1,349)

	Parkinson disease	Controls
No.	433	916
Age, median, y	64	56
Age at onset, median, y	60	
Men/women, %	56/44	60/40

exception of rs4698412 (*BST1* locus), which showed a slight deviation ($p = 0.022$).

Ten SNPs belonging to *PARK16*, *PARK8*, and *PARK1* loci were found to be associated with PD (table 2). *PARK16* SNPs were associated with a decreased risk while *PARK1* and *PARK8* SNPs were associated with an increased risk of PD. A multivariate logistic regression analysis with disease/control group as the outcome measure and adjusting for age and gender revealed significant association with *PARK16*, *PARK8*, and *PARK1* but not the *BST1* locus (table 3). Stratification by family history revealed a trend toward a stronger protective effect of SNPs at the *PARK16* locus in sporadic PD compared to familial cases and in the older compared to younger subjects (table e-1 on the *Neurology*[®] Web site at www.neurology.org). At the *PARK8* locus, there was a trend toward a higher risk in familial PD compared to sporadic PD and in the older age group compared to the younger ones (stratified at age at onset <50 years or <55 years) (table e-2, A and B).

Pooled analysis of Asian subjects. There was no heterogeneity among the Chinese and Japanese datasets

except rs11931532 (*BST1*) (table e-3). As the frequency of the studied SNPs was similar in the Japanese and Chinese control populations, a combined analysis of previously published 2 case-control replication cohorts in Japanese¹⁴ and our cohorts was carried out (1,366 subjects with PD and 16,669 controls). Robust association with *PARK16*, *PARK8*, and *PARK1* was observed in addition to *BST1* (table e-4).

DISCUSSION A PD GWAS study in an American population identified 11 SNPs using a family-based design in tier 1 and a case-control design in tier 2.² A consortium from 14 centers which pooled 5,526 patients with PD and 6,682 controls was unable to replicate any significant association with the PD-associated SNPs.¹²

More recently, in a PD GWAS study conducted in Asia, investigators¹⁴ identified 2 new susceptibility loci (*PARK16*, *BST1*) and also strong associations at *PARK1* and *PARK8*, 2 known loci implicated in autosomal dominant forms of parkinsonism. In the same study, the findings were replicated in 2 Japanese cohorts. The signal at the *PARK16* locus was less robust in the GWAS study in Caucasians¹⁵ and did not surpass correction for multiple testing. However, the investigators subsequently conducted an analysis in their replication sample and found an association of SNP (rs823128) at the *PARK16* locus. The minor allele frequency (3%–4%) of the implicated *PARK16* SNP in Caucasians is low and this probably accounts for the relatively weaker association.

We conducted a replication study in the Chinese population. In the combined analysis of our 2 cohorts, we were able to independently demonstrate an

Table 2 SNP frequency and summary of analysis in subjects with Parkinson disease and controls

Gene	SNP	Chromosome	Allele (minor/major)	Minor allele frequency (case/control)	p Value ^a	OR	95% CI
<i>BST1</i>	rs11931532	4	C/T	0.49/0.50	0.5719	0.95	0.81-1.12
<i>BST1</i>	rs4698412	4	A/G	0.40/0.39	0.8269	1.02	0.86-1.21
<i>PARK8</i>	rs1994090	12	G/T	0.07/0.04	0.0004	1.92	1.33-2.77
<i>PARK8</i>	rs2046932	12	A/G	0.06/0.03	0.0013	1.85	1.26-2.69
<i>PARK8</i>	rs7304279	12	T/C	0.07/0.03	0.0003	1.95	1.35-2.81
<i>PARK16</i>	rs11240572	1	A/C	0.15/0.18	0.0135	0.75	0.60-0.94
<i>PARK16</i>	rs16856139	1	T/C	0.12/0.14	0.1447	0.83	0.65-1.06
<i>PARK16</i>	rs823156	1	G/A	0.18/0.22	0.0161	0.77	0.63-0.95
<i>PARK16</i>	rs823128	1	G/A	0.12/0.15	0.0316	0.76	0.60-0.98
<i>PARK16</i>	rs947211	1	A/G	0.39/0.42	0.1250	0.88	0.74-1.04
<i>PARK1</i>	rs11931074	4	G/T	0.39/0.45	0.0028	0.78	0.66-0.92
<i>PARK1</i>	rs6532194	4	C/T	0.39/0.45	0.0072	0.80	0.68-0.94
<i>PARK1</i>	rs894278	4	G/T	0.41/0.36	0.0102	1.25	1.05-1.48

Abbreviations: CI = confidence interval; OR = odds ratio; SNP = single nucleotide polymorphism.

^a Corrected p value; significance at <0.01.

Table 3 Multivariate analysis adjusted for age and gender

SNP	Crude OR (95% CI)	Adjusted OR (95% CI)	p (Wald test)
rs16856139	0.84 (0.62-1.15)	0.84 (0.62-1.15)	0.287
rs823128	0.66 (0.48-0.89)	0.65 (0.48-0.89)	0.006
rs947211	0.7 (0.56-0.87)	0.71 (0.57-0.88)	0.002
rs823156	0.67 (0.52-0.88)	0.68 (0.52-0.89)	0.005
rs11240572	0.63 (0.48-0.84)	0.64 (0.48-0.85)	0.002
rs11931532	1.09 (0.88-1.34)	1.09 (0.88-1.35)	0.423
rs4698412	0.9 (0.72-1.11)	0.89 (0.72-1.11)	0.307
rs11931074	0.72 (0.58-0.88)	0.7 (0.56-0.86)	<0.0001
rs894278	1.27 (1.02-1.58)	1.27 (1.02-1.59)	0.032
rs6532194	0.77 (0.63-0.94)	0.76 (0.62-0.93)	0.008
rs1994090	1.64 (1.01-2.66)	1.67 (1.03-2.72)	0.039
rs7304279	1.64 (1.01-2.66)	1.67 (1.03-2.72)	0.039
rs2046932	1.55 (0.94-2.56)	1.57 (0.95-2.61)	0.078

Abbreviations: CI = confidence interval; OR = odds ratio; SNP = single nucleotide polymorphism.

association of *PARK16*, *PARK8*, and *PARK1* loci, except for *BST1*. While not all the SNPs located in *PARK 16* reached significant association, the trend and the effect size difference (odds ratio 0.8 to 0.9) between cases and controls was largely similar between our Chinese subjects and the Japanese subjects in the discovery GWAS sample and 2 replication Japanese cohorts. The frequency of the 13 SNPs (10%–50%) was also similar in these 2 Asian races. We observed a more robust association of the SNPs at the *PARK8* and *PARK1* loci. As there was no evidence of genetic heterogeneity between Chinese and Japanese at these GWAS-linked loci, we conducted a pooled evaluation of our cohorts and the 2 replication Japanese cohorts to increase the power of analysis. The pooled analysis further reaffirms the results across each independent dataset and the discovery dataset. As we did not observe any positive trend with the *BST1* locus in our cohort, replication in an independent Chinese cohort is advised. Though the median age of the controls in our study was 4 years younger compared to median age at onset of 60 years in subjects with PD, multivariate analysis after taking into account the effects of age and gender revealed significant associations similar to those observed in the univariate analysis. Furthermore, comparison with a selected set of 428 controls with median age at onset of 60 years showed similar results (data not shown).

Consistent and independent replication of genetic association studies remains the litmus test of the validity of the findings. While identification of susceptibility alleles is an important step, unraveling the biologic basis of their actions, if any, remains a chal-

lenging task as many of these variants are not located in the coding regions. Among the identified loci, *PARK16* is interesting as it contains a few candidate genes (*SLC41A1*, *RAB7L1*, *NUCKS1*). rs947211 is associated with transcript level of *NUCKS1*,¹⁴ though Simón-Sánchez et al.¹⁵ did not find any association of the SNPs and expression levels of the genes at *PARK16*, *PARK8*, and *PARK1*. The association of a lower risk with PD suggests that protective gene variants or genes are located within this region or they need interaction with unknown genes to exert their effect. It is possible that these SNPs are not the actual causative variants based on their biologic plausibility and the varied strength of their association. The original GWAS study¹⁴ did not analyze the strength of association between familial and sporadic PD and between older and younger subjects. Despite the limitation of a smaller sample size in the subset analysis, our data revealed a consistent trend toward a stronger protective effect of SNPs at the *PARK16* locus in sporadic PD compared to familial cases and in the older compared to younger subjects (table e-1). The significance of this is unclear, though it suggests that common variants at *PARK16* locus may be more relevant to a general group of patients with PD. There was also a consistent trend toward a higher risk in familial PD compared to sporadic PD and in the older age group compared to the younger ones at the *PARK8* locus (table e-2). As *PARK8* is responsible for autosomal dominant parkinsonism and is associated with late-onset disease, this observation may not be unexpected. Since these SNPs are located from intron 2 of *SLC2A13* to the upstream region of *LRRK2*, our finding strengthens the suggestion that these are likely to be risk variants of the *LRRK2* gene.

While current evidence suggests that common genetic variants play a role in the etiology of typical PD, GWAS studies by their inherent design may not be able to detect rare variants.^{9,17} It is also possible that cases selected for GWAS studies may not be particularly enriched with genetic susceptibility alleles, and other compounding factors like reduced penetrance of PD genes and gene-environmental interaction were unaccounted for. Thus multiple approaches including linkage analysis, sequencing, and sibpair analysis would be needed to uncover additional variants/causative genes and susceptibility loci.

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DISCLOSURE

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CORRECTION

High insulinlike growth factor I is associated with cognitive decline in Huntington disease

In the article “High insulinlike growth factor I is associated with cognitive decline in Huntington disease” by Saleh et al. (*Neurology*® 2010;75:57–63), there were errors in the Acknowledgment and Coinvestigator list. The Acknowledgment should have included the Centre d'Investigation Clinique Groupe Hospitalier Chenevier-Mondor Créteil for assistance in recruiting patients. The Coinvestigator list should have included Graça Morgado (Centre d'Investigation Clinique Groupe Hospitalier Chenevier-Mondor Créteil, Nurse, Patient Recruitment and Investigation). The following members of the Ethical Committee were listed in error as coinvestigators: Franc Bellivier, MD, PhD (Groupe Hospitalier Chenevier-Mondor, Scientific Committee); Catherine Bertrand, PhD (Groupe Hospitalier Chenevier-Mondor, Scientific Committee); Christian Danesi, PhD (UDAF, Patient's Representative); Frédéric Galacteros, MD, PhD (Groupe Hospitalier Chenevier-Mondor, President of the Committee); Hebert Geschwind, MD, PhD (Paris 12 University, Ethical Committee); Isabelle Guigon, PhD (Groupe Hospitalier Chenevier-Mondor, Director of the INSERM); Brigitte Langlois, PhD (Paris 12 University, Ethical Committee); Patrick Pollissard-Pasbecq, PhD (Paris 12 University, Jurist); Aurore Renversade (Groupe Hospitalier Chenevier-Mondor, Nurse); Françoise Roudot-Thoraval, MD, PhD (Groupe Hospitalier Chenevier-Mondor, Scientific Committee); Brigitte Tastet, MD (Paris 12 University, Advisory Committee); and Anick Tibi, PharmD (AGEPS, Scientific Committee).

CORRECTION

Analysis of GWAS-linked loci in Parkinson Disease reaffirms PARK16 as a susceptibility locus

In the article “Analysis of GWAS-linked loci in Parkinson disease reaffirms PARK16 as a susceptibility locus” by E.-K. Tan et al. (*Neurology*® 2010;75:508–512), the joint first author's name should have been listed as H.-H. Kwok, PhD. The authors regret the error.

ORIGINAL ARTICLE

Genetic and clinical analysis in a Chinese parkinsonism-predominant spinocerebellar ataxia type 2 family

Hao Sun¹, Wataru Satake², Changjun Zhang³, Yoshitaka Nagai², Youyong Tian⁴, Shouzhi Fu⁴, Jiankun Yu¹, Yaping Qian¹, Yuan Qian¹, Jiayou Chu¹ and Tatsushi Toda²

Parkinson's disease is a degenerative central nervous system disorder that often impairs motor skills, speech and other functions. We discovered a large Chinese family showing primarily parkinsonism symptoms with autosomal dominant inheritance. Six affected individuals in the family showed typical parkinsonism symptoms, including pill-rolling tremor. Two other affected individuals showed cerebellar ataxia symptoms. A whole-genome scan using the 50K single nucleotide polymorphism array with three different linkage methods detected two positive regions on chromosome 12q24.1 and 5q13.3. The *ATXN2* gene, responsible for spinocerebellar ataxia type 2 (SCA2) was located precisely in the center of the positive region on chromosome 12. Further analysis of SCA2 revealed heterozygous pathological CAG expansions in the family. The affected individuals' symptoms were typical of parkinsonism, but complex. Inverse correlation between CAG repeat size and age of onset is not obvious in this pedigree. This parkinsonism-predominant SCA2 family shared the same disease gene locus with other 'standard' SCA2 families, but it is possible that variations in one or more modifier genes might account for the parkinsonism-predominant SCA2 predisposition observed in this pedigree.

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Keywords: 5q13; genome-wide scan; linkage study; single nucleotide polymorphism chip

INTRODUCTION

Parkinson's disease (PD), a degenerative central nervous system disorder, often impairs motor skills, speech and other functions.¹ It is found worldwide, with incidence rates varying from country to country. The prevalence of PD increases with age. In Europe, PD affects about 1–2% of individuals over 60 years of age.² Although there is no cure for PD, further understanding of its genetic risks can improve neuroprotective or preventive approaches. Causative genes for Mendelian-inherited parkinsonism have been identified. Point mutations and multiplications in the *SNCA* gene have been found in some families with autosomal dominant inheritance.^{3,4} To date, mutations in the *LRRK2* gene are the most common cause of Mendelian PD. In studies across several populations, 5–15% of autosomal dominant PD families carried mutations in *LRRK2* (see refs 5, 6). Mutations in three genes, *PARK2* (encoding parkin), *PINK1* (*PARK6*) and *DJ-1* (*PARK7*), have been identified in autosomal recessive PD, which is characterized by an early age at onset and

therefore referred to as autosomal recessive juvenile parkinsonism.^{7–9} The expanded *ATXN2* gene, which causes spinocerebellar ataxia type 2 (SCA2), was found in some families with only or mainly typical parkinsonism.^{10,11} Although some parkinsonism clinical signs such as dystonia and tremor have been described in SCA2, dopamine-responsive parkinsonism has been infrequently described in SCA2 (see ref. 12). The sign of dopamine-responsive just has been seen in some Chinese families^{13,14} and some white families.^{15,16}

We described here a large family from Hubei, China, that showed primarily autosomal dominant inheritance of parkinsonism symptoms acrossing four generations. Affected family members exhibited typical clinical features of PD, such as pill-rolling tremors and levodopa responsiveness. However, some family members showed cerebellar symptoms. The patients who showed the atypical phenotypes opposed to the typical cerebellar ataxia maybe have more complex genetic causes than normal SCA2 patients. So, we performed a whole-genome linkage study to identify possible genetic causes in

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this family. At same time, the molecular and clinical features of this family were analyzed.

MATERIALS AND METHODS

Clinical information

The proband (IV-3) was initially diagnosed as PD in 2001, and therefore the family was classified as a PD pedigree. In 2007, we performed neurological examinations for eight patients in the family, and we examined four patients using magnetic resonance imaging (MRI). Blood samples were obtained from patients and unaffected relatives with informed consent. Approval for the study was obtained from the Ethical Committees of participating institutions.

Whole-genome linkage analysis

Genomic DNA was isolated from blood using QIAamp DNA Blood Mini Kits (Qiagen, Shanghai, China). Single nucleotide polymorphism genotyping was performed for 27 individuals from the family (Figure 1) using the Human Mapping 50K Xba 240 SNP array (Affymetrix, Santa Clara, CA, USA). Signal intensity data were analyzed using GeneChip DNA analysis software GDAS v.3.0.2.8 (Affymetrix). The genotype data were converted to linkage format using ALOHOMORA software¹⁷ and subjected to quality control routines, including gender check and graphical representation of relationship errors.¹⁸ Mendelian errors were detected with PedCheck,¹⁹ and non-informative markers

were deleted before further analysis. Genome-wide non-parametric multipoint linkage, single parametric and single non-parametric linkage analysis were performed using GeneSpring GT software (Agilent, Santa Clara, CA, USA).

Trinucleotide repeat analysis

We screened for mutations in the *ATXN2* gene using PCR amplification with previously published SCA-2A and SCA-2B oligonucleotide primers.²⁰ PCR products were sized precisely using capillary electrophoresis with an ABI 3730xL DNA analyzer (Applied Biosystems, Foster city, CA, USA) and compared with known samples using GeneMapper V3.5 (Applied Biosystems, Foster city, CA, USA). Some samples were isolated from agarose gels and used as DNA templates for sequencing with the Big-dye terminator kit (Applied Biosystems) on the ABI 3730xL analyzer.

RESULTS

Clinical information

The family (Figure 1) consisted of 39 members in four generations, with 16 affected members. All family members reside in Hubei Province, China. The inheritance pattern is autosomal dominant. We collected peripheral blood from 27 family members, including 10 affected members. Clinical data were shown in Table 1 for 8 of 10 patients collected in 2007. Detailed clinical data were unavailable for

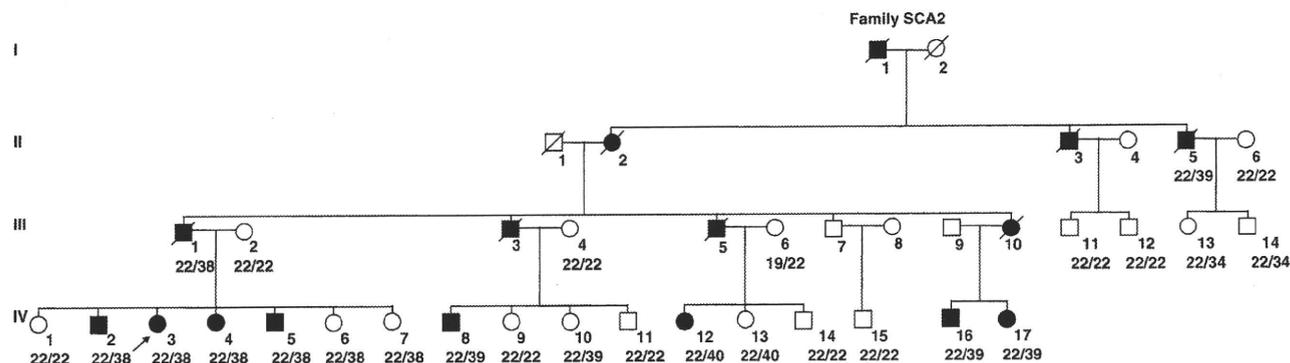


Figure 1 Pedigree of a Chinese family ascertained with parkinsonism-predominant spinocerebellar ataxia type 2 (SCA2). Squares indicate males; circles, females. A slash through the symbol indicates deceased and an arrow points to the proband. The pedigree contains 16 known affected individuals; eight patients are living. SCA2 CAG repeat allele sizes are listed below the pedigree symbols of the 27 individuals who have been genotyped.

Table 1 Clinical and genetic features of the SCA2 family

No.	IV-2	IV-3	IV-4	IV-5	IV-8	IV-12	IV-16	IV-17
Age at onset	33	36	37	22	20	37	39	35
Age at examination	46	44	42	39	50	45	51	38
Resting tremor	2	1	0	2	0	0	2 ^a	0
Bradykinesia	3	2	0	3	0	1	2	2
Rigidity	3	2	0	2	0	1	2	1
Postural instability	2	1	0	2	0	2	2	1
Masked face	3	2	0	3	1	2	2	2
Levodopa response	+	+	—	NT	NT	+	+	NT
Gait ataxia	0	0	2	1	0	0	0	0
Limb ataxia	1	1	2	1	0	0	0	0
Slow saccade	2	1	1	2	3 ^b	1	1	0
Vertical gaze palsy	2	0	0	2	3 ^b	0	0	0
Hyporeflexia	3	3	1	0	1	0	0	0
Cerebellar atrophy on MRI	2	1	3	1	NT	NT	NT	NT
CAG repeats	22/38	22/38	22/38	22/38	22/39	22/40	22/39	22/39

Abbreviations: MRI, magnetic resonance imaging; SCA2, spinocerebellar ataxia type 2.

^aPill-rolling tremor.

^bDifficulty initiating pursuit movements.

0 indicates that the individual was tested and the symptom was absent. 1, mild; 2, moderate; and 3, marked. NT indicates that the individual could not be tested. A (+) indicates that the finding was present; a (–) indicates absent.

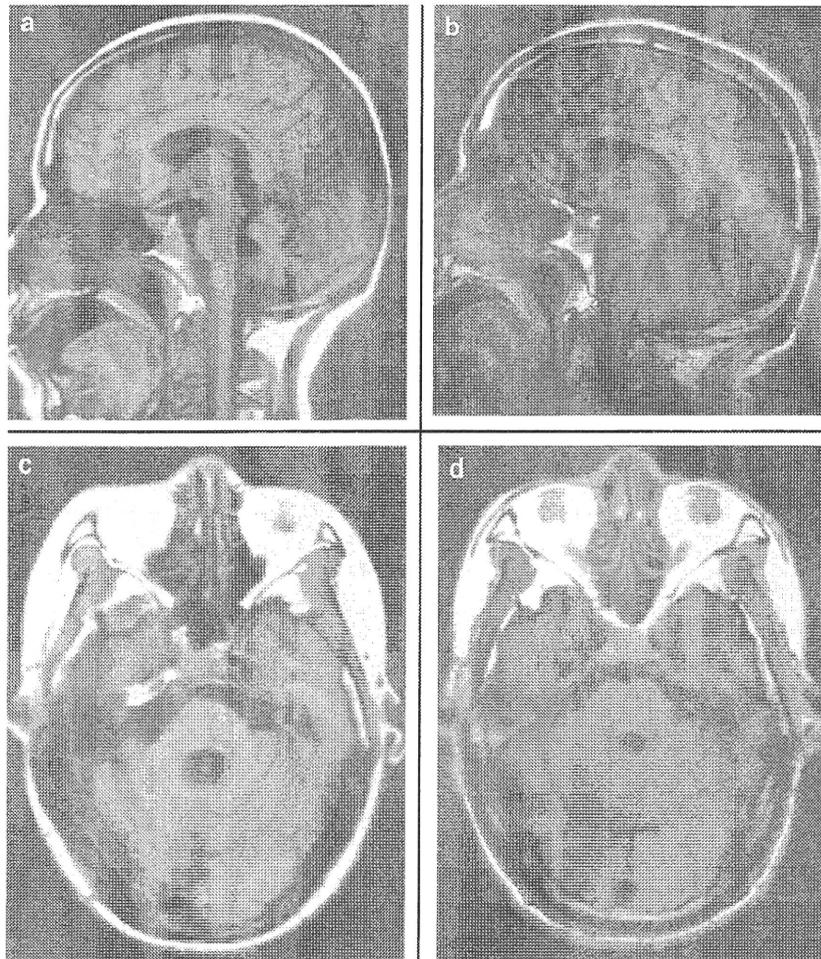


Figure 2 T1-weighted magnetic resonance imaging (MRI) of IV-4 (a, c) and IV-5 (b, d). Patient IV-4 showed marked cerebellar atrophy, and patient IV-5 showed no cerebellar atrophy.

the other two deceased patients (II-5 and III-1). Family members of the two patients provided ambiguous clinical data, and their preliminary diagnosis indicated that the two patients had parkinsonism symptoms. Blood samples, collected in 2001, were provided by their neurologist. Age of symptomatic disease onset varied from 20 to 39 years, with an average age at onset of 32.4 years.

Most patients showed typical parkinsonism symptoms, such as resting tremor, bradykinesia, rigidity and postural instability (IV-2, IV-3, IV-5, IV-12, IV-16 and IV-17). Patient IV-16 had pill-rolling tremors. However, two patients (IV-4 and IV-8) showed cerebellar symptoms such as limb ataxia and slow saccade, but no typical parkinsonism symptoms.

Five of eight patients were treated with levodopa. Only one (Patient IV-4) of them showed no response. This patient also lacked typical parkinsonism symptoms. Patients IV-2, IV-3, IV-4 and IV-5 were examined using MRI analysis. MRI images from Patients IV-4 and IV-5 were shown in Figure 2. Marked cerebellar atrophy was found in Patient IV-4, and no cerebellar atrophy was found in Patient IV-5.

Whole-genome linkage analysis

Genome-wide analysis revealed two positive regions for linkage, on chromosomes 12 and 5. On chromosome 12, the highest single-point parametric log of odds (LOD) score (2.59) was detected at rs2695281 (100.5 Mb, NCBI Build 36). The highest multi-point non-parametric

LOD score on chromosome 12 was 3.5, with a multi-point non-parametric LOD score >3 at 94.9–115.6 Mb (NCBI Build 36). On chromosome 5, the highest single-point parametric LOD score (2.73) was detected at rs10491487 (80.4 Mb, NCBI Build 36). The highest multi-point non-parametric LOD score on chromosome 5 was 3.5 with a multi-point non-parametric LOD score >3 at 79.8–81.2 Mb (NCBI Build 36). Multi-point non-parametric linkage results were compatible with the single-point parametric linkage results on both chromosomes 5 and 12. Single-point non-parametric linkage analysis detected no positive result. Most of single-point non-parametric LOD scores were smaller than 2, and the highest was 2.1, on chromosome 21 (Figure 3). Gender and relationships in the single nucleotide polymorphism array data were accurate, and PedCheck detected no Mendelian errors.

The *ATXN2* gene, responsible for SCA2, was located at 110.3 Mb (NCBI Build 36) on chromosome 12, within the linkage-positive region of 94.9–115.6 Mb.

Trinucleotide repeat analysis

We performed SCA2 molecular analysis in all 27 family members. Three types of heterozygous pathological CAG expansion (38, 39 and 40 repeats) and two types of normal repeats (19 and 22) were identified. A borderline 34 CAG repeat was found in the two family members. Genotypes with repeat numbers for individual family

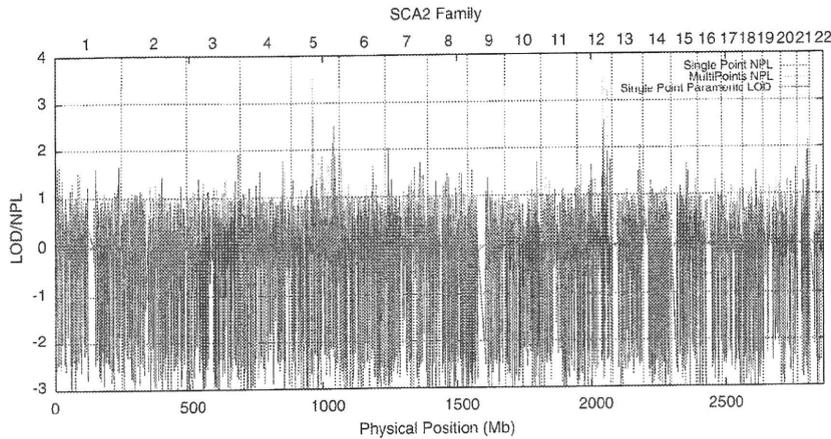


Figure 3 Three types of log of odds (LOD) scores were calculated from the genome-wide scan. The LOD score plot was created with GNPLOT 4.0 (<http://www.gnplot.info>) using the LOD plot-drawing Perl script included in the ALOHOMORA package. On chromosome 12, the highest MultiPoint non-parametric LOD score was 3.5 and the highest SinglePoint parametric LOD score was 2.59. On chromosome 5, the highest MultiPoint non-parametric LOD score was 3.5 and the highest SinglePoint parametric LOD score was 2.73. The MultiPoint non-parametric linkage results were compatible with the SinglePoint parametric linkage results on chromosomes 5 and 12. LOD scores < -3 are not shown.

members were shown in Figure 1. Sequence analysis of expanded alleles from IV-5, IV-8, IV-12 and III-14 revealed interruptions of CAG base pair repeats with CAA. The 38-repeat expansion in IV-5 contained 29 CAGs, followed by one CAA and eight CAGs. The 39-repeat expansion IV-8 contained 30 CAGs, one CAA and eight CAGs. The 40-repeat expansion in IV-12 contained 31 CAGs, one CAA and eight CAGs. The normal 34-repeat CAG expansion in III-14 contained 25 CAGs, one CAA and eight CAGs.

All affected individuals in the branch of III-1 carried 38 trinucleotide repeats. IV-6 and IV-7 also carried the 38 trinucleotide repeats, but as of 2007 no parkinsonism or ataxia had been observed in these individuals. The 39 trinucleotide repeats occurred in the branches of II-5, III-3 and III-10. Similar to IV-6 and IV-7, individual IV-10 carried the 39 trinucleotide repeats, but had shown no parkinsonism or ataxia symptoms in 2007. In the branch of II-5, the 39 repeats were shortened to 34 in transmission. III-13 and III-14, who carried 34 trinucleotide repeats, showed no symptoms.

DISCUSSION

We described a levodopa-responsive parkinsonism Chinese family with SCA2 trinucleotide expansions. The symptoms observed in this family were primarily parkinsonism, but complex. Some affected family members showed typical clinical manifestations of parkinsonism. Four of five patients responded to levodopa treatment. One affected individual (IV-4) lacking parkinsonism symptoms was unresponsive to levodopa; MRI analysis of this patient showed marked cerebellar atrophy. Patient IV-8 showed just mild masked face, but his cerebellar symptoms were severe. In other patients (IV-12, IV-16 and IV-17), cerebellar symptoms were minor or absent. Overall, the clinical signs in this family appear most similar to parkinsonism.

Molecular analysis of SCA2 expansion in the family revealed three types of expanded CAG repeats. An inverse correlation has been established between age of SCA2 onset and CAG repeat length, with repeat length accounting for 54–80% of variance.^{21,22} However, such inverse correlation was not observed in our pedigree. In the branches of III-1 and III-2, the age of onset in affected individuals who carried the 38 CAG repeats ranged from 22 to 37. Two other individuals with 38 CAG repeats showed no clinical signs at the time of examination (IV-6, age 37 years in 2007; and IV-7, age 35 years in 2007). The age of onset in the three affected individuals who carried 39 CAG repeats

(II-5, IV-16 and IV-17) ranged from 20 to 39 years. Another carrier of 39 CAG repeats (IV-10) showed no clinical signs in 2007, at age 43 years. One of the two individuals who carried 40 CAG repeats had an age at onset of 37 years (IV-12). The other (IV-13) had no clinical signs in 2007, at age 43 years. These observations showed that repeat length alone cannot account for age of onset in this family. Conversely, it is not possible to predict when, or if, the unaffected carriers of expanded CAG repeats will eventually show clinical signs.

Some researches suggested that CAA interruption can lead to phenotypic variation.^{23,24} The results of Sobczak *et al.*²⁵ showed that the CAA interruptions are major determinants of the CAG repeat folding in the SCA2 transcripts. The SCA2 transcripts interrupted by the CAA should generate shorter branched hairpins and the uninterrupted repeats transcripts form single slippery hairpins. The patients who carried SCA2 expansions with and without interruptions show two different phenotypes.^{23,24} It may be caused by the different CAG repeat folding that would interact differently with double-stranded RNA binding proteins and interfere with mRNA transcription or translation.²⁴ That structural organization of CAG expansions with interruption associate with phenotypic variation has been also reported in other neurodegenerative disorders such as SCA1 (see ref. 26). In our family, all patients carried the CAG expansions with one CAA interruption, but showed two different phenotypes. The patients IV-4 and IV-8 showed more ataxia symptoms than the other patients. Especially, patient IV-4 had no response to the levodopa treatment and had marked cerebellar atrophy on MRI. The symptoms of IV-4 made him look more like the typical SCA2. Therefore, the phenotypic variation in our family may be caused by other unknown reason such as co-effect of SCA2 gene and some modifier gene, rather than the different CAA interruption.

The SCA2 CAG repeat is highly unstable through intergenerational transmission, with a tendency to expand. One study reported that there are 27 families' SCA2 CAG repeats changed in length among 32 SCA2 families, with a mean increase of 2.2 repeat units.²⁷ In this family, we observed eight transmissions of an expanded SCA2 CAG repeat with no increase in repeat length. Six transmissions yielded no change, whereas two transmissions yielded contractions. As blood samples from several older patients were not collected, we cannot know exactly which SCA2 CAG repeat increased through intergenerational transmission. The explanation for the relative instability in the

family may include genetic or epigenetic factors. A previous study defined the range of the normal SCA2 allele size as 17–31 CAG repeats, whereas full pathogenic mutations had 36–64 repeats.²⁸ Two unaffected family members who carried the contracted CAG repeat had borderline mutations of 34 CAG repeats (III-13, 45 years of age in 2007; and III-14, 38 years of age in 2007), although 34 CAG repeats were found in some SCA2 patients.²⁹

Our linkage analysis revealed two positive regions, one at chromosome 12q24.1 and one at chromosome 5q13.3. On chromosome 12, the mutated *ATXN2* gene, which causes SCA2, is located in the middle of the linkage-positive region. This demonstrates the accuracy of the detection methods and shows that the positive regions are reliable. As the disease gene of SCA2 was located to chromosome 12q23-24.1 (see ref. 30), most of the reports about parkinsonism-predominant SCA2 was based on case cohorts. The linkage study in our family strengthened that the parkinsonism-predominant SCA2 carried the same pathogenic gene as the typical SCA2 from the whole genome perspective. We assume that this parkinsonism-predominant SCA2 family shares a disease locus with other standard SCA2 families, but it is possible that one or more modifier genes interact with *ATXN2* to produce clinical signs more similar to parkinsonism. It is difficult to predict whether the other positive region at 5q13.3 is a real positive region or an artifact. If it is not an artifact, perhaps there would be a modifier gene harbored in the region. To prove it, more detailed gene mutation analyses in the region or other linkage analyses for additional parkinsonism-predominant SCA2 families need to be performed.

The prevalence of SCA2 among patients with familial parkinsonism ranged from 1.5 to 10% (ref. 12). It is seen occasionally in German²⁸ and Japanese¹⁰ populations. After Gwinn-Hardy *et al.* described a Chinese American family with only or mainly typical parkinsonism in 2000 (see ref. 11), similar families have been reported. Surprisingly, Lu *et al.*²³ reported that four families with SCA2 were identified among 41 families with familial parkinsonism, about 10% of familial parkinsonism carried the expanded SCA2 CAG repeats in Taiwan people. Therefore, it is possible that the mutation rate of potential modifiers might account for the ethnic differences in the predisposition of parkinsonism-predominant SCA2. Better understanding of factors that determine a predominant parkinsonism phenotype in SCA2 may shed light on the pathogenesis of PD.

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Phenotypic spectrum of patients with *PLA2G6* mutation and *PARK14*-linked parkinsonism

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ABSTRACT

Background: *PLA2G6* is the causative gene for infantile neuroaxonal dystrophy, neurodegeneration associated with brain iron accumulation, and Karak syndrome. Based on previous reports, patients with *PLA2G6* mutations could show axonal dystrophy, dystonia, dementia, and cerebellar signs. Recently, *PLA2G6* was also reported as the causative gene for early-onset *PARK14*-linked dystonia-parkinsonism.

Methods: To clarify the role of *PLA2G6* mutation in parkinsonism, we conducted mutation analysis in 29 selected patients with very early-onset (≤ 30 , mean 21.2 ± 8.4 years, \pm SD) parkinsonism. These patients had other clinical features (e.g., mental retardation/dementia [14/29], psychosis [15/29], dystonia [11/29], and hyperreflexia [11/29]).

Results: Two novel compound heterozygous *PLA2G6* mutations were detected (patient A: p.F72L/p.R635Q; patients B1 and B2: p.Q452X/p.R635Q). All 3 patients had early-onset L-dopa-responsive parkinsonism with dementia and frontotemporal lobar atrophy. Disease progression was relatively rapid. SPECT in patient B1 showed frontotemporal lobar hypoperfusion. MRI in patient A showed iron accumulation in the substantia nigra and striatum.

Conclusions: Although the clinical presentation of *PLA2G6*-associated neurodegeneration was reported to be homogeneous, our findings suggest patients with *PLA2G6* mutation could show heterogeneous phenotype such as dystonia-parkinsonism, dementia, frontotemporal atrophy/hypoperfusion, with or without brain iron accumulation. Based on the clinical heterogeneity, the functional roles of *PLA2G6* and the roles of *PLA2G6* variants including single heterozygous mutations should be further elucidated in patients with atypical parkinsonism, dementia, or Parkinson disease. *PLA2G6* mutations should be considered in patients with early-onset L-dopa-responsive parkinsonism and dementia with frontotemporal lobar atrophy.

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GLOSSARY

INAD = infantile neuroaxonal dystrophy; **NBIA** = neurodegeneration associated with brain iron accumulation; **PD** = Parkinson disease; **PLAN** = *PLA2G6*-associated neurodegeneration.

PLA2G6 is the causative gene for infantile neuroaxonal dystrophy (INAD), neurodegeneration associated with brain iron accumulation (NBIA), and Karak syndrome.¹⁻³ INAD is a rare autosomal recessive progressive neurodegenerative disorder that culminates in death by 10 years of age. INAD and NBIA share distinctive pathologic features of neuroaxonal degeneration with distended axons (spheroid bodies) throughout the CNS.² Some patients with NBIA show very early onset and rapid psychomotor regression, early cerebellar signs, pyramidal signs, and visual disturbances. These clinical entities have been also collectively termed *PLA2G6*-associated neurodegeneration (PLAN).⁴

Supplemental data at
www.neurology.org

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PLA2G6 was also reported recently as the causative gene for *PARK14*, a form of autosomal recessive early-onset dystonia-parkinsonism.⁵ The 2 reported unrelated families with homozygous *PLA2G6* mutation had early-onset L-dopa-responsive dystonia-parkinsonism, pyramidal signs, and cognitive/psychiatric features, together with mild generalized cerebral atrophy on MRI but no iron accumulation.⁵ So far, there is no other *PLA2G6* mutation analysis in parkinsonism, and the role of *PLA2G6* in parkinsonism and related disorders is still not clear.⁵ In this study, we conducted mutation analysis in patients with very early-onset parkinsonism and discuss the intriguing clinical pictures of PLAN and *PARK14*-linked parkinsonism.

METHODS In this study, we selected 29 (mainly Japanese) patients with very early-onset (≤ 30 years, mean 21.2 ± 8.4 years, \pm SD) parkinsonism. Two of the 29 patients were from consanguineous families. These patients had mental retardation/dementia (14/29), psychosis (15/29), dystonia (11/29), and hyperreflexia (11/29), but none had obvious cerebellar signs. This phenotype is an unusual, very severe form of parkinsonism and cognitive decline, unlike idiopathic Parkinson disease (PD). We assumed that *PLA2G6* mutation could be found in the patients with these symptoms and accordingly performed direct sequencing for all 17 coding exons and exon-intron boundaries of *PLA2G6* in these patients.⁴ The methods of direct sequencing, PCR conditions, and sequences of the primers are described in e-Methods and table e-1 on the *Neurology*[®] Web site at www.neurology.org. Known pathogenic mutations causing early-onset dystonia/parkinsonism such as *parkin*, *PINK1*, *DJ1*, *FBXO7*, and *GCH1* were excluded previously in these patients.

RESULTS We detected 2 novel compound heterozygous *PLA2G6* mutations (patient A: p.F72L/p.R635Q; patients B1 and B2: p.Q452X/p.R635Q). Haplotype analysis suggested a founder effect (figure 1). The sites of novel p.F72L and p.R635Q mutations were conserved among various species (figure e-1). These mutations were not detected in 200 chromosomes of normal Japanese controls.

All 3 patients had early-onset L-dopa-responsive parkinsonism with dementia and frontotemporal lobar atrophy. SPECT in patient B1 showed frontotemporal lobar hypoperfusion. (Direct sequencing for coding exons and exon-intron boundaries of *MAPT* and *PGRN* in the 3 patients revealed no pathogenic mutation.) MRI in patient A showed iron accumulation in the substantia nigra and striatum (table 1, figure 2). No autopsy has been conducted.

Patient A developed right resting tremor and gait disturbance at age 20. Subsequently, she developed postural instability, antepulsion, and rigidity. The parkin-

sonian symptoms deteriorated slowly, and L-dopa at 300 mg/day was initially effective, but the treatment soon induced on-off phenomenon (Hoehn & Yahr 3/5; on/off period), severe dyskinesia, and psychoses. At age 35, she developed mild dementia with 20/30 points in Hasegawa's Dementia Scale-Revised. She developed depression, personality/behavior changes, disordered social conduct, and apathy. Brain MRI showed frontotemporal lobar atrophy.

Patient B1 was the younger brother of patient B2. Parkinsonian symptoms appeared at 25 years of age in patient B1 and 30 years of age in patient B2. The disease course was similar; the initial symptom was gait disturbance in both patients, and they showed bradykinesia and generalized rigidity, but no resting tremor. Their parkinsonian motor symptoms responded initially to L-dopa, but they developed L-dopa-induced dyskinesia and wearing off within a few months, and the efficiency of L-dopa faded away within a few years. They became wheelchair-bound 4–6 years after onset. They were cooperative and not depressed. Global cognitive function as assessed by the Mini-Mental State Examination or Wechsler Adult Intelligence Scale-Revised was normal or slightly impaired, and memory and verbal functions were normal at onset. Frontal lobe function as assessed by the Wisconsin Card Sorting Test and verbal fluency test 2–3 years after onset was normal in patient B1 and mildly impaired in patient B2. Patient B2 experienced transient auditory hallucination unrelated to L-dopa. The cognitive function declined along with motor function. Hoehn & Yahr stages were over 4.5 (even in best-on period) (table 1).

DISCUSSION To date, parkinsonism-dystonia patients with *PLA2G6* mutation have been reported in only certain populations, such as Indians, Pakistanis, and Iranians.^{2,5,6} Although patients with *PLA2G6* mutation were reported to be rare, after checking for these clinical features in such disorders as early-onset parkinsonism and dementia, we detected 2 parkinsonism families with *PLA2G6* mutation without invasive biopsy.⁴ Thus, clarification of the genotype-phenotype correlation and distribution of *PLA2G6* mutation is essential for the diagnosis using efficient and noninvasive procedures. In addition to the Caucasian, Arabic, and Chinese PLAN patients,^{4,6,7} identification of the 3 Japanese patients with novel *PLA2G6* mutations suggests that parkinsonism patients with *PLA2G6* mutation have even greater worldwide distribution among various populations. Although highly selected patients with an unusually severe and atypical phenotype were included in this study, *PLA2G6* mutation was found in 6.9% (2/29) of our very early-onset (≤ 30 years) parkinsonism cohort, suggesting the frequency is not rare. Moreover,