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（難病・がん等の疾患分野の医療の実用化研究事業（難病関係研究分野））研究事業

神経系疾患の集中的な遺伝子解析及び原因究明に関する拠点研究　に関する研究

平成２３年度　総括研究報告書

研究代表者　辻　省次

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厚生労働科学研究費補助金(難病・がん等の疾患分野の医療の実用化研究事業(難病関係研究分野))

総括研究報告書

神経疾患の集中的な遺伝子解析及び原因究明に関する拠点研究に関する研究

研究代表者　辻　省次

研究要旨　本研究の目的は，次世代シーケンサーを用いた大規模ゲノム配列解析拠点，および，高度のゲノムインフォマティクス拠点を整備し，神経疾患の病因・病態機序を解明することである．次世代シーケンサー(HiSeq2000 2 台)，ライブラリー作成の自動化システム，ゲノムインフォマティクス解析のためのサーバー整備が完了し，exome 解析については，1ヶ月で 192 検体の解析，全ゲノム解析については 1ヶ月で 16 検体の解析，および必要なゲノムインフォマティクス処理が十分なスループットで行うことが可能となった．今後この拠点を最大限活用し，神経疾患の原因究明，遺伝子診断を進めていく．

A. 研究目的

本研究の目的は，次世代シーケンサーを用いた大規模ゲノム配列解析拠点，および，高度のゲノムインフォマティクス拠点を整備し，神経疾患の病因・病態機序を解明することである．また，神経系疾患のゲノム解析拠点として，本研究事業の一般研究や他の難治性疾患克服研究事業の研究班などと連携をして，ゲノム解析拠点としての機能を果たし，神経系疾患の病因，病態機序の解明，診断未確定の神経難病の解明などに貢献することを目的としている．本研究の特色は，遺伝性神経疾患，孤発性神経疾患の病因の解明を実現するために，次世代シーケンサーを用いた大規模ゲノム配列解析および高度のゲノムインフォマティクス解析に基づく研究を強力に推進するという提案にある．期待される成果は，1. 遺伝性神経疾患の病因遺伝子の発見，2. 孤発性神経疾患の発症に関与するゲノム要因を網羅的に発見すること，3. 診断未確定の疾患の病因の解明・診断の確定に貢献すること，4. 新しい研究パラダイムとして，大規模ゲノム配列解析拠点として，国内の多くの医療機関の研究者の研究の発展に貢献すること，そして，これらの成果のすべてが，疾患の病因・病態機序に基づく，病態抑止型治療の開発，実現に貢献できることである．

B. 研究方法

次世代シーケンサーを活用した大規模なゲノム配列解析拠点を整備することをめざし，Illumina HiSeq2000 を 2 台，ライブラリーの作成のためのロボティクスシステム(SureSelect 自動化システム)を整備した．また，ゲノムインフォマティクス処理のためのサーバシステム（144core ）を導入整備した．これらのシステムを用いて，全ゲノム解析，exome 解析のためのプロトコールを整備した．多系統萎縮症，遺伝性神経疾患対象として exome 解析を中心に，ゲノム配列を開始した．

（倫理面への配慮）

「ヒトゲノム・遺伝子解析研究に関する倫理指針を遵守し，研究倫理審査委員会から承認を得て実施する．次世代シーケンサーや情報解析のためのサーバーシステムなどは，入退室管理のもとに運営する．サーバーシステムについては，インターネットから隔離された環境で運用する．

C. 研究結果

exome 解析については，1ヶ月で 192 検体の解析，全ゲノム解析については 1 ヶ月で 16 検体の解析，および必要なゲノムインフォマティクス処理が十分なスループットで行うことが可能となった．ゲノムインフォマティクスにおいては，配列データの品質チェック，QV 補正，アラインメント，SNV の推定ができるパイプラインを整備した．また，日本人ゲノムの variation database の整備を進めており，195 名の日本人の variation database を作成し，疾患研究の際に常に参照できるようにした．

診断への応用としては，これまでに遺伝性脊髄小脳変性症（AOA2），Posterior column ataxia with retinitis pigmentosa，遺伝性白質脳症（Hereditary diffuse leuko-encephalopathy with axonal spheroids, Aicardi-Goutieres syndrome）の診断確定を次世代シーケンサーを用いて行うことができた．

遺伝性疾患の病因遺伝子の解明を目指した研究としては，家族性筋萎縮性側索硬化症，家族性多系統萎縮症，ALS/PDC，てんかんなどを対象とした全ゲノム配列解析を開始し，有力な候補遺伝子が見出され，validation のための研究を進めている．

疾患関連遺伝子の探索については，多系統萎縮症 231 例，コントロール 135 名の exome 解析を開始した．

一般研究拠点との連携については，鹿児島大学の高嶋先生の拠点から提供された 288 例の Charcot-Marie-Tooth 病の exome 解析を行い，その結果を，依頼側に届けることができた．

特定疾患の調査研究班との連携も積極的に進め，パーキンソン病，脊髄小脳変性症，Fahr 病，痙性対麻痺など多くの遺伝性神経疾患の病因遺伝子解明のための exome あるいは全ゲノム解析を実施した．

D. 考察

次世代シーケンサー（Illumin HiSeq2000 2 台），ライブラリー作成の自動化システム，ゲノムインフォマティクス解析のためのサーバー整備が完了し，exome 解析については，1ヶ月で 192 検体の解析，全ゲノム解析については 1 ヶ月で 16 検体の解析，および必要なゲノムインフォマティクス処理が十分なスループットで行うことが可能となった．このことにより，拠点内のプロジェクト（遺伝性神経疾患の病因探索，孤発性疾患の疾患関連遺伝子探索，難病の遺伝子診断）の推進はもちろん，一般研究拠点との連携による大量の exome 解析を実施することができた．さらに，特定疾患調査研究班との連携により，数多くの共同研究が開始され，ゲノム解析が順調に進んでいる．

診断への応用では，具体的な成果が得られており，遺伝性脊髄小脳変性症（AOA2），Posterior column ataxia with retinitis pigmentosa，遺伝性白質脳症（Hereditary diffuse leuko- encephalopathy with axonal spheroids, Aicardi-Goutieres syndrome）の診断確定が実現できた．このことは，稀少難病の診断に次世代シーケンサーを用いた網羅的なゲノム配列解析が非常に有用であることを示している．

遺伝性神経疾患については，家族性多系統萎縮症，家族性筋萎縮性側索硬化症，てんかんなどで有力な候補遺伝子が見出されており，次世代シーケンサーを用いた解析の有効性が示されている．孤発性疾患についても，多系統萎縮症について，大規模の症例，コントロールについて exome 解析を開始しており，common disease-multiple rare variants 仮説に基づき，

effect size の大きいゲノム要因の同定が期待される.

次世代シーケンサーの拠点整備が完了したことから解析のスループットは極めて大きくなっており，このシステムを十分に活かすためには，一般研究拠点，特定疾患の調査研究班との連携により，解析規模を大きくしていくことが重要である．特に孤発性神経疾患の解明のためには，大規模リソースを必要としており，多施設共同研究体制の充実が望まれる.

E. 結論

次世代シーケンサーを用いた神経疾患の集中的な遺伝子解析及び原因究明に関する拠点の整備が完了した．今後この拠点を最大限活用し，神経疾患の原因究明，遺伝子診断を進めていく.

F．健康危険情報

特になし

G．研究発表

1. 論文発表

1. Ishiura H, Fukuda Y, Mitsui J, Nakahara Y, Ahsan B, Takahashi Y, Ichikawa Y, Goto J, Sakai T and Tsuji S. Posterior column ataxia with retinitis pigmentosa in a Japanese family with a novel mutation in *FLVCR1*. ***Neurogenet*** 12:117-21, 2011
2. Matsukawa T, Wang X, Liu R, Onuki Y, Kubota A, Hida A, Kowa H, Fukuda Y, Ishiura H, Mitsui J, Takahashi Y, Aoki S, Takizawa T, Shimizu J, Goto J, Proud CG and Tsuji S. Adult-onset leukoencephalopathies with vanishing white matter with novel missense mutations in EIF2B2, EIF2B3, and EIF2B5. ***Neurogenet*** 12:259-61, 2011
3. Seki N, Takahashi Y, Tomiyama H, Rogaeva E, Murayama S, Mizuno Y, Hattori N, Marras C, Lang AE, St George-Hyslop P, Goto J and Tsuji S. Comprehensive mutational analysis of LRRK2 reveals variants supporting association with autosomal dominant Parkinson's disease. ***J. Hum. Genet.*** 56:671-5, 2011
4. Matsukawa T, Asheuer M, Takahashi Y, Goto J, Suzuki Y, Shimozawa N, Takano H, Onodera O. Nishizawa M, Aubourg P and Tsuji S. Identification of novel SNPs of *ABCD1, ABCD2, ABCD3,* and *ABCD4* genes in patients with X-linked adrenoleukodystrophy(ALD) based on comprehensive resequencing and association studies with ALD phenotypes. ***Neurogenet***,12:41-50,2011

2. 学会発表

1. Ishiura H, Fukuda Y, Mitsui J, Nakahara Y, Ahsan B, Takahashi Y, Ichikawa Y, Goto J, Sakai T, Tsuji S. Massively parallel sequence analysis reveals the causative gene of posterior column ataxia with retinitis pigmentosa 61st American Society of Human Genetics/12th International Congress of Human Genetics. October 11-15, 2011, Toronto
2. Matsukawa T, Takahashi Y, Goto J, Suzuki Y, Shimozawa N, Takano H, Onodera O, Nishizawa M, Tsuji S. Comprehensive resequencing of PEX5, PEX13 and PEX14 gene in patients with X-linked adrenoleukodystrophy (ALD) and association studies with the phenotypes of ALD. 61st American Society of Human Genetics/12th International Congress of Human Genetics. October 11-15, 2011, Toronto
3. Takahashi Y, Goto J, Tsuji S. Resequencing-based case control study of SQSTM1 for sporadic amyotrophic lateral sclerosis based on common disease-multiple rare variant hypothesis. 61st American Society of Human Genetics/12th International Congress of Human Genetics. October 11-15, 2011, Toronto

H．知的財産権の出願・登録状況

該当なし

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Hiroyuki Ishiura & Yoko Fukuda & Jun Mitsui & Yasuo Nakahara & Budrul Ahsan & Yuji Takahashi & Yaeko Ichikawa & Jun Goto & Tetsuo Sakai & Shoji Tsuji	Posterior column ataxia with retinitis pigmentosa in a Japanese family with a novel mutation in FLVCR1	Neurogenetics	12（2）	117-121	2011
Naomi Seki1, Yuji Takahashi1,Hiroyuki Tomiyama2, Ekaterina Rogaeva3, Shigeo Murayama4,Yoshikuni Mizuno2,Nobutaka Hattori2, Connie Marras5, Anthony E Lang5, Peter St George-Hyslop3,6, Jun Goto1 and Shoji Tsuji1	Comprehensive mutational analysis of LRRK2 reveals variants supporting association with autosomal dominant Parkinson's disease	Journal of Human Genetics	56	671–675	2011
Takashi Matsukawa & Xuemin Wang & Rui Liu & Noel C. Wortham & Yuko Onuki &Akatsuki Kubota & Ayumi Hida & Hisatomo Kowa &YokoFukuda & Hiroyuki Ishiura &Jun Mitsui & Yuji Takahashi & Shigeki Aoki & Shunya Takizawa & Jun Shimizu &Jun Goto & Christopher G. Proud & Shoji Tsuji	Adult-onset leukoencephalopathies with vanishing white matter with novel missense mutations in EIF2B2, EIF2B3,and EIF2B5	Neurogenetics	12	259–261	2011

Takashi Matsukawa & Muriel Asheuer & Yuji Takahashi & Jun Goto & Yasuyuki Suzuki & Nobuyuki Shimozawa & Hiroki Takano & Osamu Onodera & Masatoyo Nishizawa & Patrick Aubourg & Shoji Tsuji	Identification of novel SNPs of ABCD1, ABCD2, ABCD3, and ABCD4 genes in patients with X-linked adrenoleukodystrophy (ALD) based on comprehensive resequencing and association studies with ALD phenotypes	Neurogenetics	12	41–50	2011

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ORIGINAL ARTICLE

Posterior column ataxia with retinitis pigmentosa in a Japanese family with a novel mutation in FLVCR1

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Abstract Posterior column ataxia with retinitis pigmentosa (PCARP) is an autosomal recessive neurodegenerative disorder characterized by retinitis pigmentosa and sensory ataxia. Previous studies of PCARP in two families showed a linkage to 1q31–q32. However, detailed investigations on the clinical presentations as well as molecular genetics of PCARP have been limited. Here, we describe a Japanese consanguineous family with PCARP. Two affected siblings suffered from childhood-onset retinitis pigmentosa and slowly progressive sensory ataxia. They also showed mild mental retardation, which has not been described in patients with PCARP. Parametric linkage analysis using high-density single nucleotide polymorphism arrays supported a linkage to the same locus. Target capture and high-throughput sequencing technologies revealed a novel homozygous c.1477G>C (G493R) mutation in *FLVCR1*, which cosegregated with the disease. A recent study has identified three independent mutations in *FLVCR1* in the original and other families. Our results further confirmed that PCARP is caused by mutations in *FLVCR1*.

Keywords Posterior column ataxia with retinitis pigmentosa · Linkage analysis · Target capture · Massively parallel sequencing · FLVCR1

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Introduction

Posterior column ataxia with retinitis pigmentosa (PCARP, MIM 609033) is an autosomal recessive, childhood onset neurodegenerative disorder characterized by sensory ataxia and retinitis pigmentosa. Previous studies [1, 2] on American and Spanish families revealed a linkage to chromosome 1q31–q32 defined by D1S2692 (206.10M in NCBI36/hg18 assembly, http://genome.ucsc.edu/) and D1S2141 (213.26M). Because only two families have been reported with proven linkage to 1q31–q32, detailed investigations on the clinical presentations as well as the molecular genetics of PCARP have been limited. We have recently identified a Japanese family with PCARP with supportive linkage to 1q31–q32. Employing target capture and high-throughput sequencing technologies, we herein identified a novel mutation in *FLVCR1*.

Patients and methods

Patients

The pedigree chart of the Japanese family with PCARP is shown in Fig. 1. Two affected siblings and an unaffected sibling were born to consanguineous parents. Written informed consent was obtained from all the participants. All the participants were clinically evaluated by a neurologist (T.S.). The study was approved by the ethical committee of The University of Tokyo.

Linkage analysis

Genomic DNAs were extracted from peripheral blood leukocytes according to standard protocols. Five of the

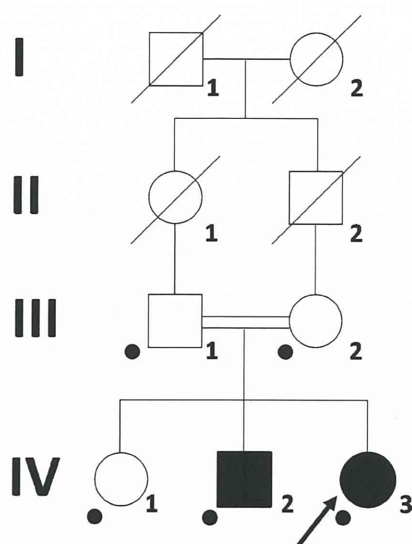

Fig. 1 Pedigree chart. Pedigree chart of a Japanese family with PCARP. *Squares and circles* indicate males and females, respectively. Affected persons are designated with filled symbols. A *diagonal line* through a symbol represents a deceased person. A person *with the arrow* is the index patient. Persons with available genomic DNAs are indicated by *dots*

family members were genotyped using Affymetrix 50K Xba and 50K Hind arrays (Affymetrix, Santa Clara, CA) following the manufacturer's instructions. Using pipeline software SNP-HiTLink [3], single nucleotide polymorphisms (SNPs) with a p value of >0.05 in the Hardy–Weinberg test, a call rate of >0.95, a confidence score of genotyping <0.1, a minor allele frequency in the controls >0, and intermarker distances of 80 to 120 kb were selected for the linkage analysis. Parametric multipoint linkage analysis (autosomal recessive model with complete penetrance) was performed with Allegro version 2 [4]. Haplotypes were reconstructed using Allegro.

Target capture

Using NimbleGen's custom human sequence capture 2.1M array (Roche NimbleGen, Madison, WI), we designed probes corresponding to the target regions (chromosome 1: 200,106,833–213,208,193 and chromosome 20: 15,311,130–32,500,997) avoiding repetitive sequences in the regions. Twenty micrograms of genomic DNA of an affected person (IV-2) was captured according to the manufacturer's instructions [5], followed by quantification of average fold enrichment of the captured sample.

Massively parallel sequencing

Since the target capture procedure was optimized for 454 Sequencer (454 Life Sciences, Branford, CT), the enrichment sample was nebulized for 16 min for further fragmentation to obtain appropriate lengths of DNA fragments suitable for sequencing using Genome Analyzer IIx (GAIIx, Illumina, San Diego, CA). We then carried out single-end library preparation for GAIIx. Massively parallel sequencing was accomplished using two lanes of GAIIx (100-bp-long single-end read).

Short read alignment and variant calling

After removing the tag sequences designed for 454 sequencing system, short reads were aligned to the reference genome (NCBI36/hg18 assembly) with bwa [6] using default parameters. After removing multiple aligned reads (mapping quality of 0), single nucleotide variants (SNVs) and short insertion/deletion variants (indels) were called with SAMtools [7]. Quality threshold for SNVs and indels were set to 20 and 50, respectively.

Annotation and confirmation of variant calls

After annotation with RefSeq (http://www.ncbi.nlm.nih.gov/projects/RefSeq/) and dbSNP130/dbSNP131 (http://www.ncbi.nlm.nih.gov/projects/SNP/), all the novel nonsynonymous variant calls were subjected to direct nucleotide sequence analysis for confirmation. Confirmed amino acid changes were then subjected to PolyPhen (http://genetics.bwh.harvard.edu/pph/) for prediction of functional effects.

Direct nucleotide sequence analysis for confirmation of mutation in *FLVCR1*

Polymerase chain reaction was performed using a primer pair of FLVCR1-F 5′-GCAATTCGCCTACCTCAACT-3′ and FLVCR1-R 5′-ACACAAGTCCTTTTGCCAGG-3′ and LATaq (TaKaRa, Ohtsu, Shiga, Japan). Direct nucleotide sequence analysis was performed using ExoSAP-IT (USB, Cleveland, OH), a BigDye Terminator v3.1 kit, and XTerminator employing an ABI PRISM3100 sequencer (Life Technologies Corporation, Carlsbad, CA).

Results

Clinical manifestations of the family

The index patient (IV-3 in Fig. 1) was a 31-year-old female, who was noted to be night-blind at the age of five by her mother. Thereafter, she developed gait disturbance. She consulted with an ophthalmologist at the age of 31. Ophthalmologic examinations revealed retinitis pigmentosa of the bone corpuscle type with optic atrophy. On neurological examination, she was found to be mildly

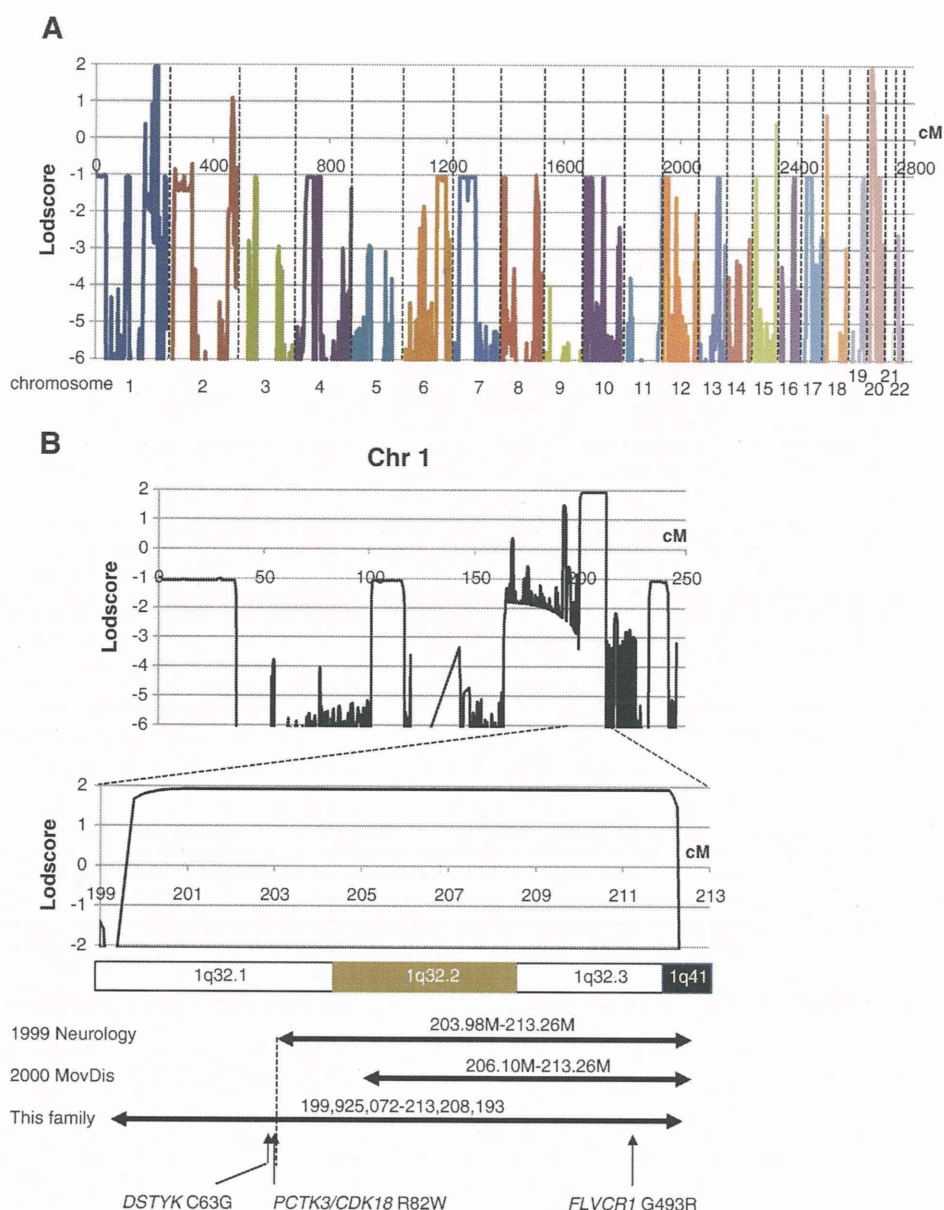

Fig. 2 Multipoint linkage analysis and candidate regions. **a** Parametric multipoint linkage analysis (autosomal recessive model) of the family revealed linked regions on chromosomes 1 and 20. Multipoint LOD scores spanning all the chromosomes are shown. The *horizontal axis* is the cumulative genetic distance (centimorgan) starting at the short arm of chromosome 1. The vertical axis represents LOD scores. Regions on chromosomes 1 and 20 give the highest multipoint LOD scores of 1.93. **b** Parametric multipoint linkage analysis of chromosome 1. Regions with a multipoint LOD score of 1.93 are enlarged below. The *horizontal axis* is the genetic distance (centimorgan) starting at the short arm of chromosome 1. The *vertical axis* shows multipoint LOD scores. *Below the graphs*, the candidate regions demonstrated by this study as well as by previous studies [1, 2] are shown along with the diagram of chromosome 1q32.1–q41. Novel non-synonymous variants detected in this study are also shown. *FLVCR1* G493R is the only variant that is located inside the minimum candidate region

retarded. Muscle tone was decreased in the limbs with normal strength. Coordination was preserved in the arms and legs, but with moderately ataxic gait and truncal titubation. Romberg's sign was positive. Deep tendon reflexes were decreased in the arms and absent in the legs with flexor plantar responses. Superficial sensations were intact, whereas vibratory and position senses were lost in the toes. Normal values were found in the following tests: complete blood count, blood vitamin E level, and plasma phytanic acid level. Her peripheral blood smears showed no acanthocytes. Axial T2-weighted images of the cervical spinal cord on magnetic resonance imaging demonstrated a hyperintense signal in the posterior half of the cord. Her brother (IV-2 in Fig. 1) was examined early in his thirties and was found to have mental retardation, retinitis pigmentosa, and posterior column ataxia. The other family members were neurologically normal.

Table 1 Variants in target regions of chromosomes 1 and 20

	No. of variants	No. of variants in exon/SS	No. of novel variants in exon/SS	No. of novel nonsynonymous variants in exon/SS
chr1	13,616	60	5	4
chr20	10,545	30	1	1

SS splice site (splice donor and acceptor sites including two adjacent nucleotides in introns)

Table 2 Novel nonsynonymous variants detected in target regions

Chr	Physical position	Variant	Gene	Amino acid change	Polyphen
1	203447100	A>C (homo)	*DSTYK*	C63G	Probably damaging
1	203759347	C>T (homo)	*PCTK3/CDK18*	R82W	Possibly damaging
1	211129174	G>C (homo)	*FLVCR1*	G493R	Possibly damaging

Chr chromosome, *homo* homozygous

Linkage analysis

Multipoint parametric linkage analysis revealed the highest LOD scores of 1.93 spanning regions on chromosome 1 (defined by rs950114 and rs10494988) and chromosome 20 (defined by rs2876404 and rs6082269, Fig. 2a). The region on the chromosome 1 overlapped the previously defined locus of PCARP (Fig. 2b).

Massively parallel sequencing analysis

Average fold enrichment for QC loci of the captured library was 129. From two lanes of GAIIx, we obtained 37,165,950 reads. Of these, 15,865,704 reads (42.7%) had tag sequences for 454 in the first 20 bases. In these reads, tag sequences were eliminated and we used them as 80 bp sequences. Aligned uniquely to the reference genome were 32,332,900 reads (87.0%), and 29,693,695 reads (79.9%) were aligned to the target region. The average coverage of target regions was 89.6X.

In the 30.3 Mb of target region on chromosomes 1 and 20, 24161 variants were called. Of these, 90 were located in coding regions and splice sites in the target regions, six of which were not registered in dbSNP131 (http://www.ncbi.nlm.nih.gov/projects/SNP/), and five of which were concluded to be novel nonsynonymous SNV (Tables 1 and 2). Two of the five novel variant calls were heterozygous, and direct nucleotide sequence analysis revealed that they were false positives.

Considering previous linkage studies [1, 2], two of the three novel nonsynonymous SNVs were located outside the overlapping candidate region (Fig. 2b and Table 1). Thus, the only novel nonsynonymous variant within the minimum candidate region was a homozygous c.1477G>C (G493R) of *FLVCR1* (Fig. 3a). The mutation was further confirmed by direct nucleotide sequence analysis (Fig. 3b). The two

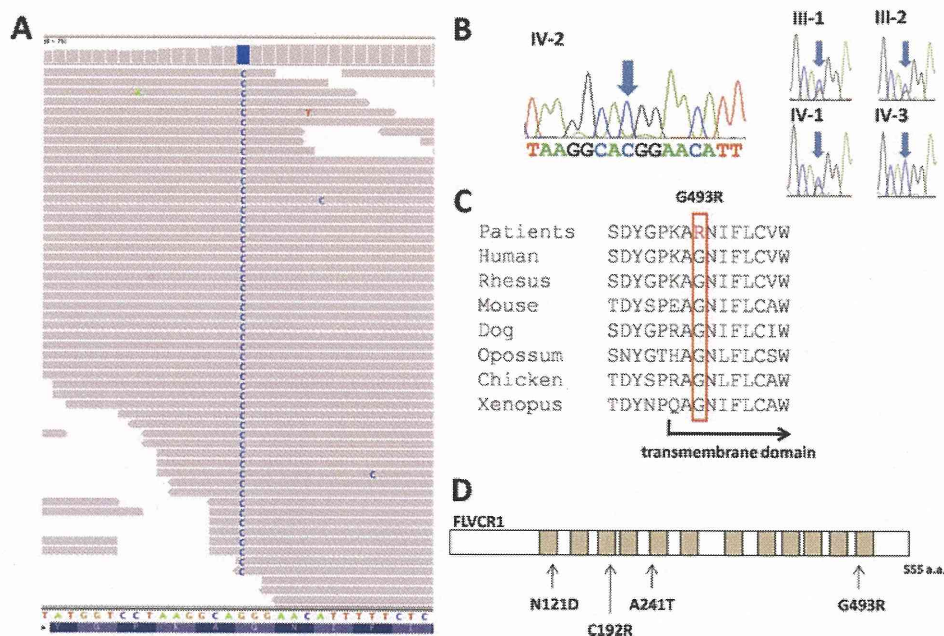

Fig. 3 Identification of causative mutation in *FLVCR1*. **a** Aligned short reads showing homozygous *FLVCR1* c.1477G>C mutation. Aligned reads are viewed using Integrative Genomic Viewer (http://www.broadinstitute.org/igv/). Each short read is represented as a horizontal bar. Only mismatched bases are explicitly shown. All the 52 reads aligned in the position show the C allele, suggesting a homozygous mutation. **b** Direct nucleotide sequence analysis confirms the mutation, which cosegregates with the disease. **c** Partial FLVCR1 amino acid sequence alignment reveals that G493 is evolutionally conserved among species. A putative transmembrane domain is also indicated by an arrow. **d** Schematic representation of FLVCR1 protein. Mutations detected to date are shown. Putative transmembrane domains are *shaded*

affected individuals carried the homozygous mutation, whereas the parents and the unaffected sibling carried the heterozygous mutation. Because R493 is evolutionally well conserved (Fig. 3c) and the amino acid change was not observed in 192 control chromosomes, we concluded it as a pathogenic mutation of PCARP.

Discussion

We described two cases of a Japanese family with PCARP. Linkage analysis supported the linkage to the previously defined locus, and we identified a novel mutation in *FLVCR1* employing targeted capture and massively parallel sequencing as the cause of PCARP. Very recently, Rajadhyaksha et al. have conducted massively parallel sequencing and found independent mutations in *FLVCR1* (N121D, A241T, and C192R) in three families [8]. Our report further confirmed that PCARP is caused by mutations in *FLVCR1*.

FLVCR1 is a 555 amino acid protein that has 12 transmembrane domains. Intriguingly, three previously reported mutations are located in the first, third, and fifth putative transmembrane domains of FLVCR1. The mutation which we found is also located in the 12th transmembrane domain (Fig. 3d). Moreover, all the mutations in FLVCR1 found in PCARP are substitution of a hydrophilic amino acid for a hydrophobic amino acid (A214T) or substitutions of charged amino acids for uncharged amino acids (N121D, C192R, and G493R). These finding suggest a possibility that disruption of transmembrane domains of FLVCR1 is involved in the pathogenesis of PCARP.

Although childhood-onset retinitis pigmentosa and sensory ataxia found in the affected siblings were characteristics of PCARP, they also had mild mental retardation. Because no cognitive deficits have been reported in the original PCARP families [2], careful interpretation would be necessary. One possibility is that the clinical presentations can be more heterogeneous depending on mutations and G493R mutation in *FLVCR1* is associated with mental retardation. Another possibility is that other gene(s) are responsible for mental retardation. Because there are at least two other novel homozygous amino acid changes in the candidate regions as determined on the basis of the linkage analysis of this family under an autosomal recessive model (Table 2), some of these substitutions may contribute to mental retardation.

Previous studies suggested that FLVCR1 is a heme transporter, and *FLVCR1* null mice present a phenotype with a lack of erythropoiesis and craniofacial and limb deformities resembling Diamond–Blackfan anemia [9]. Because neither changes in the shape of erythrocytes nor anemia was observed in the index patient, the discrepancy between human disease and mouse model should be further investigated in the future.

In conclusion, we identified a novel mutation in *FLVCR1* in a Japanese PCARP family. The study showed that target capture and massively parallel sequencing technologies enable us to identify causative genes even in a small family and they are expected to further unveil molecular pathogeneses of neurodegenerative disorders.

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References

1. Higgins JJ, Morton DH, Loveless JM (1999) Posterior column ataxia with retinitis pigmentosa (AXPC1) maps to chromosome 1q31–q32. Neurology 52:146–150
2. Higgins JJ, Kluetzman K, Berciano J, Combarros O, Loveless JM (2000) Posterior column ataxia and retinitis pigmentosa: a distinct clinical and genetic disorder. Mov Disord 15:575–578
3. Fukuda Y, Nakahara Y, Date H, Takahashi Y, Goto J, Miyashita A, Kuwano R, Adachi H, Nakamura E, Tsuji S (2009) SNP-HiTLink: a high-throughput linkage analysis system employing dense SNP data. BMC Bioinformatics 10:121
4. Gudbjartsson DF, Thorvaldsson T, Kong A, Gunnarsson G, Ingolfsdottir A (2005) Allegro version 2. Nat Genet 37:1015–1016
5. Hodges E, Xuan Z, Balija V, Kramer M, Molla MN, Smith SW, Middle CM, Rodesch MJ, Albert TJ, Hannon GJ, McCombie WR (2007) Genome-wide in situ exon capture for selective resequencing. Nat Genet 39:1522–1527
6. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 25:1754–1760
7. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R (1000) Genomes Project Data Processing Subgroup (2009) The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078–2079
8. Rajadhyaksha AM, Elemento O, Puffenberger EG, Schierberl KC, Xiang JZ, Putorti ML, Berciano J, Poulin C, Brais B, Michaelides M, Weleber RG, Higgins JJ (2010) Mutations in FLVCR1 cause posterior column ataxia and retinitis pigmentosa. Am J Hum Genet 87:643–654
9. Keel SB, Doty RT, Yang Z, Quigley JG, Chen J, Knoblaugh S, Kingsley PD, De Domenico I, Vaughn MB, Kaplan J, Palis J, Abkowitz JL (2008) A heme export protein is required for red blood cell differentiation and iron homeostasis. Science 319:825–828

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ORIGINAL ARTICLE

Comprehensive mutational analysis of *LRRK2* reveals variants supporting association with autosomal dominant Parkinson's disease

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by neurodegeneration, most notably of dopaminergic neurons in the substantia nigra. To date, six causative genes have been identified including *LRRK2*, whose mutations are the most frequent in autosomal dominant PD (Ad-PD). We conducted a comprehensive mutational analysis of *LRRK2* in 30 Ad-PD (11 Japanese and 19 Caucasian) families employing a DNA microarray-based resequencing system and direct nucleotide sequence analysis, and identified 23 variants including two known mutations, p.G2019S and p.I1371V, in three Caucasian families and one Caucasian family, respectively, a novel putative pathogenic mutation, p.N1221K, in one Japanese family, and a known nonsynonymous variant, p.G2385R, in two Japanese families. Detailed analysis of the frequency of p.G2385R among 100 Japanese Ad-PD, 73 sporadic PD (sPD) and 238 controls revealed that the frequency of the p.G2385R variant was significantly higher in Ad-PD than in controls (allele frequency, 9.0 vs 2.1%) (χ^2=16.32, P=5.34$\times$10^{-5}). The p.G2385R variant, however, did not show complete cosegregation with PD. In addition, the frequency of p.G2385R was also higher in sPD than in controls, although not significant (allele frequency, 3.4 vs 2.1%) (χ^2=0.76, P=0.38). These observations support the possibility that p.G2385R is associated with an increased risk of PD.
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Keywords: comprehensive mutational analysis; LRRK2; microarray-based resequencing; Parkinson's disease; p.G2385R

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder characterized by rigidity, tremor, akinesia and postural instability. Its pathological features include the progressive degeneration of dopaminergic neurons primarily in the substantia nigra, with Lewy bodies as the pathological hallmark. Although the majority of PD patients have sporadic PD (sPD), various forms of familial PD (FPD) have been recognized. Recent advances in the search for genes causing PD have contributed to a better understanding of the molecular pathophysiology of PD. To date, six genes (*SNCA*, *PARK2*, *DJ1*, *PINK1*, *UCHL1* and *LRRK2*) have been identified as genes causing FPD.[1–6] The genetic heterogeneity necessitates a comprehensive mutational analysis of these genes for not only molecular diagnosis but also studies of the molecular epidemiology of FPD.

Among the genes causing FPD, *LRRK2* is of particular interest, because *LRRK2* is by far the most common cause of autosomal dominant PD (Ad-PD). Approximately 4% of Ad-PD patients and 1% of sPD patients have been reported to harbor causative mutations of *LRRK2*, the most frequent of which is the mutation substituting serine for glycine at codon 2019 (p.G2019S).[7] It is mandatory to carry out mutational analysis of *LRRK2* to conduct molecular diagnosis and to investigate the molecular epidemiology of Ad-PD. Analysis of the entire 51 exons of *LRRK2* by the conventional direct nucleotide sequencing method, however, is very laborious. Therefore, the majority of previous studies have focused on particular exons for mutational analysis,[8–12] making it difficult to obtain accurate data on the molecular epidemiology of Ad-PD caused by *LRRK2*.

We herein applied a DNA microarray-based resequencing system to the comprehensive mutational analysis of all *LRRK2* exons in 30 families with Ad-PD from two cohorts with different ethnic backgrounds.

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MATERIALS AND METHODS

Subjects

To directly compare the *LRRK2* epidemiologies across ethnic groups in two similarly ascertained cohorts, we conducted a comprehensive resequencing analysis of *LRRK2* focusing on 11 Japanese and 19 Caucasian families with Ad-PD employing resequencing DNA microarrays (Table 1a). These families were diagnosed as having Ad-PD when their pedigree members in at least two generations were diagnosed as having PD. Two Caucasian probands were assessed both by the direct sequencing approach and by resequencing microarray analysis.[13] To evaluate the molecular epidemiology of *LRRK2* variants identified by the resequencing of *LRRK2*, DNA samples from index patients from 89 Ad-PD families and 73 sPD patients, and samples from 233 Japanese normal controls were further analyzed (Table 1b). All the genomic DNA samples were obtained with the written informed consent of the subjects, and this research project was approved by the Institutional Review Board of the University of Tokyo.

Mutational analysis

The mutational analysis of all 51 *LRRK2* exons was accomplished using the DNA microarray-based resequencing system as described elsewhere.[14,15] Briefly, specific PCR was conducted for each exon using previously reported primers.[6] After quantification of the PCR products, they were pooled equimolarly, fragmented with DNase I, labeled with biotin, hybridized to microarrays, stained with streptoavidin-phycoerythrin, washed and scanned. Direct nucleotide sequence analysis was also conducted using an automated DNA sequencer and BigDye Terminator ver. 3.1 (Applied Biosystems, Foster City, CA, USA).

RESULTS

Mutational analysis of *LRRK2* in 30 Ad-PD families

Twenty-three variants were identified by the resequencing of the 51 exons and the splice sites of *LRRK2* in 30 Ad-PD families (Table 2). Of the 10 nonsynonymous variants identified in the 11 Japanese Ad-PD families, two novel variants (p.N1221K and p.N1320S), both of which were identified in one family, respectively, and the previously reported nonsynonymous variant p.G2385R, which was identified in two families, were of interest. Of the 8 nonsynonymous variants identified in the 19 Caucasian Ad-PD families, the previously known mutation p.G2019S, which was identified in three families, and two previously reported nonsynonymous variant p.R1514Q and p.I1371V, both of which were identified in one family, respectively, were of interest. The remaining seven nonsynonymous variants were previously been registered in the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/index.html) and not considered as mutations causing Ad-PD.

The two novel nonsynonymous variants found in the Japanese Ad-PD families are a variant substituting lysine for asparagine at codon 1221 (p.N1221K) (Figure 1a) and that substituting serine for arginine at codon 1320 (p.N1320S). p.N1221K was located in a highly conserved region (Figure 1b) and not found in 233 controls (466 chromosomes). The index patient with p.N1221K developed levodopa-responsive parkinsonism including asymmetric rigidity and postural instability at age 47. The other novel nonsynonymous variant p.N1320S was found in one of the 11 Japanese Ad-PD families, but was also present in eight controls (allele frequency, 1.7%). In addition, the variant was also found in three additional Ad-PD patients of the 89 Ad-PD index patients (allele frequency, 1.7%) and three of the 73 sPD patients (allele frequency, 2.2%). The previously reported nonsynonymous variant p.G2385R was identified in 2 of the 11 Japanese Ad-PD families (allele frequency, 9%), but not found in the 19 Caucasian families. Because recent studies in Taiwan have shown a higher frequency of p.G2385R in sPD than in controls (5.0 vs 2.5%, P=0.012),[16] the frequencies of p.G2385R in controls, sPD and Ad-PD were further analyzed as described below.

Among the variants identified in Caucasian families, two (p.I1371V and p.G2019S) were initially missed in the analysis using GDAS software and subsequent visual inspection of the signals of undetermined

Table 1 Demographic characteristics of patients who participated in (a) comprehensive analysis of *LRRK2*; (b) frequency analysis of G2385R variant

	Japanese Ad-PD	Canadian Ad-PD
(a)		
Number of probands	11	19
Male	3 (27.3%)	9 (47.3%)
Female	8 (72.7%)	10 (52.7%)
Age at onset (years)	48.7±9.8 (32–62)	51.6±9.5 (26–70)
Male	44.3±11.6 (32–55)	52.6±11.2 (37–70)
Female	50.4±9.3 (32–62)	50.6±8.0 (26–70)

	Ad-PD	sPD	Controls
(b)			
Number of probands	89	73	233
Male	33 (37.1%)	45 (61.6%)	123 (52.8%)
Female	56 (62.9%)	28 (38.4%)	110 (47.2%)
Age at onset (years)	53.5±13.0 (25–76)	52.1±14.8 (18–80)	
Male	51.5±15.4 (25–67)	49.4±15.3 (18–80)	
Female	54.0±12.7 (34–76)	57.2±12.4 (28–71)	

Abbreviations: Ad-PD, autosomal dominant PD; PD, Parkinson's disease; sPD, sporadic PD.

Table 2 Variations identified by comprehensive analysis of *LRRK2*

Exon	*SNP ID*[a]	*Base change*	*Amino-acid change*	*Number of alleles (allele frequencies)*	
				Japanese	*Canadian*
1	rs2256408	g to a	R50H	22 (100)	38 (100)
5	rs10878245	t to c	p.L153L	0 (0)	15 (39.5)
7	rs28365216	a to t	p.N238I	1 (4.5)	0 (0)
14	novel	g to a	p.A521A	0 (0)	1 (2.6)
	rs7308720	c to g	p.K551N	3 (13.6)	1 (2.6)
18	rs10878307	a to g	p.I723V	2 (9)	5 (13.2)
22	rs7966550	t to c	p.L953L	3 (13.6)	2 (5.3)
27	novel	c to g	p.N1221K	1 (4.5)	0 (0)
29	novel	g to t	p.R1320S	1 (4.5)	0 (0)
	rs17466213	a to g	p.I1371V	0 (0)	1 (2.6)
30	rs7133914	g to a	p.R1398H	3 (13.6)	0 (0)
	rs11175964	g to a	p.K1423K	3 (13.6)	1 (2.6)
32	rs35507033	g to a	p.R1514Q	0 (0)	1 (2.6)
34	rs1427263	a to g	p.G1624G	4 (18.2)	29 (76.3)
	rs11176013	t to c	p.K1637K	6 (27.3)	15 (39.5)
	rs11564148	t to a	p.S1647T	3 (13.6)	12 (31.5)
37	rs10878371	t to c	p.G1819G	10 (45.5)	14 (36.8)
41	rs34637584	g to a	p.G2019S	0 (0)	3 (7.9)
43	rs10878405	g to a	p.E2108E	4 (18.2)	10 (26.3)
48	rs34778348	g to a	p.G2385G	0 (0)	6 (15.8)
	rs33962975	g to a	p.G2385R	2 (9)	0 (0)
49	rs3761863	t to c	p.T2397M	6 (27.3)	14 (36.8)
IVS49 -43	novel	t to c		2 (9)	2 (7.9)

[a]SNP ID denotes single-nucleotide polymorphism identification obtained from dbSNP database.

base calls, but were independently identified by direct nucleotide sequence analyses. Retrospective analysis of the microarray signals of these two variants showed that the signals for p.I1371V could have been detected as a heterozygous variant, whereas the signals for p.G2019S would have been difficult to determine considering that one of the three patients with p.G2019S was called as having a homozygous wild-type sequence using GDAS software.

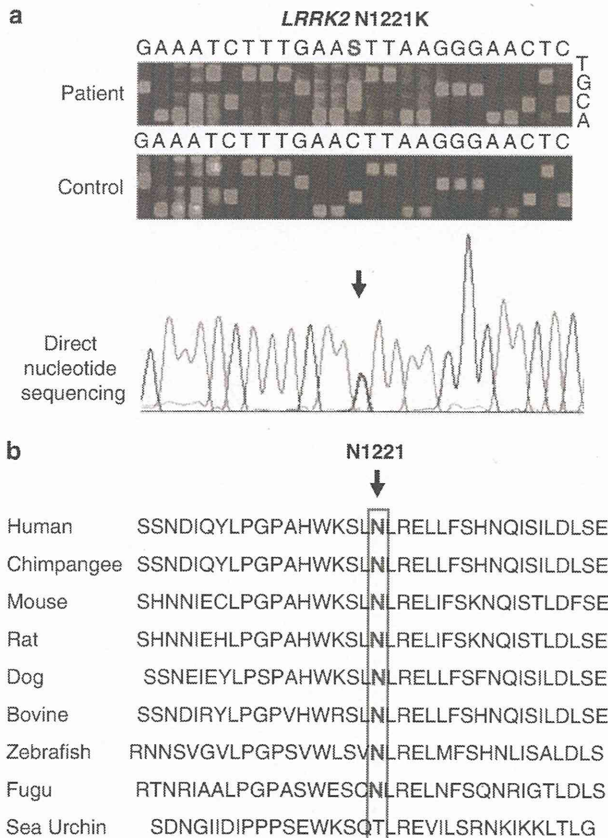

Figure 1 (**a**) Scan images of heterozygous *LRRK2* p.N1221K putative pathogenic mutation. Each column shows a base position, and each row shows a base call. In the center position, the base call was C in the control, whereas in the patients, the base calls were heterozygous C and G (upper panel). The mutation was confirmed by direct nucleotide sequencing. (**b**) Conservation of *LRRK2* amino-acid sequences among different animal species. The arginine residue at codon 1221 is highly conserved among species (shown in red). A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Association of p.G2385R with Ad-PD

As mentioned above, we identified p.G2385R in two Japanese Ad-PD families. We first analyzed the frequency of p.G2385R in another data set of 89 Japanese Ad-PD families independent of the 11 Ad-PD families. We found that the seven index patients from the 89 Ad-PD families had p.G2385R. Among the seven Ad-PD families with p.G2385R, DNA samples were available from other pedigree members including affected patients in two families (families 2938 and 3045). In the two families (families 2955 and 2932), DNA samples from only other unaffected family members were available (Figure 2). p.G2385R did not show complete cosegregation with PD. Although cosegregation was suggested in family 3045, one affected patient did not carry p.G2385R in the family 2938. Furthermore, unaffected parents of the two other families (families 2955 and 2932) also carried the p.G2385R variant in a heterozygous state. Nevertheless, the frequency in 18 index patients of the 100 Ad-PD families of p.G2385R was significantly higher than that in controls (allele frequency, 9.0 vs 2.1%) (χ^2=16.32, P=5.34$\times$10^{-5}) (Table 3). p.G2385R has recently been reported to be a polymorphism present in the Japanese control population, but overrepresented in sPD patients compared with controls (6.0 vs 2.4%, P=1.24$\times$10^{-4}).[17] Given this report, we then analyzed the frequencies of p.G2385R in 73 sPD patients and 233 controls (Table 3). The frequency of the p.G2385R variant in sPD also

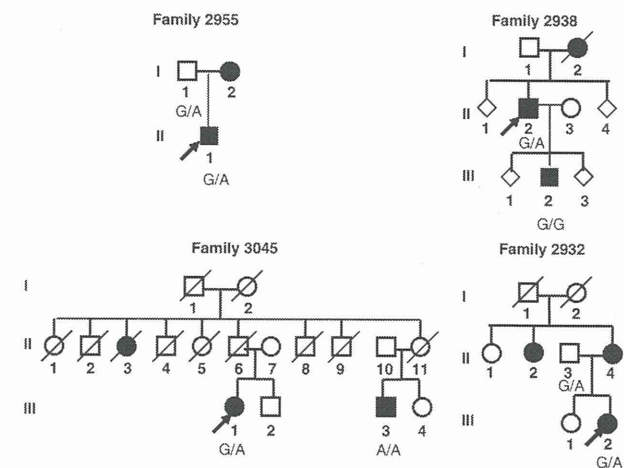

Figure 2 Pedigree charts in which cosegregation of *LRRK2* p.G2385R was examined. Affected individuals are indicated with black symbols. The proband is indicated with an arrow. Unaffected individuals are indicated with open symbols. Slashed symbols indicate deceased subjects. Genotype of each member available for cosegregation analysis is shown at each position. G: wild-type allele, A: p.G2385R allele (rs34778348:G>A).

Table 3 Genotype and allele frequencies of p.G2385R variant in Ad-PD, sPD and controls

		Genotype (%)			*Allele (%)*		*χ^2 (P-value)*	
	Number of cases	*G/G*	*G/A*	*A/A*	*G*	*A*	*vs Control*	*vs sPD*
Ad-PD	100	82 (82.0)	18 (18.0)	0 (0)	182 (91.0)	18 (9.0)	16.32 (5.34×10^{-5})*	4.22 (3.97×10^{-3})*
sPD	73	67 (90.5)	5 (6.8)	0(0)	141 (96.6)	5 (3.4)	0.76 (0.38)	
Control	233	223 (95.7)	10 (4.3)	0 (0)	456 (97.9)	10 (2.1)		

Abbreviations: Ad-PD, autosomal dominant PD; PD, Parkinson's disease; sPD, sporadic PD.
*These values reached statistical significance.

tended to be higher than that in controls (allele frequency, 3.4 vs 2.1%), although the difference did not reach statistical significance (χ^2=0.76, P=0.38) (Table 3). The mean age at onset of Ad-PD in patients carrying p.G2385R was 55.3 ± 6.1 (18–67), which was not significantly different from that in patients not carrying the variant (51.8 ± 13.0, P=0.49). The mean age at onset of sPD in patients carrying the p.G2385R variant was 60.2 ± 6.9 (52–71) years, which was similarly not significantly different from that of sPD in patients not carrying the variant (51.3 ± 15.0, P=0.19). The two Ad-PD and five sPD patients with p.G2385R whose detailed clinical information was available showed a good response to levodopa therapy and no atypical features.

DISCUSSION

This study demonstrated that the spectrum and frequency of *LRRK2* variants vary with ethnic background. In Caucasians, p.G2019S is the most frequent in both Ad-PD and sPD, accounting for ~5% of Ad-PD patients and 1.6% of sPD patients.[8,9,18] Many of the patients from European populations share a common haplotype, suggesting that they have a common founder originating in the Near East at least 4000 years ago.[19,20] In contrast, no single mutation has been reported as being predominant in Asian populations. Although three Japanese index patients with p.G2019S were reported,[21,22] we did not detect p.G2019S in our Japanese Ad-PD cohort, consistent with previous reports, showing that p.G2019S is rare in Asian cohorts.[10,11,18,23] Indeed, the substantial differences in allele frequencies of the synonymous *LRRK2* polymorphisms (rs10878245, rs1427263, rs11176013, rs17466213 and rs3761863) highlight the genetic diversity between the Japanese and Caucasian Ad-PD patients (Table 2). These findings raise the possibility that mutational analysis focusing on previously identified mutation hotspots may underestimate the incidence of mutations; therefore, a comprehensive analysis of every coding exon is warranted.

Our comprehensive analysis of *LRRK2* revealed 22 variants in 30 Ad-PD families, supporting the usefulness of the DNA microarray-based resequencing system. However, it should also be noted that data analysis using GDAS software and subsequent visual inspection of the signals of undetermined base calls may miss variants, as experienced in the cases of p.I1371V and p.G2019S. Improvement of software and careful inspection of signals are important for accurate mutational detection.

In the Japanese Ad-PD families, we identified a novel nonsynonymous variation, p.N1221K. Although cosegregation could not be confirmed, it is possibly a pathogenic mutation for the following reasons: (1) p.N1221K was not found in 466 control chromosomes, (2) the amino-acid residue N1221 was highly conserved among different animal species and (3) it is located in the LRR domain, where some likely pathogenic mutations are clustered.[24–26] The LRR domain resides in many proteins with diverse functions and provides a structural framework for the formation of protein–protein interactions.[27] The substitution of a charged amino-acid lysine for a neutral asparagine may disrupt protein–protein interactions, resulting in the dysfunction of the LRRK2 protein. Identification of additional pedigrees harboring the mutation, cosegregation analysis and functional studies would be necessary to confirm the pathogenicity of the mutation.

The age at onset of the patient with p.N1221K was earlier than those of patients with previously reported mutations of *LRRK2* (the mean age at onset was in the range of 57–59 years).[7,9,25] Considering that p.G2019S is the most frequent among the known mutations, the overall clinical presentation of patients with *LRRK2* mutations may largely reflect that of patients with p.G2019S. Interestingly, a previously reported case of a patient with the p.S1228T mutation, located in the same exon and domain as in p.N1221K, showed a similarly early age at onset to the patient with p.N1221K, who developed symptoms at the age of 49.[25] It will be important to accumulate further detailed clinical information on patients with *LRRK2* mutations to evaluate the genotype–phenotype correlations.

In the Caucasian Ad-PD families, we identified two nonsynonymous variants, p. I1371V and p. R1514Q, both of which were initially reported as a putative pathogenic mutation.[28,29] However, subsequent studies have revealed that these variants were identified in controls or not cosegregated within Ad-PD families, implying that they are not associated with an increased risk of the disease.[30–32] It is often difficult to interpret the pathogenicity of each rare nonsynonymous variant in *LRRK2* because this gene is highly polymorphic and numerous nonsynonymous variants have been identified with diverse frequencies among different ethnic backgrounds. In this study, we could not conclude that the two nonsynonymous variants were pathogenic because large families appropriate for cosegregation analysis were not obtained and control data were not available, and further studies are required to evaluate the relevance of the variants to the pathogenesis of PD.

Of note, a known nonsynonymous variant R50H was found homozygously in all the pedigrees with the allele frequency of 100% in this study. Although the variant was initially registered as a nonsynonymous single-nucleotide polymorphism, a subsequent study on the comprehensive sequencing of LRRK2 concluded that this variant should be a mistake in the consensus human genome sequence because it was present in all the cases and controls in their study.[33] The present study further supports the above conclusion that the consensus amino acid at position 50 in LRRK2 protein is histidine instead of arginine. Future studies on the mutational analysis for *LRRK2* should take these results into account.

Heterozygosity for the p.G2385R variant has recently been demonstrated to be significantly more frequent in PD patients than in controls in Asian populations.[16,17,34–39] One of these studies included 26 patients with a familial history of PD, among which six patients were p.G2385R carriers.[40] A meta-analysis on the basis of these studies involving 2205 PD patients and 1817 controls demonstrated average carrier rates of 9.3% in PD and 4% in controls.[41] In this study, the frequency of the p.G2385R variant did not reach statistical significance between sPD patients and controls, probably due to relatively small sample size.

Intriguingly, we observed a significant overrepresentation of the p.G2385R variant in Ad-PD index patients compared with that in controls, although no complete cosegregation was observed. Note that the frequency of the p.G2385R variant in our control cohort was the same as that in a previous report (2.4%).[17] In addition, the frequency of the p.G2385R variant also tended to be higher in Ad-PD patients than in sPD patients in accordance with a previous study.[40] Taken together, this study reinforced the notion that p.G2385R is a potential risk factor for PD. However, lack of evidences on the cosegregation suggested that other genetic factors should also be involved in the disease pathogenesis in the pedigrees with p.G2385R mutation. Analyses of p.G2385R in combination with recently identified genetic risk factors for PD such as *SNCA*[42–44] and *GBA*[45,46] would be important for evaluating the combined effects of these factors as well as for elucidating the molecular pathophysiology of PD.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A. *et al.* Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* **276**, 2045–2047 (1997).
2 Kitadam, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S. *et al.* Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* **392**, 605–608 (1998).
3 Leroy, E., Boyer, R., Auburger, G., Leube, B., Ulm, G., Mezey, E. *et al.* The ubiquitin pathway in Parkinson's disease. *Nature* **395**, 451–452 (1998).
4 Bonifati, V., Rizzu, P., van Baren, M. J., Schaap, O., Breedveld, G. J., Krieger, E. *et al.* Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science* **299**, 256–259 (2003).
5 Paisán-Ruíz, C., Jain, S., Evans, E. W., Gilks, W. P., Sim, J., van der Brug, M. *et al.* Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron* **44**, 595–600 (2004).
6 Zimprich, A., Biskup, S., Leitner, P., Lichtner, P., Farrer, M., Lincoln, S. *et al.* Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron* **44**, 601–607 (2004).
7 Healy, D. G., Falchi, M., O'Sullivan, S. S., Bonifati, V., Durr, A., Bressman, S. *et al.* Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. *Lancet Neurol.* **7**, 583–590 (2008).
8 Di Fonzo, A., Rohe, C. F., Ferreira, J., Chien, H. F., Vacca, L., Stocchi, F. *et al.* A frequent LRRK2 gene mutation associated with autosomal dominant Parkinson's disease. *Lancet* **365**, 412–415 (2005).
9 Gilks, W. P., Abou-Sleiman, P. M., Gandhi, S., Jain, S., Singleton, A., Lees, A. J. *et al.* A common LRRK2 mutation in idiopathic Parkinson's disease. *Lancet* **365**, 415–416 (2005).
10 Lu, C. S., Simons, E. J., Wu-Chou, Y. H., Fonzo, A. D., Chang, H. C., Chen, R. S. *et al.* The LRRK2 I2012T, G2019S, and I2020T mutations are rare in Taiwanese patients with sporadic Parkinson's disease. *Parkinsonism Relat. Disord.* **11**, 521–522 (2005).
11 Tan, E. K., Shen, H., Tan, L. C., Farrer, M., Yew, K., Chua, E. *et al.* The G2019S LRRK2 mutation is uncommon in an Asian cohort of Parkinson's disease patients. *Neurosci. Lett.* **384**, 327–329 (2005).
12 Kay, D. M., Zabetian, C. P., Factor, S. A., Nutt, J. G., Samii, A., Griffith, A. *et al.* Parkinson's disease and LRRK2: frequency of a common mutation in U.S. movement disorder clinics. *Mov. Disord.* **21**, 519–523 (2006).
13 Paisan-Ruiz, C., Lang, A. E., Kawarai, T., Sato, C., Salehi-Rad, S., Fisman, G. K. *et al.* LRRK2 gene in Parkinson disease: mutation analysis and case control association study. *Neurology* **65**, 696–700 (2005).
14 Cutler, D. J., Zwick, M. E., Carrasquillo, M. M., Yohn, C. T., Tobin, K. P., Kashuk, C. *et al.* High-throughput variation detection and genotyping using microarrays. *Genome Res.* **11**, 1913–1925 (2001).
15 Warrington, J. A., Shah, N. A., Chen, X., Janis, M., Liu, C., Kondapalli, S. *et al.* New developments in high-throughput resequencing and variation detection using high density microarrays. *Hum. Mutat.* **19**, 402–409 (2002).
16 Di Fonzo, A., Wu-Chou, Y. H., Lu, C. S., van Doeselaar, M., Simons, E. J., Rohe, C. F. *et al.* A common missense variant in the LRRK2 gene, Gly2385Arg, associated with Parkinson's disease risk in Taiwan. *Neurogenetics* **7**, 133–138 (2006).
17 Funayama, M., Li, Y., Tomiyama, H., Yoshino, H., Imamichi, Y., Yamamoto, M. *et al.* Leucine-rich repeat kinase 2 G2385R variant is a risk factor for Parkinson disease in Asian population. *Neuroreport* **18**, 273–275 (2007).
18 Funayama, M., Hasegawa, K., Ohta, E., Kawashima, N., Komiyama, M., Kowa, H. *et al.* An LRRK2 mutation as a cause for the parkinsonism in the original PARK8 family. *Ann. Neurol.* **57**, 918–921 (2005).
19 Lesage, S., Patin, E., Condroyer, C., Leutenegger, A. L., Lohmann, E., Giladi, N. *et al.* Parkinson's disease-related LRRK2 G2019S mutation results from independent mutational events in humans. *Hum. Mol. Genet.* **19**, 1998–2004 (2010).
20 Zabetian, C. P., Hutter, C. M., Yearout, D., Lopez, A. N., Factor, S. A., Griffith, A. *et al.* LRRK2 G2019S in families with Parkinson disease who originated from Europe and the Middle East: evidence of two distinct founding events beginning two millennia ago. *Am. J. Hum. Genet.* **79**, 752–758 (2006).
21 Tomiyama, H., Li, Y., Funayama, M., Hasegawa, K., Yoshino, H., Kubo, S. *et al.* Clinicogenetic study of mutations in LRRK2 exon 41 in Parkinson's disease patients from 18 countries. *Mov. Disord.* **21**, 1102–1108 (2006).
22 Zabetian, C. P., Morino, H., Ujike, H., Yamamoto, M., Oda, M., Maruyama, H. *et al.* Identification and haplotype analysis of LRRK2 G2019S in Japanese patients with Parkinson disease. *Neurology* **67**, 697–699 (2006).
23 Cho, J. W., Kim, S. Y., Park, S. S., Kim, H. J., Ahn, T. B., Kim, J. M. *et al.* The G2019S LRRK2 mutation is rare in Korean patients with Parkinson's disease. *Can. J. Neurol. Sci.* **34**, 53–55 (2007).
24 Paisán-Ruíz, C., Nath, P., Washecka, N., Gibbs, J. R. & Singleton, A. B. Comprehensive analysis of *LRRK2* in publicly available Parkinson's disease cases and neurologically normal controls. *Hum. Mutat.* **29**, 485–490 (2008).
25 Berg, D., Schweitzer, K., Leitner, P., Zimprich, A., Lichtner, P., Belcredi, P. *et al.* Type and frequency of mutations in the LRRK2 gene in familial and sporadic Parkinson's disease. *Brain* **128**, 3000–3011 (2005).
26 Skipper, L., Shen, H., Chua, E., Bonnard, C., Kolatkar, P., Tan, L. C. *et al.* Analysis of LRRK2 functional domains in nondominant Parkinson disease. *Neurology* **65**, 1319–1321 (2005).
27 Kobe, B. & Kajava, A. V. The leucine-rich repeat as a protein recognition motif. *Curr. Opin. Struct. Biol.* **11**, 725–732 (2001).
28 Mata, I., Kachergus, J., Taylor, J., Lincoln, S., Aasly, J., Lynch, T. *et al.* Lrrk2 pathogenic substitutions in Parkinson's disease. *Neurogenetics* **6**, 171–177 (2005).
29 Lesage, S., Condroyer, C., Lannuzel, A., Lohmann, E., Troiano, A., Tison, F. *et al.* Molecular analyses of the LRRK2 gene in European and North African autosomal dominant Parkinson's disease. *J. Med. Genetics* **46**, 458–464 (2009).
30 Toft, M., Mata, I., Ross, O. A., Kachergus, J., Hulihan, M. M., Haugarvoll, K. *et al.* Pathogenicity of the Lrrk2 R1514Q substitution in Parkinson's disease. *Mov. Disord.* **22**, 389–392 (2007).
31 Nichols, W. C., Marek, D. K., Pauciulo, M. W., Pankratz, N., Halter, C. A., Rudolph, A. *et al.* R1514Q substitution in Lrrk2 is not a pathogenic Parkinson's disease mutation. *Mov. Disord.* **22**, 254–256 (2007).
32 Jasinska-Myga, B., Kachergus, J., Vilarino-Guell, C., Wider, C., Soto-Ortolaza, A. I., Kefi, M. *et al.* Comprehensive sequencing of the LRRK2 gene in patients with familial Parkinson's disease from North Africa. *Mov. Disord.* **25**, 2052–2058 (2010).
33 Paisán-Ruiz, C. LRRK2 gene variation and its contribution to Parkinson disease. *Hum. Mutat.* **30**, 1153–1160 (2009).
34 Tan, E. K., Zhao, Y., Skipper, L., Tan, M. G., Di Fonzo, A., Sun, L. *et al.* The LRRK2 Gly2385Arg variant is associated with Parkinson's disease: genetic and functional evidence. *Hum. Genet.* **120**, 857–863 (2007).
35 Kim, J. M., Lee, J. Y., Kim, H. J., Kim, J. S., Shin, E. S., Cho, J. H. *et al.* The LRRK2 G2385R variant is a risk factor for sporadic Parkinson's disease in the Korean population. *Parkinsonism Relat. Disord.* **16**, 85–88.
36 Tan, E. K., Peng, R., Teo, Y. Y., Tan, L. C., Angeles, D., Ho, P. *et al.* Multiple LRRK2 variants modulate risk of Parkinson disease: a Chinese multicenter study. *Hum. Mutat.* **31**, 561–568 (2010).
37 An, X. K., Peng, R., Li, T., Burgunder, J. M., Wu, Y., Chen, W. J. *et al.* LRRK2 Gly2385Arg variant is a risk factor of Parkinson's disease among Han-Chinese from mainland China. *Eur. J. Neurol.* **15**, 301–305 (2008).
38 Chan, D. K., Ng, P. W., Mok, V., Yeung, J., Fang, Z. M., Clarke, R. *et al.* LRRK2 Gly2385Arg mutation and clinical features in a Chinese population with early-onset Parkinson's disease compared to late-onset patients. *J. Neural. Transm.* **115**, 1275–1277 (2008).
39 Zabetian, C. P., Yamamoto, M., Lopez, A. N., Ujike, H., Mata, I. F., Izumi, Y. *et al.* LRRK2 mutations and risk variants in Japanese patients with Parkinson's disease. *Mov. Disord.* **24**, 1034–1041 (2009).
40 Farrer, M. J., Stone, J. T., Lin, C. H., Dachsel, J. C., Hulihan, M. M., Haugarvoll, K. *et al.* Lrrk2 G2385R is an ancestral risk factor for Parkinson's disease in Asia. *Parkinsonism Relat. Disord.* **13**, 89–92 (2007).
41 Tan, E. K. The role of common genetic risk variants in Parkinson disease. *Clin. Genet.* **72**, 387–393 (2007).
42 Satake, W., Nakabayashi, Y., Mizuta, I., Hirota, Y., Ito, C., Kubo, M. *et al.* Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat. Genet.* **41**, 1303–1307 (2009).
43 Simon-Sanchez, J., Schulte, C., Bras, J. M., Sharma, M., Gibbs, J. R., Berg, D. *et al.* Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat. Genet.* **41**, 1308–1312 (2009).
44 Mizuta, I., Satake, W., Nakabayashi, Y., Ito, C., Suzuki, S., Momose, Y. *et al.* Multiple candidate gene analysis identifies alpha-synuclein as a susceptibility gene for sporadic Parkinson's disease. *Hum. Mol. Genet.* **15**, 1151–1158 (2006).
45 Mitsui, J., Mizuta, I., Toyoda, A., Ashida, R., Takahashi, Y., Goto, J. *et al.* Mutations for Gaucher disease confer high susceptibility to Parkinson disease. *Arch. Neurol.* **66**, 571–576 (2009).
46 Sidransky, E., Nalls, M. A., Aasly, J. O., Aharon-Peretz, J., Annesi, G., Barbosa, E. R. *et al.* Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N. Engl. J. Med.* **361**, 1651–1661 (2009).

Adult-onset leukoencephalopathies with vanishing white matter with novel missense mutations in EIF2B2, EIF2B3, *and* EIF2B5

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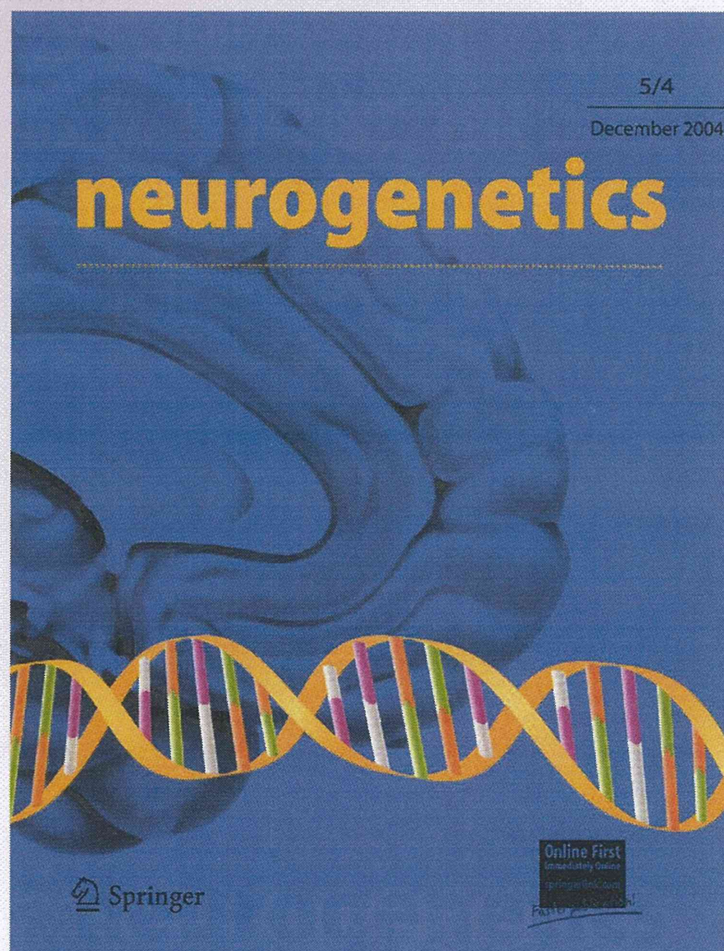

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LETTER TO THE EDITORS

Adult-onset leukoencephalopathies with vanishing white matter with novel missense mutations in *EIF2B2*, *EIF2B3*, and *EIF2B5*

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Leukoencephalopathy with vanishing white matter (VWM) is a type of leukoencephalopathy with autosomal recessive inheritance. Magnetic resonance imaging (MRI) reveals diffuse leukoencephalopathy with lesions having cerebrospinal fluid (CSF)-like signals. The clinical presentations include progressive cerebellar ataxia, spasticity, and mental decline. The course is chronic progressive with episodes of rapid deterioration following a minor head trauma. Mutations in the five gene-encoding subunits of the translation initiation factor eIF2B, *EIF2B1-5*, have been identified as the causative mutations for VWM. Although the age at onset of VWM is usually 2–6 years, patients with adult onset have been described. All adult-onset cases except one have been found to be associated with mutations in *EIF2B5* [1]. We report cases of adult-onset VWM with novel missense mutations in *EIF2B2*, *EIF2B3*, and *EIF2B5*, which showed decreased eIF2B activities.

Case 1: A Japanese woman aged 56 who experienced secondary amenorrhea and juvenile cataracts during her early 20s. Clumsiness in her hands and gait unsteadiness appeared at age 43. Leg weakness and gait unsteadiness worsened, occasionally in a phased manner after a minor trauma. Forgetfulness appeared at age 54.

Case 2: A Japanese man aged 53 who noticed gait unsteadiness and miscalculation at age 50. Gait unsteadiness worsened, sometimes in a phased manner after a minor trauma.

Case 3: A Japanese woman aged 30 who experienced secondary amenorrhea at age 28. She noticed hemianopia on the left at age 29. She noticed weakness in her left leg after a fall.

All of three cases had parents who were first cousins. MRI of the three cases showed leukoencephalopathy with lesions having CSF-like signals (Supplementary Fig. 1A–I).

The multipoint parametric linkage study (autosomal recessive model) using the 100K SNP array (Affymetrix, Santa Clara, CA, USA) identified homozygous regions in

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