Perry（ペリー）症候群の診断および治療方法の更なる推進に関する研究

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研究要旨

　Perry 症候群は常染色体優性遺伝を呈する神経変性疾患で、原因遺伝子は p150glued である。本分担研究では変異型 p150glued 強制発現によるミトコンドリア形態およびミトコンドリア膜電位を評価し、凝集体形成とミトコンドリアとの関連を検討した。

　野生型及び病因変異 p150glued 強制発現細胞を夫々固定し、電子顕微鏡にて評価したところ、変異型 p150glued 強制発現細胞においては核周囲に内部構造が破壊され、膨化した異常な形態と持つミトコンドリアを多数認めたが、コントロールでは同所見を認めなかった。さらに変異型 p150glued 強制発現細胞におけるミトコンドリア膜電位を MitoTracker-RedCMXRos によってフローサイトメーターを用いて検討したところ、強制発現細胞において膜電位の低下したミトコンドリア量が増大していることを確認した。以上の所見から変異型 p150glued は、凝集体形成を形成すると同時に異常ミトコンドリア集積を招来することを確認した。

A．研究目的

　神経難病である Perry 症候群における原因遺伝子 dynactin (p150glued)の遺伝子変異によるミトコンドリア機能への影響を評価すること。

B．研究方法

本研究では主に培養細胞（HeLa 細胞）を用いた。以下にそれぞれの実験の詳細を述べる。

1．プラスミド DNA

　Dynactin (p150glued)野生型に FLAG および EGFP をタグしたプラスミド DNA を作製し、Mutagenesis® (Stratagene 社製)を用いて、病因変異を挿入した変異型 dynactin（G59S、G71A、G71E、G71V、T72P、Q74P）を作製した。

2．ミトコンドリア形態評価（電顕による）

HeLa 細胞に、Lipofectamine 2000® (Invitrogen 社製) を用いて、dynactin(p150glued)-GFP 野生型・変異型を夫々強制発現し、24 時間後にグルタールアルデヒドおよび酸化オスミウムにて固定し、電子顕微鏡にて評価した

3．ミトコンドリア膜電位評価

HeLa 細胞に、Lipofectamine 2000® (Invitrogen 社製)を用いて、dynactin(p150glued)-GFP 野生型・変異型を夫々強制発現し、24 時間後に MitoTracker-Red CMXRos 100nM に 20 分間 incubation し、GFP 陽性細胞における MitoTracker-Red CMXRos 蛍光強度をフローサイトメーター(FACSFortessa, BD Biosciences)を用いて評価した。
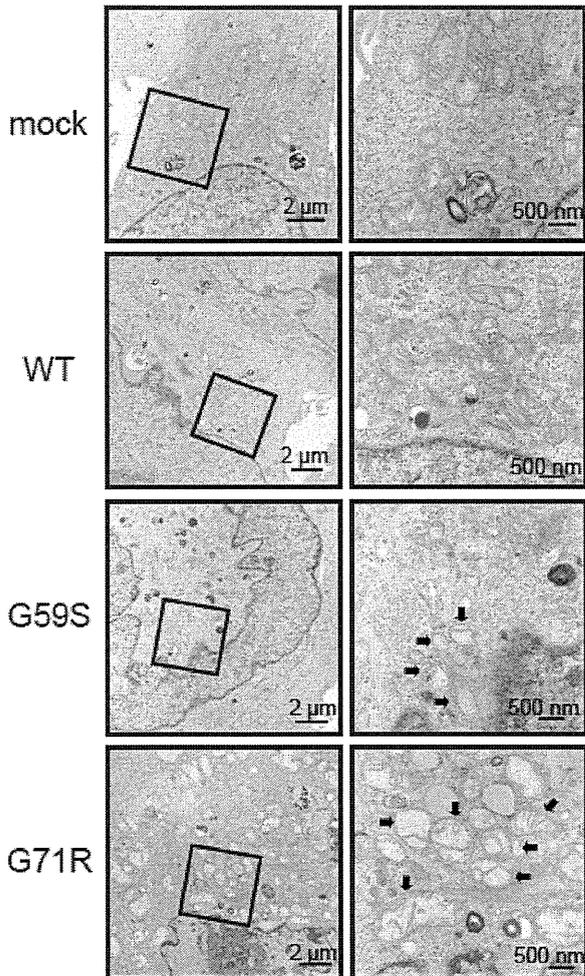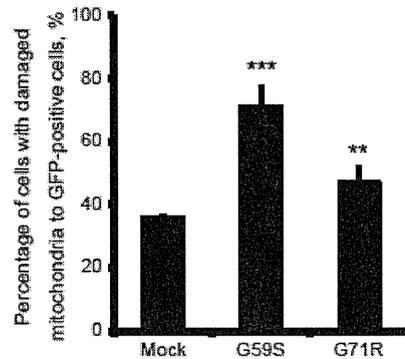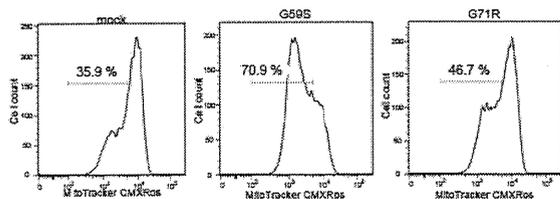
（倫理面への配慮）

特記すべきことなし。

C．研究結果

　結果 1 ：　野生型、変異型 p150glued 強制発現細胞において、それぞれミトコンドリア形態を電子

顕微鏡にて比較した。下図のように、コントロールベクター又は野生型強制発現細胞では、ミトコンドリアのクリステは保たれ、大きさに変化を認めなかった。しかし変異型強制発現細胞では、内部構造が破壊され、膨化したミトコンドリアが核周囲を中心に認められた（矢印）。
—

結果２：　変異型 p150glued の強制発現により、右図のように、Mitotracker-Red CMXRos の蛍光強度が減少した変異型導入細胞が著明に増加した。同現象は、GFP-p150glued 陽性細胞のみで評価した。
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D．考察

　初年度の検討により変異型 dynactin(p150glued) 強制発現により細胞質内凝集物が形成されることを確認していたが、同現象とミトコンドリア機能との関連について検討を行ったが、MitoTracker-Red CMXRos の取り込みが低下（膜電位が低下していることを示す）し、かつ異常ミトコンドリアが凝集することから、機能を喪失したミトコンドリアの凝集と判断された。分担研究者斉木の結果と併せて考察し、この異常ミトコンドリア凝集が内因性アポトーシス経路の原因になっていると推察された。

E．結論

　変異型 dynactin の強制発現は dynactin 凝集物を細胞質内に形成させると同時に、異常ミトコンドリアの蓄積を招く。

F．健康危険情報
分担研究者報告書のため記載の必要なし。

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波田野　琢, 久保　紳一郎, 高梨　雅史, 王子　悠, 森　聡生, 板谷　昌子, 服部　信孝

第 52 回日本神経学会（東京国際フォーラム）　平成 24 年 5 月 22-25 日

H．知的財産権の出願・登録状況（予定を含む。）

1. 特許取得

なし

　2. 実用新案登録

なし

　3.その他

なし。

研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表（服部　信孝）

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研究成果の刊行物・別刷

ELSEVIER

Brief communication

Analysis of *C9orf72* repeat expansion in 563 Japanese patients with amyotrophic lateral sclerosis

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Abstract

Recently, a hexanucleotide repeat expansion in *C9orf72* was identified as the most common cause of both sporadic and familial amyotrophic lateral sclerosis (ALS) and frontotemporal dementia in Western populations. We analyzed 563 Japanese patients with ALS (552 sporadic and 11 familial) using fluorescent fragment-length analysis of *C9orf72* and repeat-primed polymerase chain reaction analysis. Haplotype analysis was performed for 42 single nucleotide polymorphisms in patients with *C9orf72* repeat expansion. *C9orf72* repeat expansion was found in 2 patients with sporadic ALS (2/552 = 0.4%) and no patients with familial ALS (0/11 = 0%). In the probands' families, 1 primary progressive aphasia patient and 1 asymptomatic 76-year-old individual exhibited *C9orf72* repeat expansion. All of the patients with the *C9orf72* repeat expansion carried the 20-single nucleotide polymorphism consensus risk haplotype. The frequency of the *C9orf72* repeat expansion among Japanese patients is much lower than in Western populations. The existence of a 76-year-old asymptomatic carrier supported the notion of incomplete penetrance. The *C9orf72* mutation should be analyzed in sporadic ALS patients after determining their family histories not only of frontotemporal dementia but also of primary progressive aphasia.
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Keywords: Amyotrophic lateral sclerosis; *C9orf72*; Incomplete penetrance; Sporadic; Aphasia; Frontotemporal dementia

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder that primarily affects motor neurons in the spinal cord, brain stem, and cerebral cortex, typically leading to death within a few years. Five to ten percent of ALS cases are familial, and the remaining cases are believed to be sporadic (Valdmanis et al., 2009). A number of genes causing ALS with a dominant mode of inheritance have

been discovered, such as *SOD1*, *TARDBP*, *FUS*, *VAPB*, *ANG*, *VCP*, *OPTN* (Ticozzi et al., 2011), and *UBQLN2* (Deng et al., 2011). Moreover, there is increasing clinical and pathological evidence for the hypothesis that ALS and frontotemporal dementia (FTD) constitute an overlapping continuum of diseases (Lomen-Hoerth et al., 2002; Neumann et al., 2006). Recently, the expansion of a noncoding GGGGCC hexanucleotide repeat in the *C9orf72* gene has been reported to be a major cause of both ALS and FTD (DeJesus-Hernandez et al., 2011; Gijselinck et al., 2012; Renton et al., 2011) and the most common genetic abnormality in familial and sporadic forms of both ALS and FTD, particularly in Western populations (Chiò et al., 2012; DeJesus-Hernandez et al., 2011; Gijselinck et al., 2012; Renton et al., 2011; Sabatelli et al., 2012; Stewart et al., 2012). In the present study, we describe the incidence and demographic and clinical features associated with the *C9orf72* mutation in a large cohort of Japanese ALS patients. We also perform haplotype analysis to investigate whether Japanese patients have the same risk haplotype as European patients (Gijselinck et al., 2012; Laaksovirta et al., 2010; Mok et al., 2012).

2. Methods

2.1. Subjects

We obtained a total of 760 DNA samples from the Japanese Consortium for Amyotrophic Lateral Sclerosis Research (JaCALS; Appendix A). A total of 563 (11 familial and 552 sporadic) patients were diagnosed with ALS according to the El Escorial revised criteria (Brooks et al., 2000) and classified as bulbar-onset, spinal-onset, FTD-ALS, or other (see Supplementary Table 1 for details). We had determined the family histories of ALS but not FTD or primary progressive aphasia (PPA) in all of the patients when they were enrolled as patients with sporadic ALS (SALS). We recruited 197 control subjects, none of whom had a medical or family history of neurodegenerative disorders. The mean age at onset of the patients with ALS was 60.4 ± 11.7 years (range 20–86), and the mean age at sampling of the controls was 60.6 ± 10.3 years (range 26–83). All of the subjects were unrelated Japanese individuals. Written informed consent was obtained from all of the subjects. The ethical committees at the participating institutions approved this study.

2.2. Fluorescent fragment-length analysis of C9orf72 and repeat-primed PCR analysis

The normal repeat number of the GGGGCC hexanucleotide was determined in all of the patients and control subjects using genotyping primers, as previously described (DeJesus-Hernandez et al., 2011). To provide a qualitative assessment of the presence of *C9orf72* repeat expansions, we performed repeat-primed polymerase chain reaction

(PCR), as previously described (DeJesus-Hernandez et al., 2011).

2.3. Haplotype analysis

We genotyped 42 single nucleotide polymorphisms (SNPs) across 232 kilobase of Chromosome 9p21, which were first described as the founder haplotype in the Finnish ALS population (Laaksovirta et al., 2010), using primers (Supplementary Table 2) to determine whether our Japanese patients carried the haplotype associated with a risk of ALS. These 42 SNPs included the 20-SNP consensus risk allele that had recently been detected in genome-wide association studies in several populations (Mok et al., 2012). We also performed haplotype analysis with 4 microsatellites (D9S1121, D9S169, D9S270, and D9S104) flanking the *C9orf72* GGGGCC repeat, as previously described (Gijselinck et al., 2012) (Fig. 1).

3. Results

3.1. Detection of C9orf72 repeat expansion

The *C9orf72* repeat expansion was found in 2 of 522 Japanese patients (2/552 = 0.4%) with SALS and none of the 11 patients (0/11 = 0%) with familial ALS (FALS) using repeat-primed PCR (Table 1). Patient A-I with a *C9orf72* mutation was classified as SALS in this study, but after detecting the mutation, we found that patient A-II (a brother of patient A-I) developed aphasia and dementia and had a *C9orf72* mutation (Fig. 1). The average repeat number based on fluorescent fragment-length analysis was 3.65 ± 2.43 (range 2–13 repeats) in 561 ALS patients without the *C9orf72* mutation. A subsequent analysis of 197 healthy controls did not detect any *C9orf72* mutation. The average repeat number was 3.69 ± 2.46 (range 2–14 repeats) in the 197 controls. The mean age at disease onset in patients with *C9orf72* mutation, including patient A-II, was 64.7 ± 6.1 years (range 57–72). The genotypes of all individuals with the *C9orf72* mutation were detected for the 20 SNPs spanning a 140-kilobase segment concordant with the recently identified risk haplotype on chromosome 9p (Mok et al., 2012) and 24 or 25 consecutive SNPs in the 42-SNP Finish risk haplotype (Laaksovirta et al., 2010) (Fig. 1, Supplementary Table 3).

3.2. Clinical presentations of individuals with C9orf72 mutation

3.2.1. Patient A-I (family A)

Patient A-I was a 65-year-old man who reported weakness in the left leg. The weakness progressed, and he developed fasciculation. At age 66, a neurological examination revealed dementia. His Mini Mental State Examination score was 23/30, and his Frontal Assessment Battery score was 13/18. He also exhibited dysarthria and weakness, atrophy, and fasciculation in the tongue and all 4 modalities. His tendon reflexes were diminished, and the plantar re-

A)

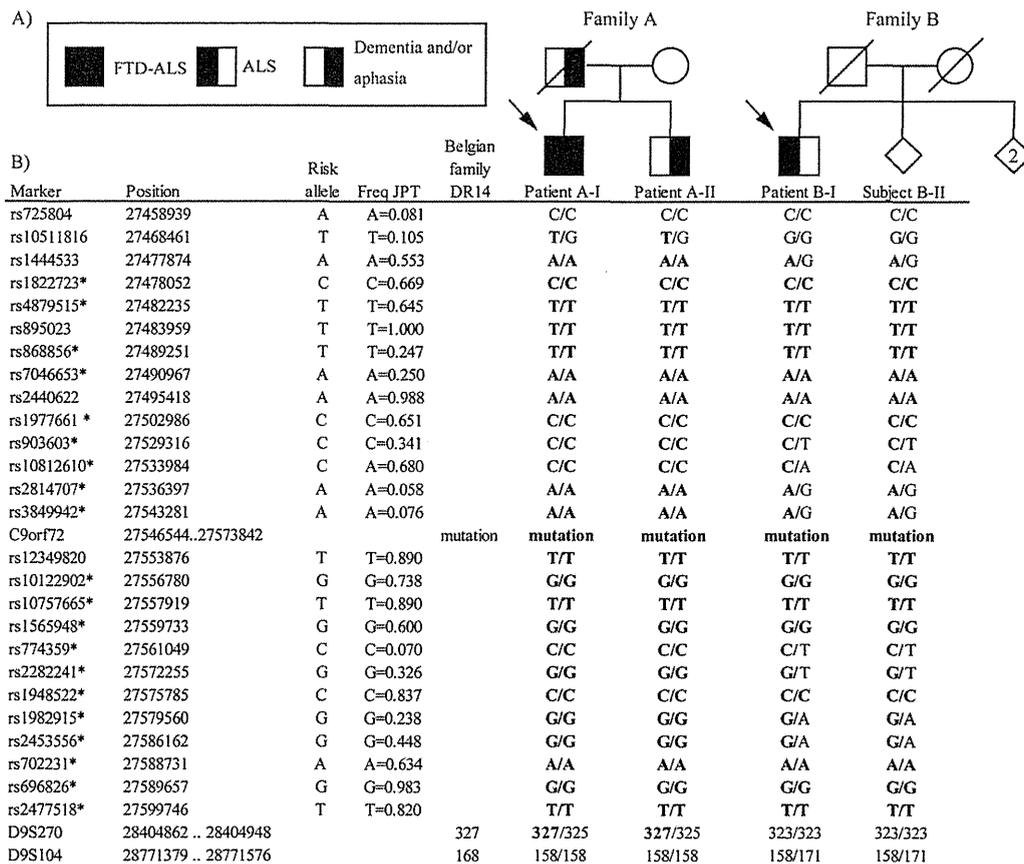| B) Marker	Position	Risk allele	Freq JPT	Belgian family DR14	Patient A-I	Patient A-II	Patient B-I	Subject B-II
rs725804	27458939	A	A=0.081		C/C	C/C	C/C	C/C
rs10511816	27468461	T	T=0.105		T/G	T/G	G/G	G/G
rs1444533	27477874	A	A=0.553		A/A	A/A	A/G	A/G
rs1822723*	27478052	C	C=0.669		C/C	C/C	C/C	C/C
rs4879515*	27482235	T	T=0.645		T/T	T/T	T/T	T/T
rs895023	27483959	T	T=1.000		T/T	T/T	T/T	T/T
rs868856*	27489251	T	T=0.247		T/T	T/T	T/T	T/T
rs7046653*	27490967	A	A=0.250		A/A	A/A	A/A	A/A
rs2440622	27495418	A	A=0.988		A/A	A/A	A/A	A/A
rs1977661 *	27502986	C	C=0.651		C/C	C/C	C/C	C/C
rs903603*	27529316	C	C=0.341		C/C	C/C	C/T	C/T
rs10812610*	27533984	C	A=0.680		C/C	C/C	C/A	C/A
rs2814707*	27536397	A	A=0.058		A/A	A/A	A/G	A/G
rs3849942*	27543281	A	A=0.076		A/A	A/A	A/G	A/G
C9orf72	27546544..27573842			mutation	mutation	mutation	mutation	mutation
rs12349820	27553876	T	T=0.890		T/T	T/T	T/T	T/T
rs10122902*	27556780	G	G=0.738		G/G	G/G	G/G	G/G
rs10757665*	27557919	T	T=0.890		T/T	T/T	T/T	T/T
rs1565948*	27559733	G	G=0.600		G/G	G/G	G/G	G/G
rs774359*	27561049	C	C=0.070		C/C	C/C	C/T	C/T
rs2282241*	27572255	G	G=0.326		G/G	G/G	G/T	G/T
rs1948522*	27575785	C	C=0.837		C/C	C/C	C/C	C/C
rs1982915*	27579560	G	G=0.238		G/G	G/G	G/A	G/A
rs2453556*	27586162	G	G=0.448		G/G	G/G	G/A	G/A
rs702231*	27588731	A	A=0.634		A/A	A/A	A/A	A/A
rs696826*	27589657	G	G=0.983		G/G	G/G	G/G	G/G
rs2477518*	27599746	T	T=0.820		T/T	T/T	T/T	T/T
D9S270	28404862 .. 28404948			327	327/325	327/325	323/323	323/323
D9S104	28771379 .. 28771576			168	158/158	158/158	158/171	158/171

Fig. 1. (A) The pedigrees of the 2 families with *C9orf72* repeat expansion. To maintain confidentiality, several unaffected individuals who died early in families A and B are not shown. Probands are indicated by arrows. (B) The genotyping data of the single nucleotide polymorphisms (SNPs) and microsatellites. Twenty SNPs, which comprised a recently identified consensus risk haplotype (Mok et al., 2012), are shown with an asterisk. See Supplementary Table 3 for details of the analyses of 42 SNPs (Laaksovirta et al., 2010) and microsatellites (Gijselinck et al., 2012). Alleles possibly shared between our subjects and patients in Western populations are shown in bold. The genotypes of all 4 subjects with respect to the 20 SNPs were found to be concordant with the risk haplotype (Mok et al., 2012). All of the positions of SNPs and microsatellites were from NC_000009.11. Abbreviations: ALS, amyotrophic lateral sclerosis; Freq JPT, Frequency in Japanese in Tokyo from International HapMap project (International HapMap Consortium, 2003); FTD, frontotemporal dementia.

sponse was extensor on the left. He had neither dysphagia nor dyspnea. No sensory abnormalities were noted. Extensive screening for causes of motor neuropathy was negative. The diagnosis was clinically probable ALS-laboratory supported (Brooks et al., 2000) and FTD-ALS.

3.2.2. Patient A-II (family A)

This patient was a 57-year-old man who presented with difficulty speaking. He was believed to have suffered from a mental disease after being imprisoned because of his involvement in a fatal car accident. At age 64, he was severely dysfluent and could barely speak. Logoclonia was particularly prominent. However, he did not exhibit any violent behavior or other behavioral abnormalities. He also did not display any clinical features of motor neuron disease. Brain magnetic resonance imaging revealed severe frontotemporal lobar atrophy. PPA was considered the most likely diagnosis.

3.2.3. Patient B-I (family B)

Patient B-I was a 72-year-old man who presented with gait disturbance and weakness in the proximal lower extremity muscle. His family history was negative for motor neuron disease and dementia (Fig. 1). The muscle weakness and atrophy progressed and spread to the other parts of his body despite treatment with intravenous gamma globulin. At age 74, he could not roll over while sleeping. A neurological examination showed marked muscle atrophy in his arms and shoulders and prominent fasciculation in his legs. The deep tendon reflexes were decreased in his limbs, and he had no pathological reflexes. Sensations in all 4 modalities were intact. At age 75, he developed dyspnea and dysphagia and started noninvasive positive pressure ventilation and intravenous hyperalimentation. He died of respiratory insufficiency at age 76. An autopsy was not performed. The diagnosis was clinically suspected ALS (Brooks et al., 2000).

Table 1
Frequencies of ALS patients with *C9orf72* and *SOD1* mutations in different countries

Study	Population	C9orf72			SOD1	
		Familial ALS	Sporadic ALS	Mean AAO (range), years	Familial ALS	Sporadic ALS
This study, 2012	Japanese (JaCALS)	0% (0/11)	0.4% (2/552)	64.7 (57–72)	NA	NA
Akimoto et al. (2011)	Japanese (JaCALS)	NA	NA	NA	NA	1.6% (4/255)
DeJesus-Hernandez et al. (2011)	Mixed[a]	23.5% (8/34)	4.1% (8/195)***	54.5 (41–72)	11.8% (4/34)	0% (0/195)
Renton et al. (2011)	Finish	46.4% (52/112)**	21.0% (61/290)***	53 (30–71)	NA	NA
Gijselinck et al. (2012)	Flanders-Belgian	46.7% (7/15)*	4.9% (6/122)***	54.5 (38–64)	0% (0/16)	0% (0/125)
Stewart et al. (2012)	Unknown[b]	27.4% (17/62)	3.6% (6/169)**	58.2 (39–82)	Total 8.2% (19/231)	
Byrne et al. (2012)	Ireland	40.8% (20/49)*	4.9% (19/386)***	56.3 (NA)	Total 0% (0/191)	
Cooper-Knock et al. (2012)	Northern England	42.9% (27/63)*	7.0% (35/500)***	57.3 (27–74)	Total 2.5% (14/563)	
Chiò et al. (2012)	Italian	37.5% (45/120)*	NA	59.0 (NA-80)	0% (0/141)	NA
	Sardinian	57.1% (12/21)**	NA	60.4 (NA)	NA	NA
	German	22.0% (9/41)	NA	56.4 (NA)	NA	NA
Majounie et al. (2012)	England	45.9% (45/98)**	6.8% (62/916)***	NA	NA	NA
	German	21.7% (15/69)	5.2% (22/421)***	NA	NA	NA
	Italian	37.8% (34/90)*	4.1% (19/465)***	NA	NA	NA
	Sardinian	57.9% (11/19)**	7.8% (10/129)***	NA	NA	NA
	USA White	US total 36.2% (59/163)*	5.4% (48/890)***	NA	NA	NA
	USA Hispanic		8.3% (6/72)***	NA	NA	NA
	USA Black		4.1% (2/49)	NA	NA	NA
	Australian	NA	5.3% (14/263)***	NA	NA	NA
	Israeli	21.4% (3/14)	NA	NA	NA	NA
	Indian	NA	0% (0/31)	NA	NA	NA
	Asian	5.0% (1/20)	0% (0/238)	NA	NA	NA
	Pacific islander/Guam	NA	0% (0/90)	NA	NA	NA
Sabatelli et al. (2012)	Italian	NA	3.7% (60/1624)***	58.6 (49–65)	NA	NA
	Sardinian	NA	6.8% (9/133)***	62.9 (58–63)	NA	NA

Key: AAO, age at onset; ALS, amyotrophic lateral sclerosis; JaCALS, Japanese Consortium of Amyotrophic Lateral Sclerosis Research; NA, not available.
[a] Mixed included 229 ALS patients from Mayo Clinic, Florida: White (212), Asian (1), Pacific Islander (1), and Black or African American (15).
[b] Unknown included 231 ALS patients from the ALS Clinic of Vancouver Coastal Health and the University of British Columbia (Vancouver General Hospital and GF Strong Rehabilitation Centre sites).
* $p < 0.05$, compared with our results (2-tailed, Yates's χ^2 test).
** $p < 0.01$, compared with our results (2-tailed, Yates's χ^2 test).
*** $p < 0.001$ compared with our results (2-tailed, Yates's χ^2 test).

3.2.4. Subject B-II (family B)

Subject B-II, a sibling of Patient B-I, had a *C9orf72* mutation but did not have symptoms of dementia or motor neuron disease until age 76 (Fig. 1).

4. Discussion

We began this study considering patients without family histories of ALS to be SALS because our cohort included only family histories of ALS but not FTD or PPA. Although it may be difficult to describe the real frequency in SALS because 1 of the SALS patients had a family member who developed PPA, the frequencies of the *C9orf72* mutation in Japanese patients were 0.4% (2/552) in SALS and 0% (0/11) in FALS according to this classification. In contrast, the frequencies of the *C9orf72* mutation fall within the ranges of 21%–57% in FALS and 3%–21% in SALS in Western populations (Table 1), and the *C9orf72* mutation has been reported as the most common genetic cause of FALS and SALS in Western populations (Byrne et al.,

2012; Chiò et al., 2012; Cooper-Knock et al., 2012; DeJesus-Hernandez et al., 2011; Gijselinck et al., 2012; Majounie et al., 2012; Renton et al., 2011; Sabatelli et al., 2012; Stewart et al., 2012). However, the *C9orf72* mutation in this study was not more frequent than the *SOD1* mutation in Japanese SALS patients (0.4% and 1.6%, Table 1) (Akimoto et al., 2011). Considering these data, the *C9orf72* mutation is more common than the *SOD1* mutation in Western populations but not in Japan, suggesting different genetic backgrounds. Our results may explain the association study of rs2814707 on 9p21.2, which was reported to be the most significantly associated SNP with SALS in Caucasian but not in Japanese and Chinese populations (Iida et al., 2011). A recent report revealed that the rate of expansion in Asian FALS and SALS was 5% (1/20) and 0% (0/238), respectively (Majounie et al., 2012). An analysis of the SNPs on chromosome 9p revealed that all 4 subjects with the *C9orf72* mutation and another Japanese subject from the previously mentioned report (Majounie et al., 2012) share a shorter region of the risk haplotype

than Western populations. Thus, the haplotype bearing the C9orf72 mutation was only shared in a narrow region between Western and Asian populations, suggesting that the C9orf72 mutation may be an old mutation in human migration history from Western to East Asia. This mutation was estimated to be approximately 1500 years old (Majounie et al., 2012).

Bulbar onset and cognitive impairment have been reported to be more common in ALS patients with the C9orf72 repeat expansion (Chiò et al., 2012; Cooper-Knock et al., 2012; DeJesus-Hernandez et al., 2011; Sabatelli et al., 2012; Stewart et al., 2012). We did not find any patients with bulbar onset, but we identified 2 patients with dementia. Although the age at onset has been known to be lower in SALS patients with the C9orf72 mutation than in those without this mutation (Sabatelli et al., 2012), our patients exhibited a relatively older age at onset (Table 1).

Although apparently sporadic patients with C9orf72 mutation have been detected worldwide (Byrne et al., 2012; Cooper-Knock et al., 2012; Sabatelli et al., 2012), it was not known whether this phenomenon was due to incomplete penetrance or to spontaneous expansion of the GGGGCC hexanucleotide repeat from a nonpathogenic parental form (ie, a de novo expansion). In this study, we found a 76-year-old healthy individual with a C9orf72 mutation (Subject B-II), as described in previous studies (Majounie et al., 2012; Renton et al., 2011). This discovery suggests not de novo expansion but incomplete penetrance, which explains the existence of apparently sporadic patients with the C9orf72 mutation. Although it has been reported that the penetrance of the C9orf72 mutation is almost full by 80 years by Kaplan–Meier analysis of 603 mutant gene carriers and 5 neurologically healthy individuals, further studies of family members of patients with the C9orf72 mutation will be required to calculate the true penetrance and to improve genetic counseling.

Finally, we found a PPA patient with the C9orf72 mutation after detecting the mutation in a SALS patient, suggesting the importance of collecting information regarding whether SALS patients have a family history of dementia or aphasia. Therefore, the possibility of C9orf72 mutation should be investigated when clinicians meet with SALS patients after determining their family histories of FTD or PPA. Furthermore, our data supported Byrne and colleagues' suggestion that a family history of FTD should also be included in the revised definition of FALS (Byrne et al., 2012).

Disclosure statement

All of the authors disclose no conflicts of interest. The study was approved by the ethical committees of the participating centers. All participants gave written informed consent.

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Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2012.05.011.

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Analyses of the *MAPT*, *PGRN*, and *C9orf72* mutations in Japanese patients with FTLD, PSP, and CBS

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ARTICLE INFO

ABSTRACT

Background: Mutations in the microtubule associated protein tau (*MAPT*) and progranulin (*PGRN*) have been identified in several neurodegenerative disorders, such as frontotemporal lobar degeneration (FTLD), progressive supranuclear palsy (PSP), and corticobasal syndrome (CBS). Recently, *C9orf72* repeat expansion was reported to cause FTLD and amyotrophic lateral sclerosis (ALS). To date, no comprehensive analyses of mutations in these three genes have been performed in Asian populations. The aim of this study was to investigate the genetic and clinical features of Japanese patients with *MAPT*, *PGRN*, or *C9orf72* mutations.
Methods: *MAPT* and *PGRN* were analyzed by direct sequencing and gene dosage assays, and *C9orf72* repeat expansion was analyzed by repeat-primed PCR in 75 (48 familial, 27 sporadic) Japanese patients with FTLD, PSP, or CBS.
Results: We found four *MAPT* mutations in six families, one novel *PGRN* deletion/insertion, and no repeat expansion in *C9orf72*. Intriguingly, we identified a de novo *MAPT* p.S285R mutation. All six patients with early-onset PSP and the abnormal eye movements that are not typical of sporadic PSP had *MAPT* mutations. The gene dosages of *MAPT* and *PGRN* were normal.
Discussion: *MAPT* p.S285R is the first reported de novo mutation in a sporadic adult-onset patient. *MAPT* mutation analysis is recommended in both familial and sporadic patients, especially in early-onset PSP patients with these abnormal eye movements. Although *PGRN* and *C9orf72* mutations were rare in this study, the *PGRN* mutation was found in this Asian FTLD. These genes should be studied further to improve the clinicogenetic diagnoses of FTLD, PSP, and CBS.

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1. Introduction

Mutations in the microtubule-associated protein tau (*MAPT*) and the progranulin (*PGRN*) genes have been identified in families with frontotemporal dementia and parkinsonism linked to chromosome 17 [1–3]. Recently, two studies reported that the expansion of a noncoding GGGGCC hexanucleotide repeat in the *C9orf72* gene is

a major cause of both frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) [4,5].

Each of these genes can be associated with multiple clinical entities. Patients with *MAPT* mutations may receive diagnoses of frontotemporal dementia (FTD), primary progressive aphasia (PPA), or progressive supranuclear palsy (PSP). Rarely, corticobasal syndrome (CBS) or FTD with ALS (FTD-ALS) may be manifested in these patients [6]. The clinical diagnoses of patients with *PGRN* mutations include FTD, PPA, and CBS [6]. *C9orf72* repeat expansion causes FTD, ALS, FTD-ALS [4,5], PPA [5,7], and CBS [8] phenotypes. Thus, due to the complicated and often overlapping genetic and phenotypic variability in these patients, an accurate diagnosis of these clinical entities before autopsy is often difficult for clinicians.

To date, few comprehensive screening studies of these three genes have been performed in Asian populations. The aims of this study are to characterize the roles of known and, more importantly, novel disease-causing genes and to investigate the genetic and clinical features of FTLD, PSP, and CBS patients with *MAPT*, *PGRN*, and *C9orf72* mutations. In this study, we also describe the abnormal eye movements that are generally not observed in sporadic PSP but occur in early-onset PSP patients bearing *MAPT* mutations.

2. Methods

2.1. Subjects

We studied 75 Japanese patients who were diagnosed with FTLD, PSP, and CBS with or without a family history of disease. FTLD was divided into three subclasses: behavioral variant FTD (bvFTD), FTD-ALS, and PPA. The clinical diagnoses were established according to the consensus criteria for FTD [9], PPA [10], PSP [11], and CBS [12]. The characteristics of the 75 analyzed patients (69 index patients) are shown in Table 1. This study was approved by the ethics committee of the Juntendo University School of Medicine. Each subject provided written informed consent. All of the subjects in the control cohort were Japanese individuals and were evaluated by neurologists to ensure that no subjects exhibited any clinical manifestations of neurodegenerative diseases.

2.2. Genetic analyses

For direct sequence analysis, each exon was amplified by polymerase chain reaction (PCR) using published primers for *MAPT* [13] and *PGRN* [2] in a standard protocol. Dideoxy cycle sequencing was performed using Big Dye Terminator chemistry (Applied Biosystems, Foster City, CA). These products were loaded into ABI310 and 3130 automated DNA sequence analyzers and analyzed with DNA Sequence Analysis software (Applied Biosystems). To provide a qualitative assessment of the presence of an expanded (GGGGCC)$_n$ hexanucleotide repeat in the *C9orf72* gene, we performed repeat-primed PCR as previously described [4]. The normal repeat number of the GGGGCC hexanucleotide was determined in all of the patients using genotyping primers, as previously described [4]. The PCR products

Table 1
The clinical diagnoses and characteristics of 75 patients (69 index patients).

Clinical phenotype	No.	% of total	% of Male	Mean (SD) AAO (range, years)	Familial	Sporadic
FTLD	38	50.7	39.5	57.1 (±12.4), 36–78	21	17
bvFTD	29	38.7	34.5	54.5 (±12.6), 36–78	18	11
FTD-ALS	2	2.7	100	67.5 (±1.5), 66–69	1	1
PPA	7	9.3	42.9	65.0 (±7.4), 58–77	2	5
PSP	25	33.3	68.0	59.8 (±13.0), 40–76	18	7
CBS	12	16.0	33.3	58.4 (±9.52), 40–71	9	3
Total	75	100	48.0	58.2 (±12.3), 36–78	48	27
Index patients	69	92.0	46.4	58.9 (±12.4), 36–78	42	27
Relatives	6	8	66.7	50.3 (±6.6), 44–61	6	0

FTLD = frontotemporal lobar degeneration.
bvFTD = behavioral variant frontotemporal dementia.
FTD-ALS = frontotemporal dementia with amyotrophic lateral sclerosis.
PPA = primary progressive aphasia; PSP = progressive supranuclear palsy.
CBS = corticobasal syndrome; SD = standard deviation; AAO = age at onset.

were analyzed on an ABI3130 DNA Analyzer and visualized using Gene Mapper software (Applied Biosystems).

2.3. Multiplex ligation-dependent probe amplification (MLPA)

To confirm the gene dosages of *MAPT* and *PGRN*, we performed MLPA using the SALSA MLPA P275-B1 MAPT-PGRN kit (MRC-Holland, Amsterdam, The Netherlands). The DNA detection/quantification protocol was provided by the manufacturer. The products were quantified using the ABI3130 Genetic Analyzer and Gene Mapper v3.7 (Applied Biosystems). The kit contains 32 probes, including 13 *MAPT* probes (located in exons 1–13) and 5 *PGRN* probes (located in exons 1, 3, 6, 10, and 12) located within other genes on chromosome 17q21. The MLPA data were analyzed as described previously [14].

2.4. Exon-trapping analysis

To determine whether a novel *MAPT* mutation was pathogenic, we performed an exon-trapping analysis. We used a wild-type construct and constructs containing the novel *MAPT* p.S285R or the IVS10+3 intronic mutation [15]. The *MAPT* sequences included exon 10, 34 nucleotides of the upstream intronic sequence and 85 nucleotides of the downstream intronic sequence. The PCR products were subcloned into the splicing vector pSPL3 (Invitrogen, Carlsbad, CA), and exon trapping was performed as described previously [15].

2.5. Paternity testing

Microsatellite analysis with 10 markers (D2S293, D3S3521, D4S2971, D5S495, D6S16171, D7S2459, D8S1705, D16S430, D18S450, and D20S842) was performed in Patient 1 and his parents to confirm paternity.

2.6. TA cloning

The novel *PGRN* heterozygous deletion/insertion found in this study, *PGRN* p.G338RfsX23 (c.1012_1013delGGinsC), was confirmed by cloning the PCR products into the pCR4-TOPO Vector using the TOPO TA Cloning kit (Invitrogen) and sequencing the two haplotypes of the heterozygote.

3. Results

3.1. Results of MAPT analysis

3.1.1. Genetic and molecular analyses of MAPT

In this study, we identified nine patients with *MAPT* mutations from six families. Four heterozygous missense mutations in *MAPT*, p.L266V, p.N279K, p.N296N, and the novel p.S285R (Supplementary Fig. 1), were identified by direct sequencing. None of the 182 normal Japanese controls included in this study had the *MAPT* p.S285R. In addition, we examined the amino acid sequences of the MAPT protein in other species and found that the site of the p.S285R mutation was highly conserved (see Supplementary Fig. 2). The novel p.S285R mutation in *MAPT* was detected in Patient 1 but not in his parents (Fig. 1A and Supplementary Fig. 1). The parentage of this patient and the DNA authenticity were confirmed using a microsatellite panel (see Supplementary Table 1). These results suggest that p.S285R is a *de novo* mutation. To investigate whether the p.S285R mutation is pathogenic, we performed an exon-trapping analysis. The p.S285R mutation produced a marked increase in the splicing of exon 10 (Fig. 1B) and resulted in the overproduction of tau isoforms that contain 4-repeat tau, such as IVS10+3 [15]. These results indicate that the p.S285R mutation is a novel, *de novo* pathogenic mutation. Previously, p.L266V, p.N279K, and p.N296N had been reported as pathogenic mutations [16–18].

Table 2 lists the clinical features of all of the *MAPT*- and *PGRN*-positive patients in this study, and Supplementary Fig. 3 shows Pedigrees C, D, E, F, and G. The average age at disease onset of patients with a single heterozygous *MAPT* mutation was 42.3 ± 2.9 (range: 37–46) years. MLPA analysis showed no gene dosage abnormalities (multiplications or deletions) in *MAPT* in this cohort.
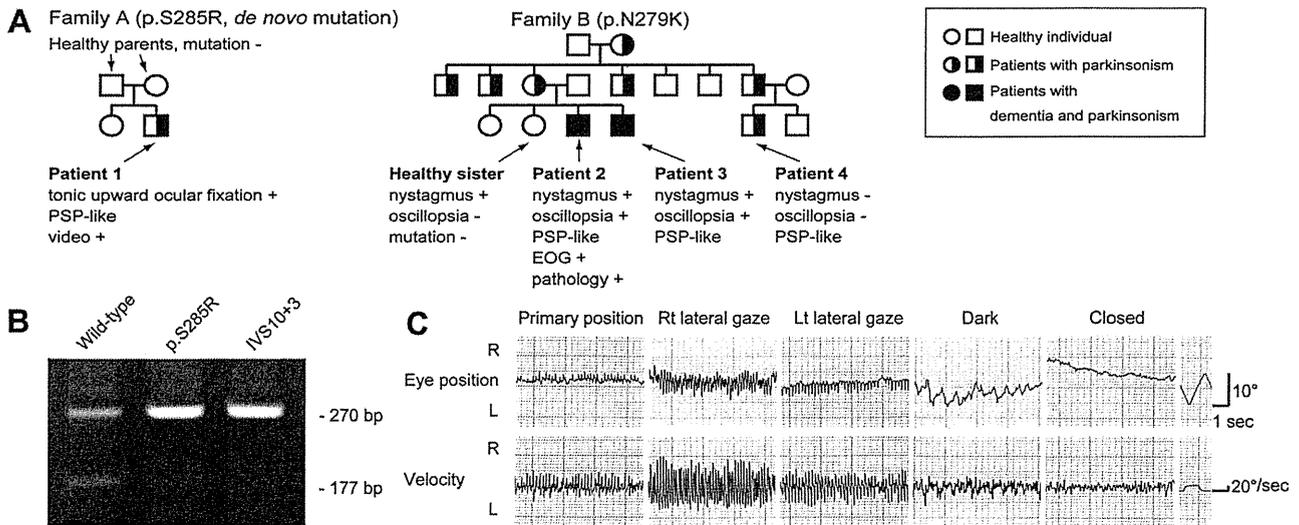
Fig. 1. (A) The pedigrees of families A and B. (B) Exon-trapping analysis for the effects of the MAPT p.S285R mutation on exon 10 splicing. (C) Horizontal electro-oculogram recordings in Patient 2.

3.1.2. Clinical presentations of MAPT-positive patients with the abnormal eye movements that are generally not observed in patients with sporadic PSP

3.1.2.1. Patient 1 (MAPT p.S285R).
This patient was a 46-year-old man who presented with difficulty speaking and breathing. The patient had no family history of dementia or movement disorders (Fig. 1A). A physical examination revealed gait disturbance, limb bradykinesia, and frequent falling. At age 47, the patient exhibited palilalia and a mild obsession with eating. The patient's Mini-Mental State Examination (MMSE) score was 28/30, but his Frontal Assessment Battery score was 12/18. The patient exhibited a slowing of saccadic eye movements with a relative preservation of smooth pursuit, vertical supranuclear gaze palsy, and tonic upward ocular fixation (see Video Supplement); when the patient's eyes opened after closing, they remained fixated upward and could not be moved voluntarily to the primary position (i.e., Bell's phenomenon remained). To overcome this disability, the patient extended his neck, which resulted in a reflex downward movement of the eyes (the vestibulo-ocular reflex), and next he slightly flexed his neck to a neutral position with his eyes in the primary position. Later, the patient developed bradykinesia and postural instability with frequent falling. L-dopa/benserazide (up to 900 mg/day) was ineffective. The patient's condition gradually deteriorated, and he developed dementia, retrocollis, vertical and horizontal supranuclear palsy, and bradykinesia. At age 49, the patient died of suffocation from the aspiration of food material. No autopsy was performed. The clinical diagnosis was probable PSP.

3.1.2.2. Patient 2 (MAPT p.N279K).
This patient was the older brother of Patient 3 (Fig. 1A). Patient 2 was a 42-year-old man who exhibited oscillopsia, micrographia, and a shuffling gait. This patient reported having had nystagmus without oscillopsia since childhood. A neurological examination revealed marked horizontal nystagmus. The patient's pupils were isocoric, and his visual acuity was normal. The patient presented with rigidity, bradykinesia, and postural tremor in the upper limbs. Electro-oculography revealed horizontal pendular nystagmus in the primary position and in all gaze directions (Fig. 1C). L-dopa/benserazide at 200 mg/day mildly alleviated his parkinsonism. Two years later, the patient developed prominent postural instability and became prone to falling. Upward and downward gaze palsy and apraxia of eyelid opening were also noted. At that time, the clinical diagnosis was possible PSP with

a family history of dementia and parkinsonism. The patient's cognitive function deteriorated gradually. At age 52, he was bedridden and required a gastrostomy. The patient died of pneumonia at age 54. A postmortem pathological examination of the brain revealed mild atrophy of the frontal lobe and the tegmentum of the midbrain and pons. Microscopic analysis showed severe degenerative changes in the substantia nigra and the subcortical nuclei. Immunohistochemistry using anti-phosphorylated tau (p-tau) antibodies revealed numerous tau-positive neuronal and glial inclusions in the frontotemporal cortex, white matter, and the subcortical nuclei (see Supplementary Fig. 4). These p-tau deposits reacted with anti-4-repeat tau antibodies but not with anti-3-repeat tau antibodies.

3.1.2.3. Patient 3 (MAPT p.N279K).
This patient was the younger brother of Patient 2 (Fig. 1A). At age 44, Patient 3 noticed clumsiness in his right hand and oscillopsia. The patient reported having nystagmus since childhood. A neurological examination revealed large, horizontal pendular nystagmus in the primary position and in all gaze directions. The patient's visual acuity, pupils, and light reflexes were all normal. Mild bradykinesia and rigidity in the neck and the right upper limb were noted. Postural tremor in both hands and the tongue and postural instability were observed. Treatment with 600 mg/day of L-dopa/carbidopa was not effective. The patient's oscillopsia gradually worsened, and eventually he was unable to read printed materials. At age 47, the patient developed upward and downward gaze palsy, slowing of saccades, and apraxia of eyelid opening. The patient had prominent postural instability and was prone to falling. The patient's first clinical diagnosis was possible PSP with a family history of dementia and parkinsonism. The patient died at age 56. An autopsy was not performed.

3.1.2.4. Patients 5, 6, and 7 (MAPT p.N279K).
The clinical presentations of these three patients have been described previously [19]. All three patients had clinical diagnoses of possible PSP (Table 2) and visual grasping [19,20].

3.2. Results of PGRN analysis

3.2.1. Genetic Analyses of PGRN
We identified one patient with a PGRN mutation (Table 2, Supplementary Fig. 3). One novel heterozygous deletion/insertion

Table 2
Clinical features of patients with MAPT and PGRN mutations.

Family	A	B			C	D		E	F	G
Patient	1	2	3	4	5	6	7	8	9	10
Gene					MAPT					PGRN
Genotyping					Heterozygous					
Nucleotide change	c.853A > C	c.837T > G	c.837T > G	c.837T > G	c.837T > G	c.837T > G	c.837T > G	c.796C > G	c.888T > C	c.1012_1013delGGinsC
Amino acid change	p.S285R	p.N279K	p.N279K	p.N279K	p.N279K	p.N279K	p.N279K	p.L266V	p.N296N	p.G338RfsX23
Exon	10	10	10	10	10	10	10	9	10	9
Mode of inheritance	de novo	AD	AD	AD	NA	AD	AD	AD	AD	AD
Age at onset, years	46	42	44	46	41	42	43	37	44	59
Age at evaluation, years	47	47	45	50	44	44	45	38	49	61
Age at death, years	49	54	56	alive	51	54	51	alive	alive	alive
Sex	M	M	M	M	F	F	F	F	M	F
Clinical syndromes	PSP	PSP	PSP	PSP	PSP	PSP	PSP	bvFTD	PSP	PPA
Clinical features										
Initial symptoms	P	P	P	P	P	P	P	dementia	P	aphasia
Personality/behavior changes	−	+	−	−	−	−	−	+	+	−
Mini mental state examination score	28/30	NA	NA	28/30	NA	NA	NA	0	24/30	29/30
Hasegawa dementia scale-revised[a]	NA	18/30	NA	NA	21/30	28/30	30/30	0	21/30	29/30
Nonfluent spontaneous speech	−	−	−	−	−	−	−	−	−	+
Apraxia of eyelid opening	−	+	+	+	+	+	+	−	−	−
Abnormal eye movements										
Supranuclear gaze palsy	+	+	+	+	+	+	+	−	+	−
Tonic upward ocular fixation	+	−	−	−	−	−	−	−	−	−
Oscillopsia with CN	−	+	+	−	−	−	−	−	−	−
Visual grasping	−	−	−	−	+	+	+	−	−	−
Parkinsonism										
Bradykinesia	+	+	+	+	+	+	+	−	+	−
Rigidity	−	+	+	+	+	+	+	−	+	−
Tremor	−	+	+	−	−	−	−	−	−	−
Postural instability	+	+	+	+	+	+	+	−	+	−
Response to L-dopa	−	partial[b]	−	partial[b]	partial[b]	partial[b]	partial[b]	NA	+	NA
Pyramidal sign	+	−	NA	−	+	−	+	+	+	−
Features of motor neuron disease	−	−	−	−	−	−	−	−	−	−
Reference					[19]	[19]	[19]			

AD = autosomal dominant.
P = parkinsonism; NA = not available.
CN = congenital nystagmus; PSP = progressive supranuclear palsy.
bvFTD = behavioral variant frontotemporal dementia; PPA = primary progressive aphasia.
  [a] The Hasegawa dementia scale-revised is a brief dementia screening scale. The maximum score of the Hasegawa dementia scale-revised is 30 points. There was a significant difference in the mean score between the demented and non-demented subjects when the cut-off point was set at 20/21 [31].
  [b] A partial response to L-dopa indicates that L-dopa was effective only in the early stages.

mutation in PGRN, p.G338RfsX23 (c.1012_1013delGGinsC), was detected by direct sequencing and TOPO TA cloning sequencing (Supplementary Fig. 1). None of the 182 normal Japanese controls included in this study had the PGRN p.G338RfsX23 (c.1012_1013delGGinsC) mutations. The age at disease onset of the patient with the heterozygous PGRN deletion/insertion was 59 years. Novel PGRN variants with unknown significance, p.R18Q and p.N118del, are listed in Table 3. MLPA analysis showed no gene dosage abnormalities in PGRN.

3.2.2. A clinical presentation of a novel PGRN mutation
3.2.1. Patient 10 (PGRN p.G338RfsX23, c.1012_1013delGGinsC). This patient, a 59-year-old woman, developed word-finding difficulties and underwent surgical clipping at age 54 for an unruptured

Table 3
Novel variants with unknown significance.

Gene	Nucleotide change	Amino acid change	Exon	Amino acid conservation	Mean AAO (years)	Frequency		P value	Clinical diagnosis
						Patients N (%)	Controls N (%)		
PGRN	c.56G > A	p.R19Q	1	not conserved	66	1/69 (1.4)	0/186 (0)	0.605	PSP (n = 1)
PGRN	c.352_354delAAC	p.N118del	4	not conserved	53	3/69 (4.3)	3/272 (1.1)	0.187	bvFTD (n = 3)

AAO = age at onset.
PSP = progressive supranuclear palsy.
bvFTD = behavioral variant frontotemporal dementia.

aneurysm of the left middle cerebral artery. The patient's mother suffered from dementia, but the details of her disease were unknown. The patient substituted words for names of people and objects. Two years after the onset of symptoms, the patient became severely disfluent. However, she did not show any violent behavior, personality changes, or other behavioral abnormalities. The patient scored 29/30 on the MMSE. On the frontal assessment battery, she scored 13/18. The patient's time to complete the Trail Making Test (TMT) A was 70 s, and she could not finish the TMT B within five minutes. Her spontaneous speech production was characterized by slow and hesitant speech, frequently interrupted by long word-finding pauses. Her motor speech abilities were within the normal limits, and no apraxia of speech was noted. No parkinsonism was observed. The patient's clinical diagnosis was PPA with a family history of dementia.

3.3. Results of C9orf72 analysis

We identified no patients with expanded hexanucleotide repeats in C9orf72 in this study. In 75 patients, the average repeat number based on fluorescent fragment-length analysis was 3.77 ± 2.56 (range 2–11 repeats). We have previously reported that an analysis of 197 Japanese healthy controls did not find any C9orf72 mutation. The average repeat number was 3.69 ± 2.46 (range 2–14 repeats) in the 197 controls [21].

4. Discussion

We identified five MAPT mutations, including a novel de novo mutation and a novel PGRN mutation, and we found no C9orf72 mutations in our 75 patients. More mutations were found in MAPT than in the other two genes evaluated in this study. The infrequent observation of PGRN and C9orf72 mutations might be partly due to the small number of FTLD patients included ($n = 38$) because the majority of PGRN and C9orf72 mutations have been described in patients with FTLD. In contrast to most other mutation screening studies, we performed MLPA analysis to ensure that exonic or larger deletions or multiplications of MAPT and PGRN would be identified. Therefore, our data also show that multiplications of MAPT and exonic or genomic deletions in PGRN are rare in Asian populations. Although mutations were detected in FTLD and PSP patients, we did not find any mutations in our CBS patients. A further larger study and investigation of the other genes are needed to clarify the genetic background of Japanese patients with CBS.

The MAPT p.S285R mutation, which we found in this study, is a novel de novo mutation. To the best of our knowledge, this report is the first description of an adult sporadic case of a de novo MAPT mutation associated with dementia and parkinsonism. All six patients (Patients 1, 2, 3, 5, 6, and 7) with PSP and the distinct eye movements described in the present study (such as tonic upward ocular fixation, oscillopsia with congenital nystagmus, and visual grasping) harbored MAPT mutations. Below, we discuss these abnormal eye movements, which are generally not observed in patients with sporadic PSP.

In Patient 1 (MAPT p.S285R), we observed tonic upward ocular fixation, which is a loss of downward saccades resembling an acquired ocular motor apraxia [22]. This condition is characterized by a loss of voluntary control of saccades and pursuit, whereas reflex movements—in particular, the vestibulo-ocular reflex—were preserved. Acquired ocular motor apraxia is usually the result of bilateral frontal or frontoparietal infarcts. Therefore, tonic upward ocular fixation due to a MAPT mutation might share "supranuclear" cerebral lesions in common with ocular motor apraxia. Brainstem functions, including the vestibulo-ocular reflex and Bell's phenomenon, were preserved in Patient 1.

In Patients 2 and 3 (MAPT p.N279K), pendular nystagmus was present since childhood and was suppressed with eyelid closure. These features are consistent with congenital nystagmus [23]. Most patients with congenital nystagmus do not complain of oscillopsia, despite having nearly continuous eye movement [23]. Notably, Patients 2 and 3 noticed oscillopsia when they developed parkinsonism. In these siblings, cerebral lesions caused solely by a MAPT mutation were unlikely to be the cause of their nystagmus; however, the co-existence of congenital nystagmus and the MAPT mutation might have caused the oscillopsia. This notion is supported in part because the patients had a sister who remained healthy – even in her late 60s – and did not complain of oscillopsia, despite having obvious pendular nystagmus (Fig. 1A). Thus, MAPT mutations might impair the visual-motion processing pathways that would normally suppress oscillopsia in patients with common congenital nystagmus. Visual grasping, which was first described by Ghika et al. [20], was observed in Patients 5, 6, and 7 (MAPT p.N279K) [19].

Although PSP is a rare manifestation of MAPT mutation [24], and the routine screening of sporadic PSP for mutations in MAPT is not recommended because of low yield [25], it is recommended that screening be considered for families in which there is an autosomal dominant history of a PSP syndrome, particularly when there are accompanying features suggestive of bvFTD [24]. The clinical difference from sporadic PSP might sometimes be difficult to detect, especially in patients without a family history [26–28]; however, an important case report indicated that an age at disease onset under 50 years combined with the absence of early falling may indicate a possible MAPT mutation in clinically diagnosed PSP, even in the absence of a positive family history [26]. Consistent with this observation, our eight MAPT-positive patients with PSP phenotype were younger than 50 years at disease onset (Table 2). We further suggest that it may be useful to test for MAPT mutations in early-onset PSP patients with the abnormal eye movements that are not typical of sporadic PSP. In fact, we identified the novel de novo mutation p.S285R in Patient 1 and p.N279K in Patient 5, who had no family history, after focusing on these clinical phenotypes.

To the best of our knowledge, the PGRN mutation has not been previously described in Asian populations [29]. We detected a novel PGRN mutation, p.G338RfsX23 (c.1012_1013delGGinsC), and thus showed that PGRN mutations may exist in Asian populations. This mutation introduces a premature termination codon at the same site as the p.G333VfsX28 (c.998delG) mutation, which was reported previously, and produced a PPA phenotype in all of the affected individuals [30]. The PPA phenotype of p.G338RfsX23 (c.1012_1013delGGinsC) in our study is remarkably similar to that of p.G333VfsX28 (c.998delG), especially in the manifestation of word-finding and object-naming difficulties and the lack of memory or personality changes during the first few years after symptom onset. We believe that the mutant RNA in both cases is most likely subjected to nonsense-mediated decay, similar to other PGRN mutations [2].

In summary, based on these findings, we recommend genetic testing for MAPT mutations not only in familial patients but also in sporadic patients, especially early-onset PSP patients with the abnormal eye movements that are generally not observed in sporadic PSP. Although PGRN and C9orf72 mutations were rare in this study, we determined that the PGRN mutation does exist in Asian patients with FTLD (PPA). Based on the clinical information, screening for MAPT, PGRN, and C9orf72 mutations should be further undertaken to improve the diagnosis of specific clinical entities of neurodegenerative disorders.

Conflicts of interest

None.