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Clinical Study

Cerebrospinal Fluid Biomarkers for Kii Amyotrophic Lateral Sclerosis/Parkinsonism-Dementia Complex

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Objective. Amyotrophic lateral sclerosis/parkinsonism-dementia complex is classified as one of the tauopathies. *Methods.* The total tau, phosphorylated tau, and amyloid β42 levels were assayed in cerebrospinal fluid from patients with Kii amyotrophic lateral sclerosis/parkinsonism-dementia complex (n = 12), Alzheimer's disease (n = 9), Parkinson's disease (n = 9), amyotrophic lateral sclerosis (n = 11), and controls (n = 5) using specific enzyme-linked immunosorbent assay methods. *Results.* Total tau and phosphorylated tau did not increase and amyloid β42 was relatively reduced in Kii amyotrophic lateral sclerosis/parkinsonism-dementia complex. Relatively reduced amyloid β42 might discriminate Kii amyotrophic lateral sclerosis/parkinsonism-dementia complex from amyotrophic lateral sclerosis and Parkinson's disease, and the ratios of phosphorylated-tau to amyloid β42 could discriminate Kii amyotrophic lateral sclerosis/parkinsonism-dementia complex from Alzheimer's disease. *Conclusions.* Cerebrospinal fluid analysis may be useful to differentiate amyotrophic lateral sclerosis/parkinsonism-dementia complex from Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease.

1. Introduction

Amyotrophic lateral sclerosis/parkinsonism-dementia complex (ALS/PDC) is a rare disorder endemic to Guam Island and the Kii Peninsula of Japan. It shows a unique combination of parkinsonism, amyotrophy, and dementia [1], and the form of dementia, which shows a phenotype similar to Alzheimer's disease (AD), is becoming predominant in the Kii Peninsula.

Although Kii ALS/PDC shows several unique clinical features, including severe atrophy of the frontal and temporal lobes on magnetic resonance imaging (MRI), decreased cerebral blood flow in the frontal and temporal lobes on single-photon emission computed tomography (SPECT) [2], pigmentary retinopathy [3], and decreased cardiac ^{123}I-metaiodobenzylguanidine uptake [4], a postmortem examination is required for a definitive diagnosis. Since biomarkers for ALS/PDC have not yet been identified, we analyzed cerebrospinal fluid (CSF) biomarkers for Kii ALS/PDC to discriminate it from other neurodegenerative disorders.

2. Material and Methods

We collected CSF samples from 12 patients with Kii ALS/PDC (6 men, 6 women, mean age 67.9 ± 3.7 years, mean illness duration 5.63 years), nine patients with AD (2 men, 7 women, mean age 61.1 ± 8.7 years, mean illness duration 1.92 years), 11 patients with ALS (8 men, 3 women, mean age 60.6 ± 12.6 years, mean illness duration 1.1 years), nine patients with Parkinson's disease (PD; 7 men, 2 women, mean age 71.3 ± 2.2 years, mean illness duration 4.42 years), and five disease control patients (C; 4 men, 1 woman, mean age 36.2 ± 20.3 years). All of the patients with Kii ALS/PDC were natives of Hohara village, which is an area of high ALS/PDC prevalence

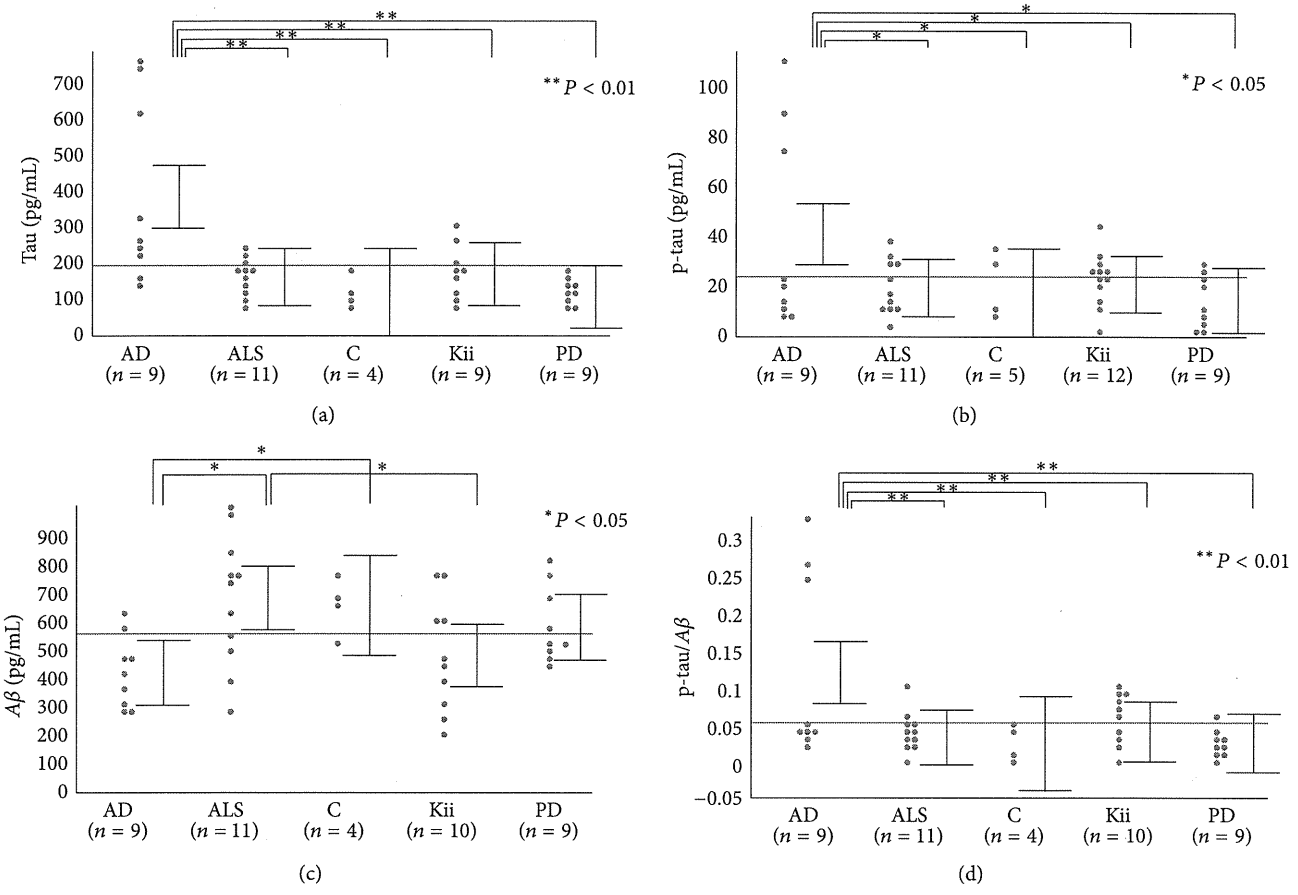

FIGURE 1: Analysis of CSF biomarkers. (a) Total tau, (b) phosphorylated tau, (c) beta-amyloid peptide (1–42), (d) the ratio of p-tau to Aβ42. AD: Alzheimer's disease, ALS: amyotrophic lateral sclerosis, C: disease control, Kii: Kii amyotrophic lateral sclerosis/parkinsonism-dementia complex, and PD: Parkinson's disease.

on the Kii Peninsula. We collected CSF samples over 10 years; therefore, the period between CSF collection and analysis was not standardized. The diagnosis of Kii ALS was made according to the Airlie House criteria, since the clinical symptoms of Kii ALS are essentially the same as those of classical ALS. The diagnosis of Kii PDC was made by a unique combination of levodopa-unresponsive parkinsonism and dementia, which are frequently accompanied by amyotrophy of the extremities and/or pyramidal tract signs. Mini-mental state examination (MMSE) was used for the evaluation of dementia and cut-off point was 23 (data not shown). The frontal lobes and/or temporal lobes of ALS/PDC patients showed atrophy on MRI and/or a decrease of cerebral blood flow on SPECT. CSF samples were immediately centrifuged at 1000 ×g for 15 min and stored at −80°C with polypropylene tube. The total tau (t-tau), phosphorylated tau (p-tau), and amyloid beta (Aβ) concentrations were measured with an enzyme-linked immunosorbent assay (ELISA) kit using a monoclonal antibody specific for t-tau, p-tau, and Aβ1–42 (INNOTEST hTAU Ag, phosphor tau(181P), and β-amyloid (1–42), Innogenetics, Ghent, Belgium). ELISA assays were carried out using several samples from each group on the same plate in a randomized manner and were repeated using randomized samples in the same manner in plural times. A factorial ANOVA was performed with CSF-t-tau, CSF-p-tau, and CSF-Aβ42, as dependent variables, with the diagnostic category (AD, ALS, C, Kii ALS/PDC, and PD) using JMP 9.0. All data were expressed as means ± SD. A P value less than 0.05 was considered statistically significant. The Ethics Committee of Mie University Graduate School of Medicine approved this study and the "Declaration of Helsinki" was followed. Informed consent was obtained from the patients or their families.

3. Results

CSF-Aβ42, CSF-t-tau, and CSF-p-tau were compared between AD, ALS, C, Kii ALS/PDC, and PD. The concentrations of CSF-t-tau and CSF-p-tau were significantly higher in AD (t-tau; 378.0 ± 41.76 pg/mL; $P < 0.001$, p-tau; 42.4 ± 6.78 pg/mL; $P < 0.028$) than in the other groups. However, the concentrations of CSF-t-tau and CSF-p-tau did not differ significantly between Kii ALS/PDC, ALS, C, and PD (Figures 1(a) and 1(b)). The concentration of CSF-Aβ42 was significantly reduced in AD (402.2 ± 56.6 pg/mL;

$P < 0.03$) compared to ALS and C and relatively reduced in Kii ALS/PDC (465.4 ± 53.69 pg/mL; $P < 0.018$) compared to ALS. Most of the ALS/PDC patients had CSF concentration values that fell below the cutoff based on C (Figure 1(c)). The ratios of CSF-p-tau to CSF-Aβ42 were significantly increased in AD (0.125 ± 0.02) compared with Kii ALS/PDC (0.043 ± 0.02; $P < 0.008$), ALS (0.035 ± 0.019; $P < 0.003$), PD (0.025 ± 0.02; $P < 0.002$), and C (0.027 ± 0.09; $P < 0.014$) (Figure 1(d)). The concentrations of CSF-t-tau, CSF-p-tau, or CSF-Aβ42 were not related to the clinical parameters (age, sex, illness duration, or dementia) in the Kii ALS/PDC patients. The number of C samples was small and the average age of control patients was low.

Generally, CSF tau level gradually increase according to age and CSF Aβ is not affected by age [5]. CSF tau level of C samples was relatively low, but it was not significant.

Thus, the CSF values of C were not comparable to those of other groups. Nevertheless, the optimal cut-off values that discriminate C from AD were similar to those in previous reports [6, 7] in which a larger number of control samples were analyzed. CSF tau level of Kii ALS/PDC samples did not increase, although the average age of Kii ALS/PDC group was older than that of AD group.

4. Discussion

The present study showed that CSF-t-tau and CSF-p-tau concentrations from patients with Kii ALS/PDC were not increased compared to those in the other disease groups, and Aβ42 concentration in the CSF was relatively decreased. The ratio of CSF-p-tau to CSF-Aβ42 segregates Kii ALS/PDC from AD. Because ALS/PDC is associated with tau pathology in the absence of amyloid plaques, the expectation was that ALS/PDC patients would not show the Alzheimer's disease (AD) profile of decreased Aβ42 but might show increased t-tau and/or p-tau in the CSF.

In general, decreased CSF-Aβ42 indicates plaque pathology, and increased CSF-t-tau and CSF-p-tau indicate axonal degeneration and tangle pathology, respectively [8]. Recently, the average age of onset of Kii ALS/PDC is increasing and Aβ deposition is conspicuous in autopsied patients. Therefore, decreased CSF-Aβ42 may reflect Aβ pathology in the most recent patients. We analyzed the precise tau isoform of over 10 patients with autopsy-proven ALS/PDC recently and identified a 3R + 4R type, 4R > 3R type, and a 4R predominant type. The glial tau pathology is particularly related to the 4R isoform, and we consider Kii ALS/PDC to be a 4R-dominant tauopathy (unpublished data). Noguchi et al. examined the concentrations of CSF-t-tau, CSF-p-tau, and CSF-Aβ42 in patients with progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD); the concentrations of CSF-t-tau and CSF-p-tau did not significantly differ between PSP, CBD, and controls, and the concentration of CSF-Aβ42 was significantly lower in PSP and CBD than in controls. The authors speculated that the absence of an increase of CSF-t-tau and CSF-p-tau concentrations might reflect 4R tau predominance and a reduction of CSF-Aβ42 might suggest deposition or mismetabolism of Aβ [6]. Taken together, CSF biomarkers of Kii ALS/PDC might have similar properties to those of 4R tauopathy, PSP, and CBD; however the relationship between tau isoform and CSF tau level remains to be resolved.

Finally, the present findings, in which CSF-t-tau and CSF-p-tau concentrations were not increased and CSF-Aβ42 concentration was relatively decreased, suggest that CSF analysis may be useful to differentiate ALS/PDC from AD, ALS, and PD. Nevertheless there is a major limitation of the interpretation of the data. The size of each group is small, the age of the control group is much younger, and there were two populations in the AD group regarding the levels tau, p-tau, and Aβ/p-tau. Further study using groups with larger size of subjects is needed to confirm the proposed utility of the CSF biomarkers.

Abbreviations

ALS/PDC: Amyotrophic lateral sclerosis/parkinsonism-dementia complex
CSF: Cerebrospinal fluid
NFTs: Neurofibrillary tangles
t-tau: Total tau
p-tau: Phosphorylated tau
AD: Alzheimer's disease
PD: Parkinson's disease
MRI: Magnetic resonance imaging
SPECT: Single-photon emission computed tomography
C: Control patients
ELISA: Enzyme-linked immunosorbent assay
3R: 3 repeat
4R: 4 repeat
PSP: Progressive supranuclear palsy
CBD: Corticobasal degeneration.

Disclosure

Y. Kokubo certifies that all my affiliations or financial involvement, within the past year and in the foreseeable future (e.g., employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, and royalties) with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the paper are completely disclosed. All authors report no disclosures and no conflicts of interest concerning the research related to the paper. There was no ghost writing by anyone not named in the authors list.

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

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abstract

Background: Mutations in the microtubule associated protein tau (MAPT) and progranulin (PGRN) have been identi ed 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 identi ed 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 rst 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.

1. Introduction

Mutations in the microtubule-associated protein tau (MAPT) and the progranulin (PGRN) genes have been identi ed in families with frontotemporal dementia and parkinsonism linked to chromosome 17 [1e3]. Recently, two studies reported that the expansion of a noncoding GGGGCC hexanucleotide repeat in the C9orf72 gene is

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

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), 36e78	21	17
bvFTD	29	38.7	34.5	54.5 (12.6), 36e78	18	11
FTD-ALS	2	2.7	100	67.5 (1.5), 66e69	1	1
PPA	7	9.3	42.9	65.0 (7.4), 58e77	2	5
PSP	25	33.3	68.0	59.8 (13.0), 40e76	18	7
CBS	12	16.0	33.3	58.4 (9.52), 40e71	9	3
Total	75	100	48.0	58.2 (12.3), 36e78	48	27
Index patients	69	92.0	46.4	58.9 (12.4), 36e78	42	27
Relatives	6	8	66.7	50.3 (6.6), 44e61	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.

2.3. Multiplex ligation-dependent probe ampli cation (MLPA)

To con rm 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/quanti cation protocol was provided by the manufacturer. The products were quanti ed using the ABI3130 Genetic Analyzer and Gene Mapper v3.7 (Applied Biosystems). The kit contains 32 probes, including 13 MAPT probes (located in exons 1e13) 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 con rm paternity.

2.6. TA cloning

The novel PGRN heterozygous deletion/insertion found in this study, PGRN p.G338RfsX23 (c.1012_1013delGGinsC), was con rmed 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 identi ed 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 identi ed 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 con rmed 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 [16e18].

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: 37e46) years. MLPA analysis showed no gene dosage abnormalities (multiplications or deletions) in MAPT in this cohort.

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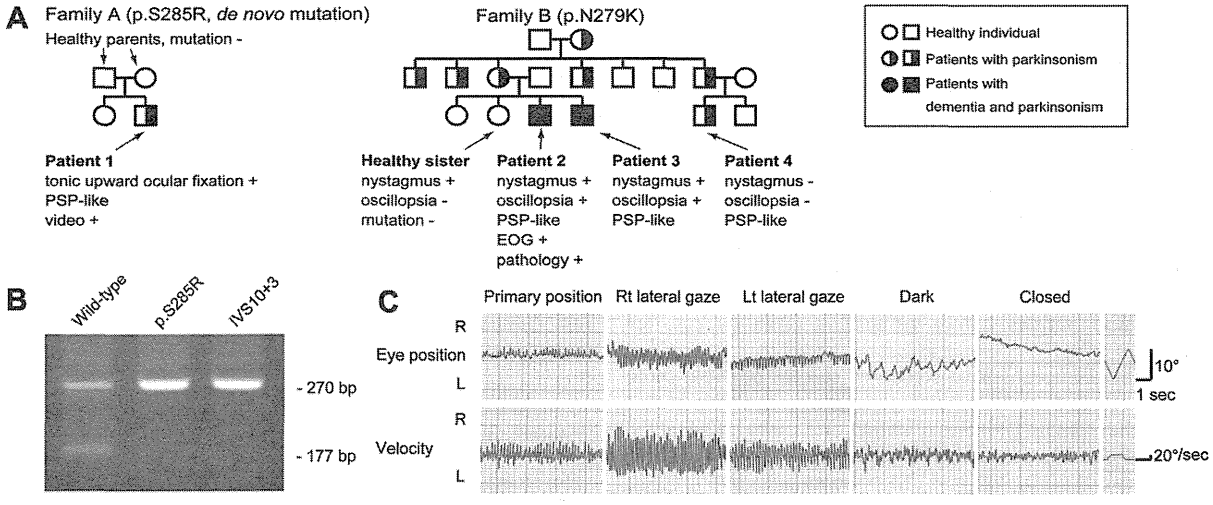

Fig. 1. (A) The pedigrees of families A and B. (B) Exon-trapping analysis for the effects of the MAPT p.S285R mutation on exon 10 splicing. (C) Horizontal electro-oculogram recordings in Patient 2.

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 dif culty 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 xation (see Video Supplement); when the patient's eyes opened after closing, they remained xated 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 re ex downward movement of the eyes (the vestibulo-ocular re ex), and next he slightly exed 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 shuf ing 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 re exes 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 rst 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 identi ed one patient with a PGRN mutation (Table 2, Supplementary Fig. 3). One novel heterozygous deletion/insertion

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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	e		e	e	e	e	e			e
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
Non uent spontaneous speech	e	e	e	e	e	e	e	e	e	
Apraxia of eyelid opening	e							e	e	e
Abnormal eye movements										
Supranuclear gaze palsy								e		e
Tonic upward ocular xation		e	e	e	e	e	e	e	e	e
Oscillopsia with CN	e			e	e	e	e	e	e	e
Visual grasping	e	e	e	e				e	e	e
Parkinsonism										
Bradykinesia								e		e
Rigidity	e							e		e
Tremor	e			e	e	e	e	e	e	e
Postural instability								e		e
Response to L-dopa	e	partial[b]	e	partial[b]	partial[b]	partial[b]	partial[b]	NA		NA
Pyramidal sign		e	NA	e		e				e
Features of motor neuron disease	e	e	e	e	e	e	e	e	e	e
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 signi cant 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 signi cance, 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

3.2.2.1. Patient 10 (PGRN p.G338RfsX23, c.1012_1013delGGinsC). This patient, a 59-year-old woman, developed word- nding dif culties and underwent surgical clipping at age 54 for an unruptured

Table 3
Novel variants with unknown signi cance.

Gene	Nucleotidechange	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.

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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 dis uent. 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 nish the TMT B within ve minutes. Her spontaneous speech production was characterized by slow and hesitant speech, frequently interrupted by long word- nding 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 identi ed no patients with expanded hexanucleotide repeats in C9orf72 in this study. In 75 patients, the average repeat number based on uorescent fragment-length analysis was 3.77 2.56 (range 2e11 repeats). We have previously reported that an analysis of 197 Japanese healthy controls did not nd any C9orf72 mutation. The average repeat number was 3.69 2.46 (range 2e14 repeats) in the 197 controls [21].

4. Discussion

We identi ed ve 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 identi ed. 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 nd 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 rst 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 xation, 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 xation, 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 re ex movements d in particular, the vestibulo-ocular re ex d were preserved. Acquired ocular motor apraxia is usually the result of bilateral frontal or frontoparietal infarcts. Therefore, tonic upward ocular xation due to a MAPT mutation might share "supranuclear" cerebral lesions in common with ocular motor apraxia. Brainstem functions, including the vestibulo-ocular re ex 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 e even in her late 60s e 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 rst 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 dif cult to detect, especially in patients without a family history [26e28]; 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 identi ed 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- nding and object-naming dif culties and the lack of memory or personality changes during the rst 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 ndings, 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 speci c clinical entities of neurodegenerative disorders.

Con icts of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.parkreldis.2012.06.019.

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