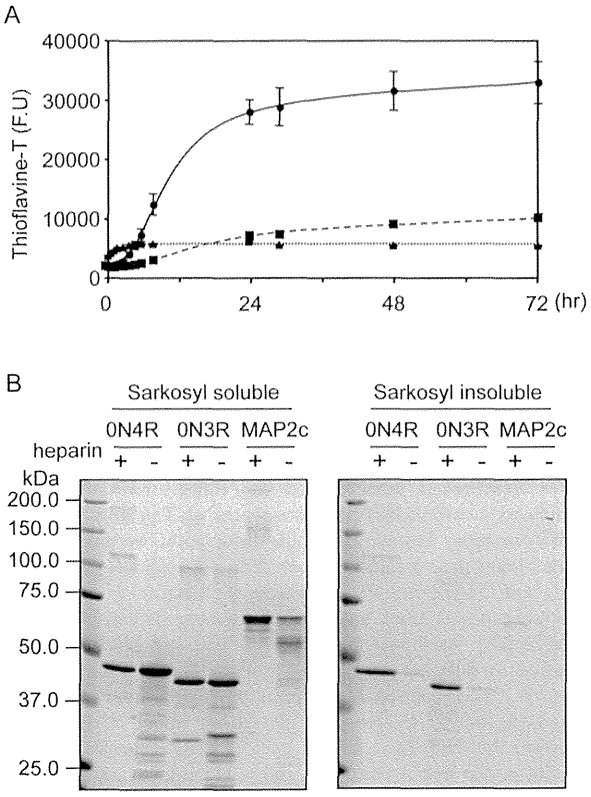

Figure 7. Tau but not MAP2c forms ThT-positive insoluble aggregates induced by heparin. (**A**) The ThT fluorescence of Tau 0N4R isoform (circles), Tau 0N3R isoform (squares), and MAP2c (triangles) aggregates were measured at the times indicated. (**B**) After the 7-day incubation, the amount of Sarkosyl-insoluble proteins in the indicated samples was analyzed by SDS-PAGE followed by Coomassie brilliant blue staining.
doi:10.1371/journal.pone.0089796.g007

raised MAP2 carboxyl-terminal site-specific antibodies used in this study.

The present results indicated that four-repeat Tau is significantly neurotoxic but three-repeat Tau is not (Figure 2). Because MAP2 is predominantly in the three-repeat form in mammalian neurons, the question arises why three-repeat MAP2 showed significantly greater neurotoxicity than three-repeat Tau. We compared the carboxyl-terminal amino acid sequences, as shown in Figure 1B, which are highly homologous between MAP2 and Tau. Aside from the homologous amino acid sequences, there are some differences in amino acids between Tau and MAP2. It is possible that these distinct amino acids make MAP2 more toxic than three-repeat Tau. The hereditary FTDP-17 form of dementia is caused by mutations in the Tau gene. Previous studies have indicated that these mutations increase Tau pathology through apparently different mechanisms, such as increasing four-repeat Tau isoforms by affecting the splicing pattern or increasing Tau aggregation. Interestingly, two perfect FTDP-17 mutations, Q336R and E342V, and two imperfect mutations, S325A (S325L in FTDP-17) and K369V (K369I in FTDP-17), are observed in MAP2 MTBDs [40–43] (Figure 1B). The presence of FTDP-17 mutations in the MAP2 sequence may increase its neurotoxicity to a greater extent.

The present study raises the possibility of a pathoetiological role of MAP2 in the generation of AD and tauopathies. The regions responsible for both aggregation and toxicity are located in the same carboxyl-terminal portions of Tau and MAP2. Thus far, aggregation has been the focus of research, and therefore Tau has been considered exclusively as a pathogenic molecule. Our present study indicates that Tau and MAP2 exhibit toxicity without forming aggregates in worms. This suggests that the involvement of MAP2 in the pathogenesis of AD cannot be excluded because of the absence of its aggregates.

Supporting Information

Figure S1 Abnormal neurites expressing Tau or MAP2. The paraffin sections of 5-day-old worms (Is388/592, DsRed/mock-transgenic(Tg) worm; Is390/592, DsRed/Tau-Tg worm; Is849/592, DsRed/MAP2-Tg worm) used in Figure 5 were colabeled with anti-DsRed and either pool 2 (anti-Tau) or MAP2N (anti-MAP2). Arrows indicate the normal neurites. Abnormal kinks (arrowheads) are observed in the neurites expressing MAP2 or Tau. Scale bar = 20 μm. n = 6–12.
(TIF)

Figure S2 The three independent site-specific MAP2 antibodies did not cross-react with Tau. Purified recombinant MAP2c and Tau (0N4R) linked with His-tags at the amino-terminals were subjected to western blotting using anti-His tag and anti-MAP2 antibodies (#39, #40, and #41).
(TIF)

Figure S3 Semiquantification of Tau and MAP2 in Sarkosyl-insoluble, SDS-soluble fractions from human autopsy samples from normal and advanced AD brains. Note that standards made of recombinant Tau and MAP2 showed similar staining levels. The amount of Tau was greater than that of MAP2 in the Sarkosyl-insoluble/SDS-soluble fractions from advanced AD brains. NC, normal brain; AD, Alzheimer's disease brain.
(TIF)

Table S1 Information of cases used in this study.
(PDF)

Text S1 Supporting Methods.
(PDF)

Author Contributions

Conceived and designed the experiments: CX TM. Performed the experiments: CX TM S. Yoshimura. Analyzed the data: CX TM. Contributed reagents/materials/analysis tools: CX TM HH S. Yoshina EKN S. Mitani S. Murayama YI. Wrote the paper: CX TM YI.

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Evaluation of *SLC20A2* mutations that cause idiopathic basal ganglia calcification in Japan

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ABSTRACT

Objective: To investigate the clinical, genetic, and neuroradiologic presentations of idiopathic basal ganglia calcification (IBGC) in a nationwide study in Japan.

Methods: We documented clinical and neuroimaging data of a total of 69 subjects including 23 subjects from 10 families and 46 subjects in sporadic cases of IBGC in Japan. Mutational analysis of *SLC20A2* was performed.

Results: Six new mutations in *SLC20A2* were found in patients with IBGC: 4 missense mutations, 1 nonsense mutation, and 1 frameshift mutation. Four of them were familial cases and 2 were sporadic cases in our survey. The frequency of families with mutations in *SLC20A2* in Japan was 50%, which was as high as in a previous report on other regions. The clinical features varied widely among the patients with *SLC20A2* mutations. However, 2 distinct families have the same mutation of S637R in *SLC20A2* and they have similar characteristics in the clinical course, symptoms, neurologic findings, and neuroimaging. In our study, all the patients with *SLC20A2* mutations showed calcification. In familial cases, there were symptomatic and asymptomatic patients in the same family.

Conclusion: *SLC20A2* mutations are a major cause of familial IBGC in Japan. The members in the families with the same mutation had similar patterns of calcification in the brain and the affected members showed similar clinical manifestations. *Neurology*® 2014;82:705-712

GLOSSARY

DNTC = diffuse neurofibrillary tangles with calcification; **FIBGC** = familial idiopathic basal ganglia calcification; **IBGC** = idiopathic basal ganglia calcification; **MMSE** = Mini-Mental State Examination; **PDGF** = platelet-derived growth factor; **PDGFRB** = platelet-derived growth factor receptor-β; **Pi** = inorganic phosphate; **PiB** = Pittsburgh compound B; **PiT** = type III sodium-dependent phosphate transporter; **PKC** = paroxysmal kinesigenic choreoathetosis.

Idiopathic basal ganglia calcification (IBGC), also known as Fahr disease, is thought to be a rare neuropsychiatric disorder characterized by symmetrical calcification in the basal ganglia and other brain regions. Clinical manifestations range widely from asymptomatic to variable symptoms including headaches, psychosis, and dementia.[1] The diagnosis of IBGC generally relies on the visualization of bilateral calcification mainly in the basal ganglia by neuroimaging and the absence of metabolic, infectious, toxic, or traumatic causes.[2,3]

The mode of inheritance of familial IBGC (FIBGC) has been thought to be autosomal dominant and, to date, 4 responsible chromosomal regions have been identified, namely 14q (IBGC1), 2q37 (IBGC2), 8p11.21 (IBGC3), and 5q32 (IBGC4).[3–14] The causative gene at the IBGC3 locus was identified as *SLC20A2* encoding type III sodium-dependent phosphate transporter 2 (PiT-2). Screening of a large series of patients with IBGC revealed that mutations in *SLC20A2* are a major cause of FIBGC[10]; moreover, other mutations in *SLC20A2* have recently been reported in China and Brazil.[11–13] The mutations of *PDGFRB* encoding platelet-derived growth factor

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Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

(PDGF) receptor-β (PDGFRB) and *PDGFB* have recently been reported to cause calcification in the brain.[14,15]

We have collected clinical information of patients with IBGC in a nationwide survey in Japan. Here, on the basis of a mutational analysis of *SLC20A2*, we aim to establish the molecular epidemiology of IBGC3 and evaluate clinically and genetically *SLC20A2* mutations in Japan.

METHODS Subjects and samples. We collected clinical information on patients with IBGC in a nationwide study. The criteria for the selection of patients in the initial survey were as follows: 1) conspicuous calcification is observed in the basal ganglia and/or dentate nucleus by CT scan; 2) calcification is bilateral and symmetrical; and 3) idiopathic (absence of biochemical abnormalities, and an infectious, toxic, or traumatic cause).[2,3] Neurologists enrolled patients in the survey. They examined the medical charts and performed the neurologic examinations again if necessary. The survey was approved by the Ethics Committee of the Gifu University Graduate School of Medicine. During the survey, some patients were found to have hypoparathyroidism, Aicardi-Goutières syndrome, and Cockayne syndrome, and these patients were excluded. For the genetic study, a total of 69 subjects from 41 hospitals provided written informed consent and were enrolled in the project. Of these patients, 46 came from families with a single affected member, and the other 23 came from 10 families with multiple affected members. We defined the former as sporadic patients and the latter as familial patients. The patients' mean age ± SD was 41.3 ± 23.6 years at registration. The patients comprised 32 males and 37 females.

Standard protocol approvals, registrations, and patient consents. All experiments on human DNA were approved by the Ethics Committees of both Gifu University and the University of Tokyo. After written informed consent was obtained, peripheral blood samples were collected.

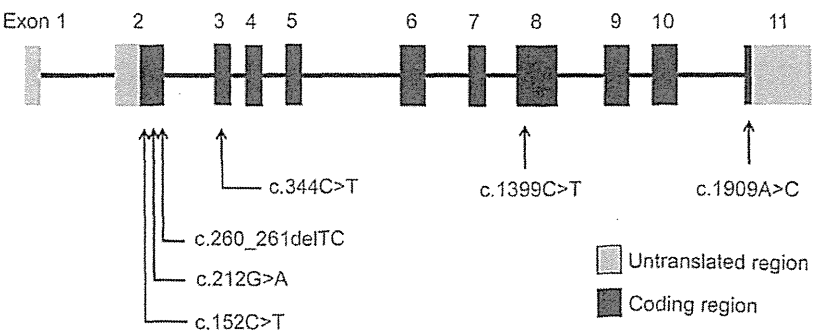
Mutational analysis. Genomic DNA was extracted from the whole blood samples. *SLC20A2* analysis was performed by Sanger sequencing of all coding regions, as described in detail in e-Methods and table e-1, A and B, on the *Neurology*® Web site at www.neurology.org. The pathologic potential of the identified variants was predicted using PolyPhen-2 (http://genetics.bwh.harvard.edu/pph/).[16]

RESULTS Mutational analysis. We screened a total of 69 subjects including 23 subjects from 10 families in which multiple affected subjects were observed and 46 subjects in sporadic cases, all of whom were Japanese. Six new mutations in *SLC20A2* were found: 4 missense mutations, 1 nonsense mutation, and 1 frameshift mutation (figure 1). Electropherograms showed the individual heterozygous mutations (figure e-1). None of them were present in an in-house exome sequencing data set (358 Japanese control subjects), dbSNP 137 (www.ncbi.nlm.nih.gov/snp/), or the National Heart, Lung, and Blood Institute "Grand Opportunity" Exome Sequencing Project (ESP6500SI-V2). In silico analysis predicted deleterious consequences, as determined from the residue changes in figures 1 and e-1. When confined to the FIBGC patients, 5 of the 10 families (50.0%) showed mutations in *SLC20A2*. In contrast, 2 of the 46 patients (4.3%) with sporadic IBGC carried mutations in *SLC20A2* in this study.

Clinical manifestations. The clinical manifestations are summarized in table 1. A positive family history of IBGC was present in 5 families. Families 1 and 2 had the same mutation.

Familial cases. ***Case 1 (in family 1).*** The proband in family 1 was a 64-year-old woman who had dysarthria and gait disturbance for 5 years. She showed no dementia. Her neurologic examination revealed dysarthria, small steppage gait, rigidity at bilateral wrist joints, bradykinesia, and a pyramidal sign. Her CT images revealed severe calcification at the bilateral globus pallidus, caudate nuclei, thalamus, subcortical white matter, and dentate nuclei (figure 2C). Her son's CT showed similar brain calcification (figure 2D), although he was clinically asymptomatic. His DNA study revealed the

Figure 1 Schematic representation of causative mutations in SLC20A2 in idiopathic basal ganglia calcification



Six new causative mutations in exon 2 (c.152C>T, c.212 G>A, c.260_261delTC), exon 3 (c.344C>T), exon 8 (c.1399C>T), and exon 11 (c.1909A>C) were found in this study.

Table 1 Clinical features of 6 individuals (probands) with *SLC20A2* mutations

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Mutation	c.1909A>C	c.1909A>C	c.344C>T	c.212G>A	c.1399C>T	c.152C>T	c.260_261delTC
	S637R	S637R	T115M	R71H	R467X	A51V	L87Hfs*6
Zygosity	Hetero	Hetero	Hetero	Hetero	Hetero	Hetero	Hetero
Exon	11	11	3	2	8	2	2
Proband information							
Age at detection of calcification, y	60	51	60	73	23	71	74
Age at onset, y	58	50	60	71	15	71	57
Onset symptom	Dysarthria	Dysarthria	Dementia	Parkinsonism	PKC	Dementia	Athetosis
Neurologic findings							
Cognitive impairment (MMSE)	27	24	20	16	30	22	22
Pyramidal sign	+	+	−	−	−	−	−
Extrapyramidal sign	+	+	−	+	−	−	+
Family information (except the proband)							
No. of other individuals with calcification	1	2	5	1	1	0[a]	0[a]
No. of other individuals with confirmed mutations	1	NE	5	NE	1	NA	NA
No. of other symptomatic individuals	0	0	2	0	0	NA	NA
Other symptoms (no.) in the family	—	—	Mental disorder (1), alcoholism (1)	—	—	NA	NA

Abbreviations: MMSE = Mini-Mental State Examination; NA = not applicable; NE = not examined; PKC = paroxysmal kinesigenic choreoathetosis.
[a] Because there was no other family member who had any neurologic symptoms, brain CT screening of other family members was not performed.

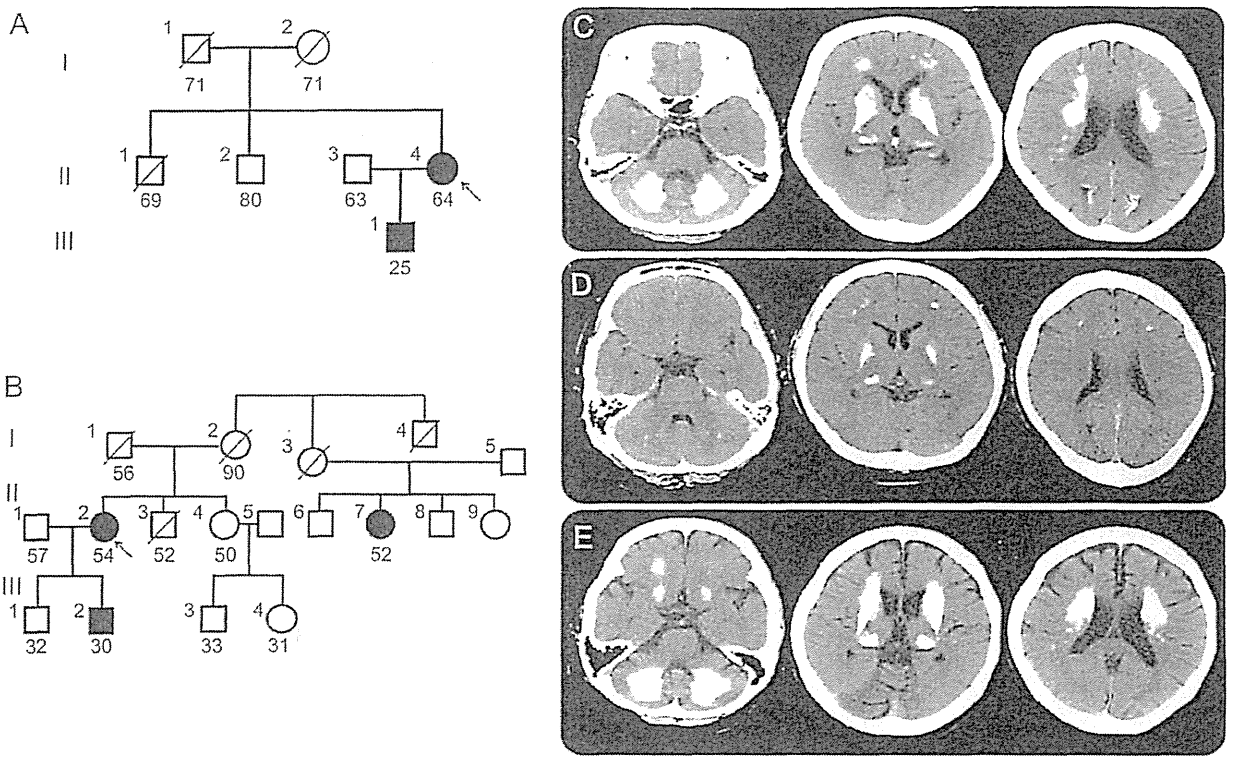
same mutation in exon 11 that had been found in his mother.

Case 2 (in family 2). The proband in family 2 was a 54-year-old woman who had dysarthria and gait disturbance for 4 years. She showed mild mental deterioration in Mini-Mental State Examination (MMSE) score of 24 points, frontal signs, dysarthria, mild parkinsonism (rigidity of bilateral wrist joints and bradykinesia), adiadochokinesis, spasticity, and small steppage gait. Her CT images revealed severe calcification at the bilateral globus pallidus, caudate nuclei, thalamus, subcortical white matter, and dentate nuclei (figure 2E). Although her son and cousin also showed calcification in CT images, they were asymptomatic. Her DNA analysis revealed the same mutation as that in family 1.

Case 3 and other symptomatic individuals (in family 3). The proband was a 69-year-old woman (II-1 in the pedigree in figure 3). She was admitted to a hospital at the age of 65 because of forgetfulness since the age of 60 years. Her MMSE score was 20, which indicated a possibility of dementia (MMSE score below 22). Decreased blood flow was detected in the bilateral basal ganglia and thalamus and the right frontal lobe in particular by SPECT. She had a positive family history of brain calcification, as shown in figure 3A. The initial clinical diagnosis had been diffuse neurofibrillary tangles with calcification (DNTC),[17] although to our knowledge familial cases of DNTC have not been reported. Her son had psychological disorders including violent behavior; unfortunately, no brain CT had yet been performed on him. In the patients in family 3, the degree of calcification was mild compared with that observed in the other families (figure 3, B–G). Her brother with calcification in the brain (II-7) had a mental disorder and another (II-8) presented with alcoholism. The 3 other relatives with calcification were asymptomatic (II-5, II-9, and III-3). The symptomatic patients (II-1, II-7, and II-8) showed more apparent brain atrophy than the others (figure 3, B, D, and E, respectively). The individuals with calcification on the CT images (II-1, II-5, II-7, II-8, II-9, and III-3) had the same mutation in exon 3 in *SLC20A2*. However, the individuals with no calcification (III-2, III-5, and IV-1) revealed no mutation in *SLC20A2*. In summary, 6 patients had calcification among the 10 individuals examined by CT scan in family 3 and all of them carrying the *SLC20A2* mutation exhibited similar calcification on CT images. However, persons without the mutation did not show calcification.

Case 4 (in family 4). Family 4 had a mutation in exon 2. The proband developed clumsiness of her hands and gait unsteadiness at the age of 71 years, and she was diagnosed as having Parkinson disease. Visual

Figure 2 CT images and family trees of families 1 and 2



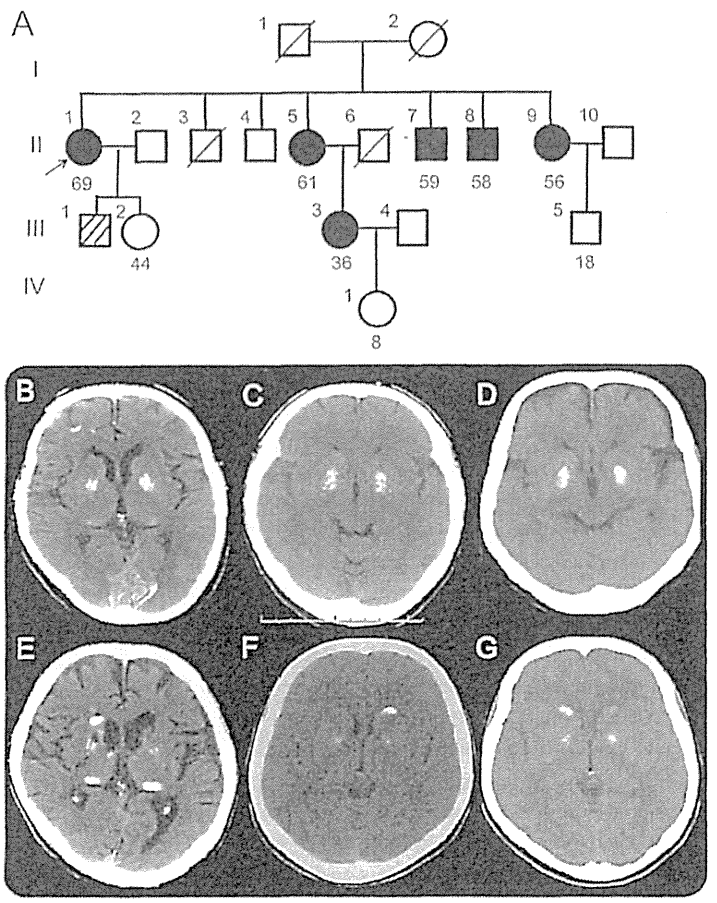
(A) Family tree of family 1. (B) Family tree of family 2. The arrow indicates the index subject. Filled symbols represent patients affected by brain calcification. We show the ages of persons under symbols in the family tree for those we could obtain. (C) CT images of proband (II-4 in pedigree of family 1, part A). (D) CT images of the proband's son (III-1 in pedigree of family 1, part A). (E) CT images of the proband (II-2 in pedigree of family 2, part B). All have mutation of S637R.

hallucinations started with the initiation of medication. She showed parkinsonism (rigidity, bradykinesia, and postural instability), which responded to levodopa. Her MMSE score was 16. Her brain CT images revealed calcification at the globus pallidus, caudate nuclei, and dentate nuclei, and her daughter, who was asymptomatic, also had intracranial calcification (figure e-2C). Brain CT was not performed in her other children. Her SPECT images showed decreased perfusion in the bilateral frontal, temporal, and parietal regions of the brain. She died of pneumonia at the age of 79. Neuropathologic examination revealed neuronal loss and Lewy bodies in the substantia nigra, locus ceruleus, amygdala, and parahippocampal gyrus indicative of Parkinson disease, and prominent deposition of calcium in the parenchyma and the wall of arteries in the globus pallidum and dentate nuclei compatible with the pathologic findings of IBGC.

Case 5 (in family 5). The proband was a 24-year-old man who had paroxysmal kinesigenic choreoathetosis (PKC). His laboratory data were normal except for CT findings. He presented with an attack of PKC after exercise and his symptom responded well to carbamazepine. His CT images revealed calcification at the globus pallidus, thalamus, subcortical white matter, and dentate nuclei (figure e-2B [A]). We had an opportunity to examine his parents, who had no symptoms or signs. Mutational analysis of *SLC20A2* of his parents with their informed consent revealed the same mutation in exon 8 in his mother as he had. Brain CT scan of his mother confirmed calcification at the globus pallidus, subcortical white matter, and dentate nuclei.

Sporadic cases. *Case 6.* The patient had a mutation in exon 2. She was a 72-year-old woman who noticed forgetfulness at the age of 71. She had no motor deficits. Her MMSE score was 22, and her score on the revised Hasegawa Dementia Scale was 24. Her Frontal Assessment Battery score at bedside was 5, indicating a frontal lobe deficit (cutoff score, 11/12). The index scores of the revised Wechsler Memory Scale were as follows: attention and concentration, 86; verbal memory, 89; general memory, 85; attention/concentration, 71; and delayed recall, 75. Her brain CT images revealed calcification at the globus pallidus, caudate nuclei, thalamus, subcortical and periventricular white matter, and dentate nuclei (figure e-2B [B]). Her SPECT images showed decreased perfusion in the left frontal,

Figure 3 Pedigree and CT images of family 3



(A) Pedigree of family 3. The arrow indicates the index subject. Filled symbols represent patients affected by brain calcification. We show the ages of persons under symbols in the family tree for those we could obtain. The striped symbol represents a symptomatic patient, although his CT image and DNA sample were not available for the study. (B) CT image of the proband (II-1 in pedigree of family 3). (C) CT image of asymptomatic II-5. (D) CT image of symptomatic II-7. (E) CT image of symptomatic II-8. (F) CT image of asymptomatic II-9. (G) CT image of asymptomatic III-3. All have mutation of T115M.

temporal, and parietal regions of the cerebrum and bilateral cerebellum. [^{11}C] Pittsburgh compound B (PiB) retention was not observed by [^{11}C]PiB PET. There were no other family members presenting with similar neurologic symptoms. CT scan was not performed for other individuals in the family.

Case 7. The patient was a 78-year-old man who had a frameshift in exon 2. Involuntary movement of the left thumb and index finger like "pill-rolling" began in his sixth decade. His family first noticed memory impairment at the age of 75. Gait disturbance appeared at the age of 77 and oral dyskinesia and left shoulder shrugging appeared at the age of 78. His scores on the MMSE and Frontal Assessment Battery were 22 and 10, respectively. His brain CT images showed calcification at the globus pallidus, thalamus, subcortical and periventricular white matter, and dentate nuclei (figure e-2B [C]). His SPECT images showed decreased perfusion in the bilateral (predominantly in the left) frontal and temporal regions of the cerebrum and bilateral cerebellum. [^{11}C]PiB retention was not observed by [^{11}C]PiB PET, which was performed at the age of 81. There were no other family members presenting with similar neurologic symptoms. CT scan was not performed for other individuals in the family.

DISCUSSION We have obtained clinical information of 161 patients with brain calcification in a nationwide study. We discovered that 3 patients had hypoparathyroidism, Aicardi-Goutières syndrome, and Cockayne syndrome during the survey. CT images revealed varying degrees of calcification, from marked calcification in the basal ganglia to patchy calcification in various regions, suggesting diversity in the etiologies. Some patients were incidentally found to have calcification by CT performed for head injury caused by accidents. Because our previous survey revealed a considerable frequency (1%–2%) of patchy calcification in the CT images of all patients in 2 university hospitals in Japan,[18] more asymptomatic IBGC patients with patchy calcification may exist than the number that we had previously assumed to be present in the population in Japan. After the examination by neurologists, we collected 69 DNA samples from patients who met the criteria for IBGC.[2,3] Symptoms and neurologic findings varied widely from asymptomatic to variable symptoms including headaches, psychosis, and dementia.

In this study, we investigated mutations in *SLC20A2* in 69 patients with IBGC in Japan and identified 4 new mutations in 10 familial cases (the same mutation in 2 families) and 2 other new mutations in 46 sporadic cases. The frequency of families with mutations in *SLC20A2* was 50% (5 of the 10 families), and that of sporadic patients was 4.3% (2 of the 46 patients). The frequency of the mutations in *SLC20A2* in FIBGC in Japan was as high as in other countries in a previous report.[10] Case 5 indicates that it is difficult to reliably determine sporadic cases without brain CT scans and genetic studies of all members in the family.

The mutations in our study existed in exons 2, 3, 8, and 11. One of these mutations (R467X) in exon 8 resulted in a substitution to a TGA stop codon, and the other (c.260_261delTC) in exon 2 was a frameshift. None of the mutations were reported previously, indicating heterogeneities of the mutations in *SLC20A2*. Taken together with other reports, causative mutations identified in *SLC20A2* include 6 mutations in exon 2, 1 in exon 3, 3 in exon 4, 1 in exon 5, 1 in exon 7, 10 in exon 8, 2 in exon 9, 4 in exon 10, and 4 in exon 11.[9–12] It does not seem that there

are mutation hot spots in *SLC20A2*. The in silico analysis using PolyPhen-2 for the missense mutations predicted all to be likely damaging, as determined from the residue changes. We drew the structure model of the PiT-2 protein using the TOPO2 software (http://www.sacs.ucsf.edu/TOPO/top.html). The schematic structure of the PiT-2 protein with the mutations is shown in figure 4.

Although the clinical features varied widely among the families with IBGC with *SLC20A2* mutations, the patients in families 1 and 2 with the same *SLC20A2* mutation exhibited similar clinical manifestations including dysarthria, mild cognitive decline, pyramidal signs, and extrapyramidal signs as well as similar ages at detection of calcification and onset of symptoms. Of note, the CT images among the affected individuals in the 2 families are similar (figure 2). In family 3, in contrast, 3 symptomatic patients presented with dementia, psychological disorder, and alcoholism, accompanied with brain atrophy in CT images. None of them showed movement disorders such as those in families 1 and 2.

Although mutational analysis and CT scan were not performed in other familial members of cases 6 and 7, concordance of the presence of mutations of *SLC20A2* and brain calcification were confirmed in 15 individuals, and we did not observe any individuals who carried the mutation and did not show brain calcification. These observations strongly support a high penetrance of the *SLC20A2* mutations regarding brain calcification.

Correlations of genotypes and neurologic phenotypes, however, have been controversial. *SLC20A2* mutations in patients with FIBGC have been described to show variability in clinical manifestations among the families. In the present study, the 2 affected individuals in families 1 and 2, who carried the same mutation, exhibited quite similar neurologic manifestations and clinical courses, suggesting a genotype-phenotype correlation of the S637R mutation. Of note, 2 individuals aged 56 and 61 years in family 3 did not exhibit any neurologic manifestations despite carrying the mutation and having brain calcification, indicating that penetrance regarding the neurologic manifestations is incomplete.

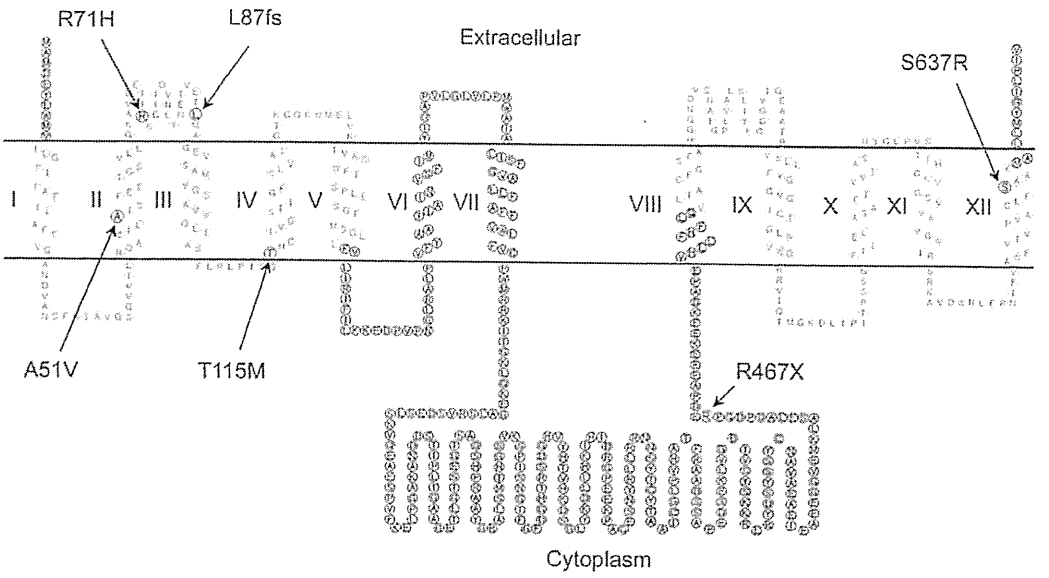
In case 4, interestingly, the proband showed pathologic findings of both IBGC and Parkinson disease. Because Parkinson disease is a common disorder in aged people, there remains a possibility that the presence of IBGC and Parkinson disease is coincidental.

Case 5 had a mutation that leads to a premature stop codon, making an incomplete structure of PiT-2. His neurologic symptom was PKC controllable by carbamazepine. Intriguingly, several patients with IBGC have been reported to present with PKC or paroxysmal nonkinesigenic dyskinesia.[19,20] For these cases of PKC or paroxysmal nonkinesigenic dyskinesia, mutational analyses of not only *SLC20A2* but also *PRRT2* and MRI will be indispensable.[21,22]

Herein, we have reported 5 cases of FIBGC and 2 cases of IBGC with *SLC20A2* mutations in Japan. We could not find any characteristic features of Japanese patients, although we had discovered that each case has a new mutation in *SLC20A2*, respectively.

The mechanisms of calcification and cell damage remain to be elucidated. Despite that the expression of PiT-2 encoded by *SLC20A2* is distributed widely in the human body,[23] mutations in *SLC20A2* cause

Figure 4 Schematic structure of PiT-2 (type III sodium-dependent phosphate transporter) with the mutations



calcification only in the brain. Mutations in *SLC34A2* have been reported to cause pulmonary alveolar microlithiasis.[24] Because Npt2b encoded by *SLC34A2* is the only phosphate transporter that is highly expressed in the lungs,[25] the mutations in *SLC34A2* are compatible with the lesion of the alveolar type II cells in the lungs.[24] Because the limitation of calcification to the brain cannot be explained by only the mutation in *SLC20A2* followed by abnormalities of inorganic phosphate (Pi) transport via PiT-2, there might be some other genes responsible for calcification in the brain, or the mutations in *SLC20A2* may take some toxic gain of function. The dysfunction of Pi transport can explain the accumulation of various metals in regions of the brain and the abnormal distribution of metals, which we observed in CSF[26] and hair in the patients with IBGC.[27] We have recently shown that PiT-2 immunopositivity was expressed predominantly in neurons, astrocytes, and vascular endothelial cells in the mouse brain.[28] PDGF-B is expressed in endothelial cells and neurons.[29] PDGF-B homodimer (PDGF-BB) enhanced the expression of PiT-1 mRNA encoded by *SLC20A1* in human aortic smooth muscle cells.[30] The hypomorph of PDGF-B in mice has recently been revealed to cause brain calcification through pericyte and blood-brain barrier impairment.[15] Recently, simple knockout of *SLC20A2* has also been shown to lead to calcification in the mouse brain.[31] PiT-2, PDGF, and as yet undetermined other molecules are considered to have pivotal roles in blood vessel–associated calcification and neuronal death in patients with IBGC. Elucidation of the molecular basis underlying IBGC will contribute to the development of therapeutic measures for patients with calcification in the brain.

AUTHOR CONTRIBUTIONS

Principal investigator: Isao Hozumi. Study supervision: Shoji Tsuji, Gen Sobue, Takashi Inuzuka, and Kortaro Tanaka. Manuscript draft preparation: Megumi Yamada and Masaki Tanaka. Acquisition and collection of data: Seiju Kobayashi, Yoshiharu Taguchi, Shutaro Takashima, Tetsuo Touge, Hiroyuki Hatsuta, and Shigeo Murayama. Analysis and interpretation: Megumi Yamada, Masaki Tanaka, Mari Takagi, Yuichi Hayashi, Masayuki Kaneko, Naoki Atsuta, Nobuyuki Shimozawa, Hiroyuki Ishiura, and Jun Mitsui.

ACKNOWLEDGMENT

The authors thank the patients and their families who supported this research. The authors also thank Societas Neurologica Japonica and the Japanese Society of Child Neurology (Professor Hideo Sugie, Jichi Medical University) for cooperation of collecting patients. The authors thank the Japanese Consortium for Amyotrophic Lateral Sclerosis research (JaCALS) and Japan MSA research consortium (JAMSAC) for kindly providing the exome sequencing data of controls.

STUDY FUNDING

This study was sponsored by a grant from the Ministry of Health, Labour and Welfare of Japan (H23-Nanbyo-Ippan-106 and H25-Nanchitoh [Nan]-Ippan-002).

DISCLOSURE

M. Yamada, M. Tanaka, and M. Takagi report no disclosures. S. Kobayashi received research funds from Research Funding Shionogi Pharma Inc., Mochida Pharmaceutical Co., Ltd., and Eli Lilly Japan K.K. Y. Taguchi and S. Takashima report no disclosures. K. Tanaka received funds from the University of Toyama, Otsuka Pharmaceutical Co., Ltd., Mochida Pharmaceutical Co., Ltd., Sanofi Co., Ltd., GlaxoSmithKline Co., Ltd., and Nippon Boehringer Ingelheim Co., Ltd. T. Touge reports no disclosures. H. Hatsuta received a fund from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant-in-Aid for Young Scientists [B] 24700371). S. Murayama received research funds from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grants-in-Aid for Comprehensive Scientific Research Network for Brain Bank; Basic Research B for Parkinson Disease), National Center for Geriatrics and Gerontology (Brain Bank), the Ministry of Health, Labour and Welfare of Japan (Grant-in-Aid for Neurodegenerative Disease, Prion Disease and Amyotrophic Lateral Sclerosis), and National Center for Neurology and Psychiatry (Brain Bank). Y. Hayashi reports no disclosures. M. Kaneko received funds from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant-in-Aid for Young Scientists [B] 23790095, Grant-in-Aid for Scientific Research on Priority Areas 22020032, and Grant-in-Aid for Scientific Research [B] 21300142), Takeda Science Foundation, the Research Foundation for Pharmaceutical Sciences, Niigata Brain Institute, and Japan Amyotrophic Lateral Sclerosis Association. H. Ishiura received funds from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant-in-Aid for Young Scientists [Start-up] 24890044) and the Cell Science Research Foundation. J. Mitsui received a fund from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant-in-Aid for Young Scientists [B] 25860700). N. Atsuta is funded by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan (grant number 25461277) and the Inochinoito Foundation of Japan. G. Sobue serves on the scientific advisory board for the Kanae Science Foundation for the Promotion of Medical Science, Takeda Science Foundation, and serves as an advisory board member of *Brain*, an editorial board member of *Degenerative Neurological and Neuromuscular Disease*, the *Journal of Neurology*, and *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*, and is funded by the Ministry of Education, Culture, Sports, Science and Technology of Japan; the Ministry of Welfare, Health and Labor of Japan; the Japan Science and Technology Agency, Core Research for Evolutional Science and Technology. N. Shimozawa reports no disclosures. T. Inuzuka received research funds from the Ministry of Health, Labour and Welfare of Japan (H23-Nanbyo-Ippan-106, H22-Nanbyo-Shitei-002, H23-Nanbyo-Shitei-001, H23-Nanti-Ippan-039), and the Ministry of Education, Culture, Sports, Science and Technology of Japan (Basic Research [C] 24591256), and Eizai Co., Ltd., Dainippon Sumitomo Pharmaceutical Co., Ltd., Takeda Pharmaceutical Co., Ltd., Otsuka Pharmaceutical Co., Ltd., and GlaxoSmithKline, LSE: GSK, NYSE: GSK. S. Tsuji received research funds from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grants-in-Aid for Scientific Research on Innovative Areas [22129001 and 22129002]), the Ministry of Health, Labour and Welfare of Japan (Grant-in-Aid H23-Jitsuyoka [Nanbyo]-Ippan-004), Sanofi K.K., Japan Blood Products Organization, Mitsubishi Tanabe Pharma Co., Pfizer Japan Inc., Ono Pharmaceutical Co., Ltd., Daiichi Sankyo Co., Ltd., Eisai Co., Ltd., Kowa Pharmaceutical, Co., Ltd., and GlaxoSmithKline, K.K. I. Hozumi received funds from the Ministry of Health, Labour and Welfare of Japan, the Ministry of Education, Culture, Sports, Science and Technology of Japan (Basic Research [C] 24590664), Niigata Brain Institute, the Community for Communication of Technology of Gifu University, and Eizai Co., Ltd. Go to Neurology.org for full disclosures.

Received May 18, 2013. Accepted in final form November 15, 2013.

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