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Letter to the Editor

# Novel *SLC20A2* mutation in a Japanese pedigree with primary familial brain calcification

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*Dear Editor,*

Primary familial brain calcification (PFBC) is a rare neurodegenerative disorder characterized by idiopathic deposits of calcium in bilateral brain structures, particularly in the basal ganglia and cerebellar dentate nuclei. Clinical manifestations include extrapyramidal, cerebellar, and psychiatric symptoms, although clinically asymptomatic patients also exist. To date, solute carrier family 20 member 2 (*SLC20A2*, MIM*158378), platelet-derived growth factor subunit B (*PDGFB*, MIM*190040), platelet-derived growth factor receptor beta (*PDGFRB,* MIM*173410), and xenotropic and polytropic retrovirus receptor 1 (*XPR1*, MIM*605237) were found to form the major genetic basis for the pathophysiology of this autosomal dominant condition [1]. We herein report a Japanese pedigree of PFBC, in which the proband presented with a rare clinical manifestation, with a novel haploinsufficient mutation in *SLC20A2*.

The proband was a 79-year-old woman, in whom brain calcification had been discovered earlier during health screening at a local clinic. Clopidogrel was administered daily because white matter lesions around the calcification in the brain were considered to reflect cerebral ischemia at the clinic (Fig. 1A). However, she later developed a transient ischemic attack (TIA) with left hemiparesis and dysarthria. Although the symptoms disappeared within 10 min and diffusion-weighted imaging of the brain demonstrated no acute cerebral infarction, further examination was performed in our hospital taking into consideration the relevance of the prominent brain calcification (Fig. 1A). She had no prevailing risk factors for ischemic stroke, such as diabetes mellitus, dyslipidemia, or atrial fibrillation, although she was mildly hypertensive with no need for medication (< 150/90 mmHg in late-phase elderly persons) according to the Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2014) [2]. Her serum levels of calcium, phosphate, and intact parathyroid hormone (i-PTH) were within normal limits when screening for known underlying causes of brain calcification such as hypoparathyroidism, pseudohypoparathyroidism, and pseudopseudohypoparathyroidism. We diagnosed her with PFBC because the family investigation revealed that her brothers (II4 and II6) also had brain calcifications (Fig. 1B–D). Brother II4 died of bile duct cancer, and brother II6 underwent hemodialysis due to chronic renal failure. They had no neurological or neuropsychiatric symptoms associated with PFBC.

In order to further confirm the diagnosis of PFBC, genetic testing was performed in this family. The ethical committee of Tokyo Women's Medical University approved the study protocol. After obtaining written informed consent, genomic DNA (gDNA) samples were obtained from four participants: the proband (II3), her affected brother (II6), and two presumed intrafamilial controls (III7 and III8) who were confirmed to have no brain calcification using head computed tomography (CT) (Fig. 1D–F). The gDNA samples from the affected siblings were subjected to exome enrichment using a SureSelect Human All Exon V5 kit (Agilent Technologies Inc., Santa Clara, CA), and whole-exome sequencing (WES) was performed using an Illumina HiSeq 2500 sequencer (Illumina, San Diego, CA). As a result, a novel frameshift mutation was identified in *SLC20A2*, which is the most common causative gene among patients with PFBC [3]. It was a heterozygous c.1349delG mutation resulting in a premature termination (p.Gly450AlafsX5, CCDS6132.1) (Fig. 1G). This termination met the criteria of the nonsense-mediated mRNA decay and, therefore, it was predicted to lead to haploinsufficiency. Furthermore, this *SLC20A2* mutation was shared among the affected siblings but was not identified in large-scale sequencing projects, such as the Human Genetic Variation Database (HGVD, http://www.genome.med.kyoto-u.ac.jp/SnpDB/index.html) and the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/) [4], or in the two intrafamilial controls (Fig. 1G). We diagnosed the *SLC20A2* c.1349delG mutation as the disease-causative mutation in this pedigree. To the best of our knowledge, this *SLC20A2* mutation has not been previously reported in the literature and was not found after screening the NCBI ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) and PubMed (http://www.ncbi.nlm.nih.gov/pubmed) databases. In this study, WES also revealed that the proband's genotype was homozygous for a loss-of-function single nucleotide polymorphism in *CYP2C19* (c.681G > A, rs4244285) which alters the intron4/exon5 splicing junction and leads to aberrant splicing. This variant is also known as the *CYP2C19* *2 allele impairing conversion from clopidogrel to its active metabolite. The *2/*2 homozygotes are classified as “poor metabolizers” constituting non-responders to clopidogrel treatment as was observed in the proband [5,6].

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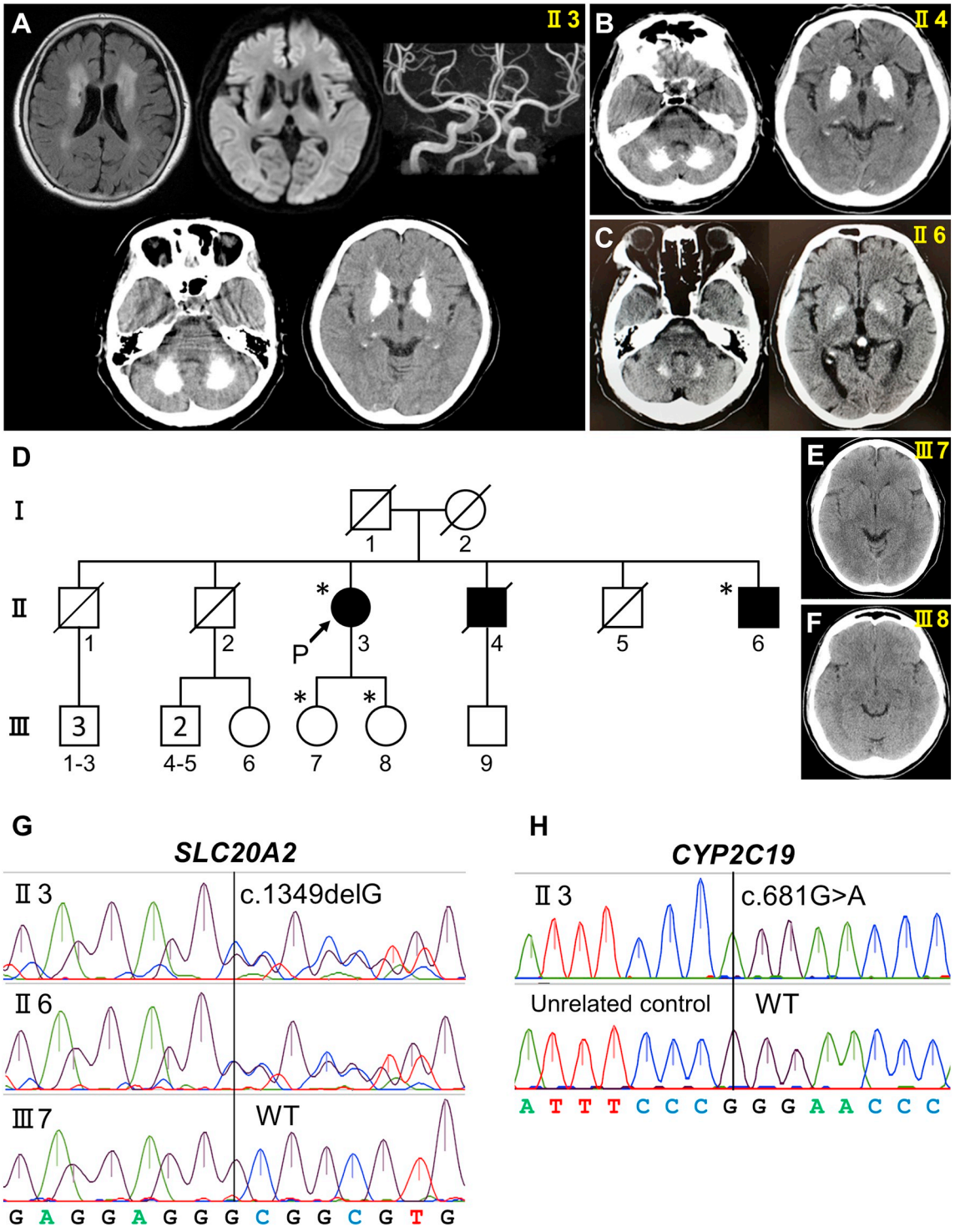


**Fig. 1.** Radiological and genetic manifestations of the present pedigree. (A) The imaging studies of the proband. Fluid-attenuated inversion recovery magnetic resonance imaging (MRI) demonstrated white matter hyperintensities around the brain calcification (upper left panel). Diffusion-weighted MRI and MR angiography (MRA) did not show acute cerebral infarction or stenosis of the major cerebral arteries when she developed a transient ischemic attack (TIA; upper middle and upper right panels). Computed tomography (CT) demonstrated brain calcification in the bilateral basal ganglia, thalamus, and dentate nucleus (lower left and lower right panels). (B) Her deceased brother and (C) the youngest brother who provided genomic DNA samples for the present study had brain calcifications. (D) Genealogical tree of the present pedigree. The black-filled symbols represent the affected individuals. Square, male; circle, female; crossed-out symbol, deceased individual; arrow, proband. DNA sequencing was carried out in individuals denoted with an asterisk. (E and F) The two daughters of the proband did not exhibit brain calcification. (G) Chromatograms of the heterozygous p.Gly450AlafsX5 mutation in *SLC20A2* shared between affected siblings. (H) Chromatograms of the *CYP2C19**2/*2 homozygous genotype of the proband and a wild-type control.

*SLC20A2* encodes the type III sodium-dependent phosphate transporter 2 (PiT2), which is expressed in brain tissues involved in the production and maintenance of cerebrospinal fluid (CSF), including neurovascular tissues as well as the choroid plexus, and serves as a major determinant of CSF phosphate concentration [1,7,8]. *Slc20a2*-depleted mice demonstrate elevated CSF phosphate levels, resulting in

calcium phosphate deposition around the cerebral perforating arterioles within glymphatic pathways [7], including recently discovered periarteriolar CSF channels in the brain parenchyma connecting to Virchow-Robin spaces [9]. Therefore, haploinsufficiency of *SLC20A2* could cause reduced elastance, vasomotor responses, and lumen diameters of the affected arterioles [10–13]. Among symptomatic cases of PFBC, the most commonly described manifestation is movement disorder including dystonia and parkinsonism [1], however, recent reports have suggested the possibility of secondary ischemic stroke, including TIA, associated with calcified vessels involved in the pathological process of PFBC [10–13].

In conclusion, genetic analysis of a Japanese PFBC pedigree revealed a novel frameshift mutation in *SLC20A2*. *SLC20A2* haploinsufficiency may also be associated with the proband's TIA; however, further studies are required because cerebrovascular events are common among elderly persons. In addition, comprehensive genetic testing using next-generation sequencing will become increasingly important in the clinical field because diagnosis and selection of therapeutic agents can be simultaneously determined along with our expanding knowledge of clinical genetics.

## Conflict of interest

We have no conflict of interest to declare.

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