Genetics of Primary Familial Brain Calcification

Subjects: Clinical Neurology

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Primary familial brain calcification (PFBC), also known as Fahr's disease, is a rare inherited disorder characterized by bilateral calcification in the basal ganglia according to neuroimaging. Other brain regions, such as the thalamus, cerebellum, and subcortical white matter, can also be affected. Among the diverse clinical phenotypes, the most common manifestations are movement disorders, cognitive deficits, and psychiatric disturbances. Although patients with PFBC always exhibit brain calcification, nearly one-third of cases remain clinically asymptomatic.

primary familial brain calcification | *SLC20A2* | *PDGFRB* | *PDGFB* | *XPR1* | *MYORG* | *JAM2* | *CMPK2*

1. Introduction

To date, two inheritance patterns have been found in PFBC patients. Heterozygous variants in *SLC20A2*, *PDGFRB*, *PDGFB*, and *XPR1* are responsible for autosomal dominant PFBC [1][2][3][4], while biallelic changes in *MYORG*, *JAM2*, and *CMPK2* are associated with autosomal recessive forms of the disease [5][6][7][8]. At present, around 50% of patients with PFBC do not have a pathogenic variant in the seven currently known genes [9], which indicates a more diverse genetic heterogeneity.

PFBC is genetically heterogeneous. To date, seven genes have been associated with PFBC, including four dominant genes and three recessive genes (**Table 1**).

Table 1. Summary of PFBC-causative genes.

Gene	Locus	Mode of Inheritance	Protein	Expression	Function	Effect of Variant	Most Common Variant Type	References
SLC20A2	Chr 8	AD	Type III sodium-dependent inorganic phosphate transporter 2 (PiT2)	Ubiquitously, higher level in the brain	Uptake of inorganic phosphate (Pi) into cells	Loss of function	Missense	[1][10][11][12]

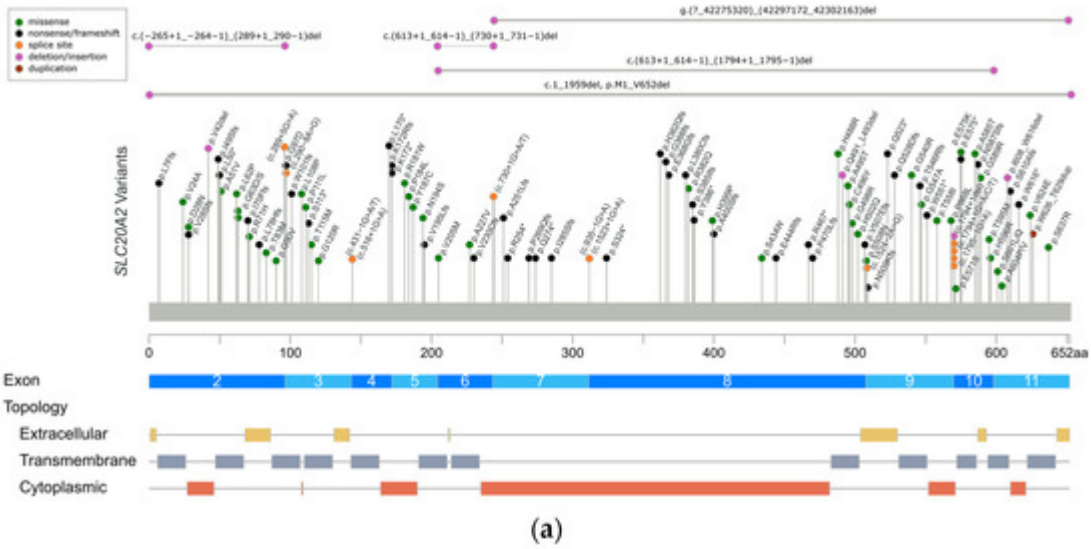
Gene	Locus	Mode of Inheritance	Protein	Expression	Function	Effect of Variant	Most Common Variant Type	References
PDGFRB	Chr 5	AD	Platelet-derived growth factor receptor-β (*PDGFRB*)	Neurons, vascular smooth muscle cells (SCMs), pericytes in the brain	Cell-surface tyrosine kinase receptors for the PDGF family, especially for homodimeric PDGF-B and PDGF-D; Essential for angiogenesis and formation of the blood–brain barrier (BBB)	Loss of function	Only missense	[2][9][10][13]
PDGFB	Chr 22	AD	Platelet-derived growth factor subunit B (*PDGFB*)	Neurons and endothelial cells in the brain	Growth factors for mesenchymal cells; Crucial role in the proliferation and recruitment of pericytes and vascular SCMs	Loss of function	Missense	[3][10][14][15]
XPR1	Chr 1	AD	Xenotropic and polytropic retrovirus receptor 1 (*XPR1*)	Ubiquitously, higher level in the brain	Pi efflux from cells	Loss of function	Missense	[4][10][16]
MYORG	Chr 9	AR	Myogenesis regulating glycosidase (*MYORG*)	Endoplasmic reticulum of the astrocytes in the brain	Member of the glycosyl hydrolase 31 family; Regulate protein glycosylation	Loss of function	Missense	[5][10]
JAM2	Chr 21	AR	Junctional-adhesion-molecule-2 (*JAM2*)	Endothelial cells and astrocytes in the brain	Member of the junctional adhesion molecules family; Crucial role in the regulation of cell polarity,	Loss of function	Nonsense	[6][7][10]

2. *SLC20A2*

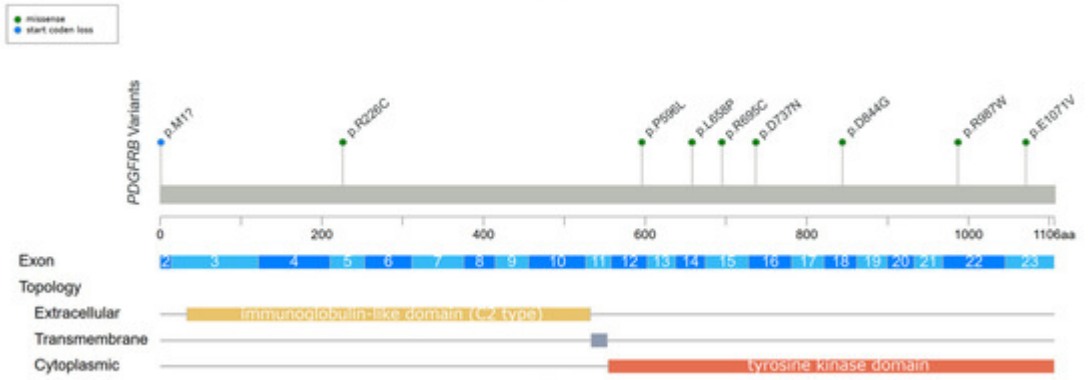
Gene	Locus	Mode of Inheritance	Protein	Expression	Function	Effect of Variant	Most Common Variant Type	References
CMPK2	Chr 2	AR	Uridine monophosphate-cytidine monophosphate kinase 2 (UMP-*CMPK2*)	Neurons and endothelial cells in the brain	Takes part in the salvage pathway for phosphorylation of dCMP, dUMP, CMP, and UMP in the mitochondria	Loss of function	Missense and start-codon loss	[8][17]

Abbreviations: Chr: chromosome; AD: autosomal dominant; AR: autosomal recessive.

mice was also found inside cells, mainly in the pericytes and astrocytes, which suggested the intracellular cytosolic initiation of calcification [63]. Moreover, increasing T-cell infiltration in the brain parenchyma was found in *Slc20a2*$^{-/-}$ mice, which is positively associated with brain calcification and aging. Impaired blood–brain barrier (BBB) permeability with the enhancement of endocytosis and transcytosis was also demonstrated, which may be explained by the dysfunction of pericytes and astrocytes due to intracellular calcification [65]. In addition, PiT2 is known to be expressed in the apical membrane of choroid plexus epithelial cells in spiny dogfish and rats, suggesting that PiT2 plays an important role in actively transporting Pi from the cerebrospinal fluid (CSF) to the blood to maintain phosphate homeostasis in the CSF [66]. The level of Pi in CSF is significantly elevated in both *Slc20a2* homozygous knockout mice and PFBC patients with *SLC20A2* pathogenic variants [63][67][68]. In summary, PiT2 dysfunction can leads to a local increase in the extracellular and CSF Pi concentrations, which then trigger cell-mediated mineralization progression, ensuing calcification [62][63].


missense
nonsense/frameshift
splice site
deletion/insertion
duplication
SLC20A2 Variants
0
100
200
300
400
500
600
652aa
Exon
Topology
Extracellular
Transmembrane
Cytoplasmic

(a)


missense
start codon loss
PDGFRB Variants
P.M1?
P.R228C
P.P596L
P.L658P
P.R695C
P.D737N
P.D844G
P.R987W
P.E1071V
0
200
400
600
800
1000
1106aa
Exon
Topology
Extracellular
Transmembrane
Cytoplasmic
immunoglobulin-like domain (C2-type)
tyrosine kinase domain

(b)

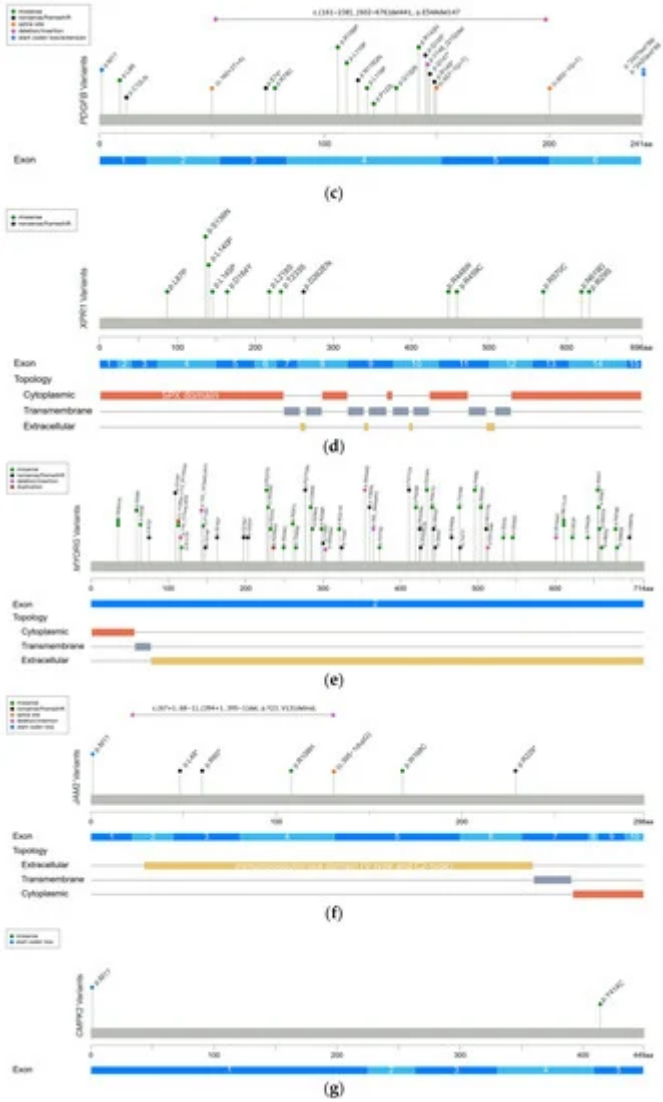


Figure 1. Reported variants in seven genes linked to PFBC along the protein sequence and their topologic protein models. (**a**) The reported variants in the *SLC20A2* gene and topology protein model of PiT2. (**b**) *PDGFRB* gene and PDGF-Rβ. (**c**) *PDGFB* gene. (**d**) *XPR1* gene and *XPR1*. (**e**) *MYORG* gene and *MYORG*. (**f**) *JAM2* gene and *JAM2*. (**g**) *CMPK2* gene. There is no obvious hotspot for *SLC20A2*, *MYORG*, and *JAM2* genes. The variants tend to cluster in the tyrosine kinase domain of the *PDGFRB* gene, the mature protein product between positions 82 and 190 of the *PDGFB* gene, and in the SPX domain of the *XPR1* gene. Meaning of symbols: *, stop codon; ?, unknown (a variant affecting the initiation codon cannot be predicted).

3. *PDGFRB*

The *PDGFRB* gene was identified in PFBC patients in 2013 [2], and it is located on chromosome 5 and encodes for platelet-derived growth factor receptor-β (PDGF-Rβ). The structure of PDGF-Rβ includes five extracellular immunoglobulin-like C2 type domains, a transmembrane domain, and a tyrosine kinase domain. PDGF-Rβ is a cell-surface tyrosine kinase receptor for members of the platelet-derived growth factor (PDGF) family, with a high affinity for homodimeric PDGF-B and PDGF-D. At the tissue level, the *PDGFRB* gene is highly expressed in the

brain, especially in the basal ganglia and dentate nucleus of the cerebellum. At the cellular level, the *PDGFRB* gene is expressed in neurons, vascular smooth muscle cells (SCMs), and pericytes. The signal transduction of PDGF-Rβ and its ligand is essential for the proliferation and migration of vascular SCMs and pericytes, and subsequently, the angiogenesis and formation of the BBB [2][9][18]. Heterozygous variants in the *PDGFRB* gene have been identified in 5% of genetically confirmed PFBC patients [10]. The missense change is currently the only variant type to be identified in the *PDGFRB* gene. The variants tend to cluster in the tyrosine kinase domain (**Figure 1**b) [2][10][21][32][69][70], and they are likely to cause haploinsufficiency and affect the kinase function of the protein [13]. The functional loss of PDGF-Rβ may lead to pericytes dysfunction, which would impact BBB integrity and secondarily induce calcium depositions in the vessel walls or perivascular space. Several studies have shown that homodimeric PDGF-B can directly induce vascular SCM calcification via enhancing the expression of inorganic phosphate transporter 1 (PiT1) [71][72]. Hence, Nicolas et al. hypothesized that activating variants in *PDGFRB* may impact the PDGF-PiT1 pathway and cause vascular calcification [2]. Interestingly, PiT1 is encoded by the *SLC20A1* gene, which is one of two type III sodium-dependent Pi transporters. The other is PiT2 encoded by *SLC20A2* [2][70]. However, there is no further evidence supporting this hypothesis [73].

4. *PDGFB*

The *PDGFB* gene was also reported in 2013 [3], and it is located on chromosome 22 and encodes for the PDGF-B precursor protein. This precursor protein is cleaved at positions 81 and 191, and subsequently forms a homodimer through disulfide bonds, which is the main ligand of PDGF-Rβ [18]. The *PDGFB* gene is expressed in neurons and endothelial cells in the brain [3]. PDGF-B is a growth factor for mesenchymal cells and plays a crucial role in the proliferation and recruitment of pericytes and vascular SMCs. [14][15]. Heterozygous variants in the *PDGFB* gene have been identified in 12% of genetically confirmed PFBC patients [10]. The missense change is the most common variant type in the *PDGFB* gene, followed by nonsense, splice site, and extension variants. The variants cluster between protein positions 82 and 190, which are retained in the mature PDGF-B protein (**Figure 1**c) [3][10][19][32][35][45][58][74][75][76][77][78][79][80][81][82]. The same as *PDGFRB* does, *PDGFB* variants also cause haploinsufficiency, either by deleting critical parts of protein or disrupting normal protein function. The loss of normal PDGF-B function leads to BBB impairment via PDGF-Rβ, and then triggers the process of calcification [3][9].

5. *XPR1*

The *XPR1* gene was identified in 2015 [4], and it is located on chromosome 1 and encodes for xenotropic and polytropic retrovirus receptor 1 (*XPR1*). XPR contains eight transmembrane domains and an amino-terminal SPX domain [18]. This protein mediates Pi efflux from cells [4][9]. *XPR1* is expressed universally, and a high *XPR1* mRNA level has been demonstrated in mouse brains, especially in the cerebellum and striatum [16]. Heterozygous variants in the *XPR1* gene have been identified in 6% of genetically confirmed PFBC patients [10]. The missense change is the most common variant in *XPR1*-related PFBC patients, which tends to cluster in the SPX domain (**Figure 1**d) [4][10][29][32][83][84][85]. *XPR1* variants may cause haploinsufficiency, leading to the intracellular accumulation of Pi and formation of calcium phosphate [4]. Interestingly, mutual interactions between *XPR1* and *PDGFRB* were found in a

recent immunoprecipitation study [16]. It is hypothesized that these two proteins may form a complex on the cell membrane, further suggesting that *PDGFRB* may be the upstream regulator of *XPR1* [16].

6. *MYORG*

In 2018, the first and most common autosomal recessive PFBC-causative gene, *MYORG*, was identified. *MYORG* is located on chromosome 9 and encodes for myogenesis-regulating glycosidase (*MYORG*). It contains a short cytoplasm domain at the N-terminal, a transmembrane domain, and a long luminal fragment with a glycosidase domain at the C-terminal. *MYORG* is a member of the glycosyl hydrolase 31 family, and its function is to regulate protein glycosylation. In the brain, the *MYORG* gene is highly expressed in the cerebellum, specifically in the endoplasmic reticulum of the astrocytes [5]. Biallelic variants in the *MYORG* gene have been identified in 13% of genetically confirmed PFBC patients [10]. The missense change is the most common variant type in the *MYORG* gene, followed by in-frame indels, nonsense, and frameshift variations. There is no obvious hotspot of pathogenic variants (**Figure 1**e) [5][10][86][87][88][89][90][91][92][93][94][95][96][97][98][99][100][101][102]. Pathogenic variants cause the loss of the glycosidase function of *MYORG*, which may lead to abnormal protein glycosylation and metabolic disturbance. It is believed that *MYORG* variants can induce astrocyte dysfunction, which then disturbs the association between astrocytes and pericytes, resulting in neurovascular unit (NVU) dysfunction and subsequently causing the formation of brain calcification [5]. However, the exact linkage between the loss of protein glycosylation and astrocyte dysfunction remains to be elucidated.

7. *JAM2*

In 2020, another autosomal recessive PFBC-causative gene, *JAM2*, was identified. *JAM2* is located on chromosome 21 and encodes for junctional-adhesion-molecule-2 (*JAM2*). The structure of *JAM2* includes two immunoglobulin-like domains (V-type and C2-type). *JAM2* is a member of the junctional adhesion molecule family, and it plays crucial roles in the regulation of cell polarity, endothelium permeability, leukocyte migration, and BBB function [6][7]. In the brain, *JAM2* is highly expressed in the caudate nuclei. At the cellular level, *JAM2* is specifically expressed in endothelial cells and astrocytes [7]. Biallelic variants in the *JAM2* gene have been identified in 2% of genetically confirmed PFBC patients [10]. The nonsense change is the most common variant type in the *JAM2* gene. Missense, frameshift, and structural variants have also been reported, without a mutation hotspot (**Figure 1**f) [6][7][10]. Variants of *JAM2* gene cause the loss of cell–cell adhesion and the dysfunction of the solute passage, which may contribute to the formation of brain calcification [6][7].

8. *CMPK2*

The *CMPK2* gene is the latest autosomal recessive PFBC-causative gene to be identified at the end of 2022 [8]. This gene is located on chromosome 2 and encodes for uridine monophosphate-cytidine monophosphate kinase 2 (UMP-*CMPK2*), which can be separated into N-terminal and C-terminal domains according to the sequence properties. *CMPK2* is a member of the nucleoside monophosphate kinase family and participates in the salvage

pathway for the phosphorylation of dCMP, dUMP, CMP, and UMP in the mitochondria [17]. In the brain, the *CMPK2* gene is highly expressed in the hippocampus and cerebellum. At the cellular level, *CMPK2* is specifically expressed in neurons and vascular endothelial cells [8]. To date, biallelic variants in the *CMPK2* gene have only been reported in two PFBC families, which carry missense and start-codon loss variants (**Figure 1**g) [8]. The loss of UMP-*CMPK2* function leads to a reduction in mitochondrial genome DNA copy numbers, the downregulation of the expression of mitochondrial protein, the decrease in ATP production, and the disturbance of mitochondrial cristae morphology. The disturbance of mitochondrial function is believed to cause impairment of energy homeostasis and the upregulation of intracellular phosphate levels, subsequently triggering the development of brain calcification [8].

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