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ARTICLE

Primary brain calcification: an international study reporting novel variants and associated phenotypes

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Abstract

Primary familial brain calcification (PFBC) is a rare cerebral microvascular calcifying disorder with a wide spectrum of motor, cognitive, and neuropsychiatric symptoms. It is typically inherited as an autosomal-dominant trait with four causative genes identified so far: *SLC20A2, PDGFRB, PDGFB*, and *XPR1*. Our study aimed at screening the coding regions of these genes in a series of 177 unrelated probands that fulfilled the diagnostic criteria for primary brain calcification regardless of their family history. Sequence variants were classified as pathogenic, likely pathogenic, or of uncertain significance (VUS), based on the ACMG-AMP recommendations. We identified 45 probands (25.4%) carrying either pathogenic or likely pathogenic variants ($n = 34$, 19.2%) or VUS ($n = 11$, 6.2%). *SLC20A2* provided the highest contribution (16.9%), followed by *XPR1* and *PDGFB* (3.4% each), and *PDGFRB* (1.7%). A total of 81.5% of carriers were symptomatic and the most recurrent symptoms were parkinsonism, cognitive impairment, and psychiatric disturbances (52.3%, 40.9%, and 38.6% of symptomatic individuals, respectively), with a wide range of age at onset (from childhood to 81 years). While the pathogenic and likely pathogenic variants identified in this study can be used for genetic counseling, the VUS will require additional evidence, such as recurrence in unrelated patients, in order to be classified as pathogenic.

Introduction

Primary familial brain calcification (PFBC) is a rare neuropsychiatric disorder characterized by abnormal calcium–phosphate deposits in the microvessels of the basal ganglia and other brain regions. Clinical manifestations can start at any age (median 31 years, range 6–77 years) [1], and include a wide spectrum of movement disorders (dystonia, parkinsonism, tremor, and chorea), neuropsychiatric symptoms (behavioral disturbances, psychosis, mood disorder, and cognitive impairment), cerebellar signs, and other symptoms [2], while up to 42% of the patients remain asymptomatic [1]. Even though the clinical presentation is variable, the neuroradiological picture (evidence of bilateral calcification affecting at least the basal ganglia) is thought to be invariably present by the age of 50. Hence, the diagnosis relies on a computerized tomography (CT) scan, in the absence of other known causes of brain calcification [2]. PFBC is typically inherited as an autosomal-dominant trait, and to date four causative genes have been identified.

These authors contributed equally: Eliana Marisa Ramos, Miryam Carecchio, Giovanni Coppola, Gaël Nicolas.

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SLC20A2 (solute carrier family 20, member 2) was the first gene to be linked to PFBC [3]. Since its discovery, many protein-truncating and deleterious missense variants have been identified, accounting for up to 40% of the familial cases [4]. *SLC20A2* encodes the transmembrane sodium-inorganic phosphate cotransporter PiT2, suggested to have a role in phosphate clearance from the cerebrospinal fluid by recent in vitro and knockout mice studies [5].

Variants in the *PDGFRB* gene [6–8], encoding the platelet-derived growth factor receptor β (PDGF-Rβ), and in the *PDGFB* gene (PDGF-Rβ's main ligand) [9–12], have been reported in more than 20 unrelated probands so far. PDGFB–PDGF-Rβ signaling mediates survival, differentiation, and migration of mesenchymal cells, including the vascular smooth muscle cells affected by calcifications in PFBC [13]. While increased signaling is associated with cancers, overgrowth, and progeria syndromes [14–18], in PFBC patients, protein-truncating *PDGFB* and missense *PDGFB* and *PDGFRB* variants lead to decreased PDGFB–PDGF-Rβ signaling [8, 19, 20]. Although PDGFB–PDGF-Rβ signaling is implicated in the regulation of inorganic phosphate transport [21], the mechanisms leading to microvascular calcification remain unknown [19].

More recently, missense variants in another phosphate transporter, encoded by the *XPR1* gene, were identified in several PFBC families [22]. Subsequent functional studies showed that XPR1 mutant proteins had severely reduced membrane localization and/or impaired phosphate efflux activity [22, 23].

The interpretation of sequence variants identified in genetic screens for rare diseases remains challenging. The American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) recently established a set of guidelines to classify genetic variants into five categories from benign (1) to pathogenic (5) [24]. While large sequence variant databases, such as gnomAD [25], are helpful in estimating allele frequencies in control populations, for rare diseases with incomplete penetrance (such as PFBC), variant recurrence in unrelated patients and family segregation data remain critical for interpretation.

In an international effort, four centers from France, USA, Italy, and Brazil gathered and analyzed sequence data from the four genes known to cause autosomal-dominant PFBC.

Materials and methods

Patients

We included patients with brain calcification who were referred to four centers of expertise: University of California, Los Angeles, USA; IRCCS Neurological Institute C. Besta, Milan, Italy; Inserm U1245, Rouen, France; and Universidade Federal de Pernambuco, Recife, Brazil. All patients presented calcifications affecting at least both lenticular nuclei, beyond the age-specific severity threshold [7], a normal phospho-calcic assessment (including at least calcium, phosphate, and PTH) in blood, and no other known etiology. Probands and, if available, family members underwent clinical examination and blood sampling. Details on clinical and family history were obtained by direct interview and/or by reviewing medical records. All individuals included in this study had a brain CT scan; for some, however, details about the extent and localization of brain calcifications were not available. Detailed inclusion criteria are reported in Supplementary Methods. All participants signed written informed consent for genetic analyses.

Genetic screening

Genomic DNA was extracted from peripheral blood by standard methods. For samples from the French, US, and Brazilian series, PCR amplification and subsequent Sanger sequencing of all protein-coding exons and exon–intron boundaries of *SLC20A2*, *PDGFB*, *PDGFRB*, and *XPR1* genes was performed as previously described [3, 6, 9, 22]. All 49 patients from the Italian series were screened with a customized gene panel (Nextera Rapid Capture Custom Enrichment), which included the PFBC genes and 55 additional genes responsible for diseases characterized by cerebral calcification (Supplementary Methods). The following genomic and transcript references were used for variant nomenclature and exon numbering: NG_032161.1 and NM_006749.4 for *SLC20A2*, NG_012111.1 and NM_002608.2 for *PDGFB*, NG_023367.1 and NM_002609.3 for *PDGFRB*, and NG_050964.1 and NM_004736.3 for *XPR1*.

Copy-number variation

Quantitative multiplex PCR of short fluorescent fragments (QMPSF) was used to assess the presence of copy-number variations (CNVs) encompassing *SLC20A2* and *PDGFB*, in the French and Brazilian series, as previously described [12, 26]. For the US series, CNVs were genotyped using TaqMan copy-number assays, following the manufacturer's instructions. Commercially available assays for the *SLC20A2* (Hs00279506_cn, Hs00383415_cn), *PDGFB* (Hs00902096_cn and Hs01735391_cn), and *PDGFRB* (Hs01615581_cn, Hs02279533_cn, and Hs02258542_cn) genes were used. For the Italian series, the cn.MOPS tool was applied to next-generation sequencing data for CNV detection [27].

Variant assessment

Variant classification was conducted following ACMG-AMP recommendations [24]. Briefly, these criteria included

Table 1 Details on *SLC20A2* variants and phenotype of variant carriers

Family number	Case ID	Study	Novel variant or ref.	ACMG class	Variant type	cDNA	Protein	Domain (missense) or predicted protein consequences	gnomAD	Mutation Taster	Polyphen2	SIFT	Ethnicity	Sex	Clinical summary	AAO	Family history	CT scan
1	EXT 1291 001	France	Novel	5	Nonsense	c.149T>G	p.(Leu50Ter)	Premature stop codon	Absent	NA	NA	NA	Caucasian	F	Psychosis and extrapyramidal syndrome	63	Negative	Pa, Pu, WM, D
2	IT-PFBC-7	Italy	32	4	Missense	c.212G>A	p.(Arg71His)	Phosphate transporter	Absent	DC (0.9)	PD (1)	D (0)	Caucasian	F	Asymptomatic	NA	Negative	Pa, Pu, Ca, T
3	EXT 878 001	France	Novel	3	Predicted splicing	c.289+5G>A	p.?	Predicted loss of 5' splicing donor site	Absent	NA	NA	NA	Caucasian	F	Pain, akinetic–rigid syndrome with tremor, gait disorder, and hypophonia	72	Negative	Pa, Pu, Ca, D, T, Co, WM, Ver
4	IT-PFBC-1	Italy	Novel	3	Predicted splicing	c.290-8A>G	p.?	Predicted loss of 3' splicing acceptor site	Absent	NA	NA	NA	Caucasian	F	Akinetic–rigid parkinsonism, LD responsive	65	Negative	Pa, Pu, D
5	EXT 1132 001	France	Novel	3	Missense/ splicing?	c.290G>A	p.(Gly97Asp)	First base of exon 3; however, splicing tools predict a minor effect on splicing (MaxEntScan score change:-7%)	Absent	DC (1)	PD (1)	D (0.02)	Polynesian	M	Anxiety, depression, apathy, somatoform signs, and attention deficit	18	Positive	Pa, Pu, Ca, T, WM, Co
6	Proband	USA	28,29	5	Nonsense	c.338C>G	p.(Ser113Ter)	Premature stop codon	Absent	DC (1)	NA	NA	NA	F	NA	NA	Positive	NA
7	IT-PFBC-6	Italy	28 [same patient]	5	Nonsense	c.338C>G	p.(Ser113Ter)	Premature stop codon	Absent	DC (1)	NA	NA	Caucasian	M	Focal unilateral chorea (hand)	53	Negative	Pa, Pu, Ca, T, D
8	EXT 945 001	France	Novel	5	Frameshift	c.382del	p.(Val128SerfsTer43)	Premature stop codon	Absent	NA	NA	NA	Caucasian	M	Cerebellar ataxia, dysarthria, memory impairment with dysexecutive signs, and depression	76	Negative	Pa, Pu, Ca, T, D, WM, Co, Ver
9	Proband	USA	Novel	4	Missense	c.541C>T	p.(Arg181Trp)	Phosphate transporter domain	4.084e −6 (4.512e −5, NFE)	DC (0.99)	PD (0.995)	T (0.06)	Caucasian	M	Progressive involuntary movements, neuropathic pain, and chronic headache	60	Positive	Pa, Pu, Ca
	Father													M	Asymptomatic	NA		Pa, Pu, Ca
10	ROU 375 004 (mother)	France	6	5	Missense	c.551C>T	p.(Pro184Leu)	Cytoplasmic	Absent	DC (1)	PD (0.98)	D (0.05)	Caucasian	F	Restless leg syndrome	55	Positive	Pa, Pu, Ca, T, D
	ROU 375 003 (sister)													F	Asymptomatic (migraine)	NA		Pa, Pu, Ca, T
	ROU 375 002 (sister)													F	Pyramidal signs	26		Pa, Pu, Ca, T, WM

Table 1 (continued)

Family number	Case ID	Study	Novel variant or ref.	ACMG class	Variant type	cDNA	Protein	Domain (missense) or predicted protein consequences	gnomAD	Mutation Taster	Polyphen2	SIFT	Ethnicity	Sex	Clinical summary	AAO	Family history	CT scan
	ROU 375 001 (proband)													F	Asymptomatic (migraine)	NA		Pa, Pu, Ca, T
11	EXT 1146 001	France	7	4	Missense	c.581A>G	p.(Asn194Ser)	Transmembrane	1.446e −5 (1.556e −4, NFE)	DC (0.99)	B (0.155)	T	NA	M	Akinetic–rigid syndrome, tremor, cerebellar ataxia, dysexecutive signs, and memory impairment	68	Positive	Pa, Pu, Ca, D, T, Co, WM, Ver
12	EXT 1180 001	France	Novel	5	Splicing	c.730+1G>T	p.?	Predicted skipping of exon 6 (in-frame) or use of alternative splice site	Absent	NA	NA	NA	Caucasian	F	Bradykinesia, tremor, and dysexecutive signs	40	Negative	Pa, Pu, T, D, Co
13	IT-PFBC-5b (first cousin)	Italy	Novel	5	Nonsense	c.739C>T	p.(Gln247Ter)	Premature stop codon	Absent	DC (1)	NA	NA	Caucasian	F	Asymptomatic	NA	Positive	Pa, Pu, Ca, T, D
	IT-PFBC-5a (proband)													M	Chorea, orofacial dyskinesia, depression, and cognitive decline	65		Pa, Pu, Ca, T, D, Co, WM
14	ROU 5028 001	France	30	5	Nonsense	c.1158C>A	p.(Tyr386Ter)	Premature stop codon with evidence of nonsense-mediated decay	Absent	DC (1)	NA	NA	Caucasian	F	Asymptomatic (migraine)	NA	Positive	Pa, Pu, D, Co
15	EXT 1118 001	France	30	5	Nonsense	c.1158C>A	p.(Tyr386Ter)	Premature stop codon with evidence of nonsense-mediated decay	Absent	DC (1)	NA	NA	Caucasian	M	Akinetic–rigid syndrome, orofacial dyskinesia and dystonia (induced by L-dopa), pyramidal signs, gait disorder, and hallucinations (induced by L-dopa)	51	Negative	Pa, Pu, Ca, D, T, Co, WM, Ver
16	1B02BR	Brazil	Novel	5	Frameshift	c.1187dup	p.(Pro397AlafsTer18)	Premature stop codon	Absent	DC (1)	NA	NA	NA	F	Parkinsonism	NA	Positive	NA
	1B01BR													M	Stroke, aphasia, and parkinsonism	NA		NA
17	EXT 1083 001	France	Novel	5	Nonsense	c.1207C>T	p.(Arg403Ter)	Premature stop codon	Absent	DC (1)	NA	NA	Caucasian	M	Akinetic–rigid syndrome	65	Negative	Pa, Pu, Ca, D, T, Co, WM, Ver
18	IT-PFBC-2	Italy	31	4	Missense	c.1301C>G	p.(Ser434Trp)	Phosphate transporter domain	3.228e −5 (6.663e −5, NFE)	DC (0.99)	PD (0.997)	D (0.00)	Caucasian	F	Parkinsonism and postural/kinetic tremor. Comorbid Down syndrome	3	Negative	Pa, Pu, D
19	EXT 1063 001	France	Novel	5	Nonsense	c.1426G>T	p.(Glu476Ter)	Premature stop codon	Absent	DC (1)	NA	NA	Caucasian	M	Akinetic–rigid syndrome, bipolar disorder. Mild cerebellar ataxia	44	Positive	Pa, Pu, Ca, D, T, Co, WM, Ver

Table 1 (continued)

Family number	Case ID	Study	Novel variant or ref.	ACMG class	Variant type	cDNA	Protein	Domain (missense) or predicted protein consequences	gnomAD	Mutation Taster	Polyphen2	SIFT	Ethnicity	Sex	Clinical summary	AAO	Family history	CT scan
20	IT-PFBC-3	Italy	Novel	3	Missense	c.1463A>G	p.(His488Arg)	Phosphate transporter	Absent	DC (0.99)	B (0.005)	T (0.83)	Caucasian	F	Subjective memory impairment, normal psychometry	59	Negative	Pa, Pu
21	Proband	USA	3	5	Missense	c.1492G>A	p.(Gly498Arg)	Phosphate transporter	Absent	DC (0.99)	PD (0.994)	D (0.00)	Caucasian	M	L-dopa-responsive parkinsonism, increased muscle tone and pain	NA	Negative	Pa, Pu, Ca, T
22	IT-PFBC-8a (proband)	Italy	3	5	Missense	c.1492G>A	p.(Gly498Arg)	Phosphate transporter	Absent	DC (0.99)	PD (0.994)	D (0.00)	Caucasian	M	Akinetic–rigid parkinsonism, dysarthria	68	Positive	Pa, Pu, Ca, T, D, Co, WM
	IT-PFBC-8b (daughter)													F	Asymptomatic	NA		Pa, Pu
23	EXT 1136 001	France	Novel	5	Splicing	c.1524-2A>G	p.?	Predicted skipping of exon 9 (in-frame) or use of alternative splice site	Absent	NA	NA	NA	Caucasian	F	Dysarthria, gait disorder, akinetic–rigid syndrome, memory impairment, and dysexecutive signs	71	Negative	Pa, Pu, Ca, T, D, Ve, Co
24	EXT 1318 001	France	Novel	3	Missense /splicing	c.1523G>A	p.(Ser508Asn)	Last base of exon 8; splicing tools predict a major effect on splicing (MaxEntScan score change:-59.5%)	Absent	DC (1)	PB (0.999)	D (0.01)	Caribbean	F	Right upper-limb dystonia, intention tremor, bradykinesia, mood disorder, and migraine	33	Positive for psychiatric signs	Pa
25	Proband	USA	Novel	5	Frameshift	c.1637_1638delCA	p.(Thr546ArgfsTer52)	Premature stop codon	Absent	NA	NA	NA	Caucasian	F	Migraine, vestibular signs	NA	Positive	Pa, Pu, Ca, and T
26	EXT 1235 001	France	4	4	Missense	c.1753G>A	p.(Ala585Thr)	Phosphate transporter	Absent	DC (1)	PD (0.999)	T (0.09)	African	F	Dementia and parkinsonism	NA	Negative	Pa, Pu, T, Ca, D, Co
27	EXT 1138 001	France	4	5	Frameshift	c.1755_1768del	p.(Asn587SerfsTer7)	Premature stop codon	Absent	NA	NA	NA	Caucasian	M	Mild-to- moderate intellectual disability, bipolar disorder, mild akinetic–rigid syndrome signs, ataxia, mild postural and intention tremor	3	Positive	Pa, Pu, Ca, T, D, T, Co
28	IT-PFBC-4	Italy	Novel	3	Missense	c.1765G>A	(p.Gly589Arg)	Phosphate transporter	Absent	DC (0.99)	PD (0.99)	D (0.01)	Caucasian	F	Dementia	81	Positive	Pa, D
29	Proband	USA	Novel	4	In-frame deletion (27 bp)	c.1822_1848del	p.(Ile608_Trp616del)	Phosphate transporter	Absent	NA	NA	NA	Caucasian	M	ADHD	NA	Positive	Pa, Pu, Ca, T, Co
	Father													M	Anxiety, dystonia	NA		Pa

Table 1 (continued)

Family number	Case ID	Study	Novel variant or ref.	ACMG class	Variant type	cDNA	Protein	Domain (missense) or predicted protein consequences	gnomAD	Mutation Taster	Polyphen2	SIFT	Ethnicity	Sex	Clinical summary	AAO	Family history	CT scan
30	EXT 1020 001	France	Novel	3	Missense	c.1871T>A	p.(Val624Glu)	Phosphate transporter	Absent	DC (1)	PossD (0.503)	T (0.15)	Caucasian	M	Tremor of the four limbs, memory impairment with dysexecutive signs. NB: tremor, beginning from age 7, is also present in two sibpairs in the absence of brain calcification	7	Negative	Pa, Pu, D, WM

ACMG class: 5—pathogenic, 4—likely pathogenic, and 3—variant of unknown significance. Novel variant refers to variants that have not been previously reported in PFBC patients. gnomAD frequency, in parentheses is the maximal subpopulation frequency for non-Finnish Europeans (NFE). Family history was considered positive if at least one first-degree relative exhibited at least one neuropsychiatric symptom by interview

Variants were submitted to the https://coppolalab.ucla.edu/lovd_pfbc/genes/SLC20A2 database. Reference sequences: NG_032161.1 and NM_006749.4

Associated references: [3, 4, 6, 7, 28–32]

AAO age at onset, *Pa* pallidum, *Pu* putamen, *Ca* caudate nuclei, *T* thalamus, *D* dentate nuclei, *Co* cerebral cortex, *WM* subcortical white matter, *Ver* vermis, *NA* not available, *DC* disease causing, *PossD* possibly damaging, *PD* probably damaging, *T* tolerated, *D* deleterious

prior identification as a PFBC-causing variant (reported in the literature, HGMD, Clinvar, and/or the PFBC variant database https://coppolalab.ucla.edu/lovd/genes), allele frequency in population databases (gnomAD [25], http://gnomad.broadinstitute.org/), computational and predictive data (Polyphen2, SIFT, MutationTaster, and splicing predictions provided by the Alamut visual software (Interactive Biosoftware, Rouen, France)), functional studies (reported in the literature), and segregation data. Each variant was first classified into one of the five ACMG-AMP classes by an investigator from the group where it was identified and then reviewed by the entire study group. All variants reported in this study were added to the PFBC database at https://coppolalab.ucla.edu/lovd/genes.

Affected relatives

Clinical and imaging data from affected relatives were collected, and genetic testing was performed on available DNA samples to ascertain variant cosegregation.

Results

Genetic screening in four series

By screening the four known PFBC-causative genes in 177 unrelated probands from four independent international series, we identified 34 probands (19.2%) carrying a variant classified as pathogenic (class 5) or likely pathogenic (class 4), while 11 carried a variant of uncertain significance (VUS) (class 3, 6.2%). In contrast, CNV analysis did not reveal any clear large deletion or duplication in the PFBC genes screened. The overall variant detection rate was therefore 25.4% (45/177) (Supplementary Table 1). Only 2 out of the 177 unrelated probands were previously reported [23, 28]. After including 11 variant-carrying affected relative members, 56 individuals are described herein.

SLC20A2 variants

We identified 27 distinct *SLC20A2* variants in 30 unrelated probands (16.9%, Table 1). Nine of these variants had previously been reported in other PFBC patients [3, 4, 6, 7, 29–32], including six missense variants for which pathogenicity was uncertain and that can now be classified as pathogenic: p.(Pro184Leu) and p.(Gly498Arg), or likely pathogenic: p.(Arg71His), p.(Asn194Ser), p.(Ser434Trp), and p.(Ala585Thr). These variants were seen in 12 of our unrelated probands, including one case already reported in the literature [28]. The remaining 18 *SLC20A2* variants were novel, of which nine were protein-truncating variants (PTV) and were therefore classified as pathogenic.

Table 2 Details on *PDGFB* variants and phenotype of variant carriers

Family number	Case ID	Study	Novel variant or ref.	ACMG class	Variant type	cDNA	Protein	Domain (missense) or predicted protein consequences	gnomAD	Mutation Taster	Polyphen2	SIFT	Ethnicity	Sex	Clinical summary	AAO	Family History	CT scan
31	EXT 929 001	France	Novel	4	Missense	c.394G>C	p.(Gly132Arg)	PDGF domain	Absent	DC (1)	PD (1)	D (0)	Caucasian	F	Asymptomatic	NA	Negative	Pa, Pu, D
32	ROU 1184 001	France	Novel	3	Missense	c.425G>A	p.(Arg142His)	PDGF domain	Absent	DC (0.97)	PD (1)	T (0.33)	Caucasian	F	Personality disorder, depressive episodes with memory impairment. Progressive cognitive decline. Akinetic–rigid syndrome, pyramidal signs, gait disorder, frontal behavioral disorder	68	Negative	Pa, Pu, D
33	EXT 1196 001	France	9	5	Nonsense	c.445C>T	p.(Arg149Ter)	Premature stop codon	Absent	DC (1)	NA	NA	Caucasian	M	Dysexecutive syndrome with memory impairment, anxiety, depression, akinetic–rigid syndrome, tremor	44	Positive	Pa, Pu, Ca, D, T (MRI)
34	Proband	USA	Novel	5	Splicing	c.456+1G>A	p.?	Predicted skipping of exon 4 introducing a frameshift	Absent	NA	NA	NA	NA	F	Severe migraine, history of depression	20	Positive	Pa, Pu, Ca, WM, D, Co
	Mother													F	Migraine, history of depression	26		Pa, Pu, Ca, T, D, WM

Table 2 (continued)

Family number	Case ID	Study	Novel variant or ref.	ACMG class	Variant type	cDNA	Protein	Domain (missense) or predicted protein consequences	gnomAD	Mutation Taster	Polyphen2	SIFT	Ethnicity	Sex	Clinical summary	AAO	Family History	CT scan
35	EXT 1251 001	France	Novel	5	Stop loss	c.724T>C	p.(Ter242GlnExtTer89)	Extended protein, loss of function	Absent	NA	NA	NA	Caucasian	F	Seizures, migraine, depression, cognitive impairment (memory, executive dysfunction)	25	Negative	Pa, Pu, Ca, D (MRI)
36	ROU 5019 001	France	9	5	Stop loss	c.726G>C	p.(Ter242TyrExtTer89)	Extended protein, loss of function	Absent	NA	NA	NA	Caucasian	M	Orofacial dyskinesia, oral tics, pyramidal signs, alcohol abuse, comorbid aneurysm of the right medial cerebral artery	NA	Positive	Pa, Pu, Ca, D, T, Co, WM

ACMG class: 5—pathogenic, 4—likely pathogenic, 3—variant of unknown significance. Novel variant refers to variants that have not been previously reported in PFBC patients. Family history was considered positive if at least one first-degree relative exhibited at least one neuropsychiatric symptom by interview

The variants were submitted to the https://coppolalab.ucla.edu/lovd_pfbc/genes/PDGFB database. Reference sequences: NG_012111.1 and NM_002608.2

Associated reference: [9]

AAO age at onset, *Pa* pallidum, *Pu* putamen, *Ca* caudate nuclei, *T* thalamus, *D* dentate nuclei, *Co* cerebral cortex, *WM* subcortical white matter, *NA* not available, *DC* disease causing, *PossD* possibly damaging, *PD* probably damaging, *T* tolerated, *D* deleterious

Two novel likely pathogenic variants were also identified. First, an in-frame deletion of 27 nucleotides (c.1822_1848del) in exon 11 of *SLC20A2* was identified in a proband and his affected father. This variant is predicted to cause a deletion of nine amino acids, p.(Ile608_Trp616del), at the C-terminal domain of Pit-2, in a transmembrane region. Second, a predicted-damaging missense variant, c.541C>T, p.(Arg181Trp) in exon 5 was identified in a patient and his affected father. This variant was found in one individual from the gnomAD database (MAF = 4.1e−06). Other missense pathogenic variants in nearby residues have been reported in PFBC patients [4], supporting evidence for pathogenicity.

Among the additional seven novel VUS identified, two were intronic (c.289+5G>A, c.290–8A>G), absent from gnomAD, and with strong in silico predictions of a splicing defect at the closest canonical site (MaxtEntScan score change of –80.7% and –54.4%, respectively, with the c.290–8A>G predicted to create a new acceptor site at position c.290–7). Two other novel missense VUS were located at exon boundaries. The c.290G>A, p.(Gly97Asp) variant, affecting the first base of exon 3, was predicted as damaging by in silico tools and to cause a slight effect in splicing (MaxEntScan score change: –7%). The c.1523G>A variant, p.(Ser508Asn), affecting the last base of exon 8, was also predicted to be damaging, in addition to a strong effect on splicing (MaxEntScan score change: –59.5%). RNA from these patients was not available to confirm the hypothesis of a protein-truncating effect through altered splicing, precluding their classification as (likely) pathogenic. The other novel VUS, p.(His488Arg), p.(Gly589Arg), and p.(Val624Glu), were not detected in gnomAD and are predicted to be damaging by in silico analysis. Even though other missense pathogenic variants in nearby residues have been reported, there was not sufficient evidence to classify these specific variants as (likely) pathogenic.

PDGFB variants

We identified six distinct *PDGFB* variants in 6 unrelated probands (3.4%, Table 2). Two of these variants had already been reported in other PFBC patients: nonsense p.(Arg149Ter) and, stop loss c.726G>C, p.(Ter242TyrExtTer89) that adds 89 residues to the protein [9]. We identified a novel stop loss variant, c.724T>C, p.(Ter242GlnExtTer89), which is also predicted to cause an elongation of the reading frame by 89 amino acids. Functional studies have shown that proteins with variants causing a C-terminal extension, namely p.(Ter242TyrExtTer89), failed to induce any detectable PDGF-Rβ autophosphorylation [19]. A novel canonical splice site variant, c.456+1G>A (Table 2), predicted to affect splicing of exon 4 in *PDGFB*, was identified in a proband and the affected mother. Both of these novel variants were absent from gnomAD. Therefore, there was enough evidence to support these variants as pathogenic for PFBC.

We also identified two novel missense variants, both absent from gnomAD and predicted damaging by in silico analysis: p.(Gly132Arg) and p.(Arg142His) (Table 2). Variant p.(Gly132Arg) was identified in an additional unrelated French patient with brain calcifications (enrolled after the data freeze, hence not included in this series) and was therefore classified as likely pathogenic.

PDGFRB variants

Three distinct *PDGFRB* variants were found in three unrelated probands (1.7%, Table 3): p.(Arg226Cys), p.(Pro596Leu), and p.(Asp844Gly), all novel missense variants, predicted damaging. Of these, only the p.(Pro596Leu) variant was present in two individuals in gnomAD (MAF = 8.1e−06). Segregation data was only available for the family carrying the p.(Asp844Gly) variant and we showed that this variant resulted in a loss of PDGFRβ autophosphorylation (Supplementary Figure 1). Based on this evidence, this variant was classified as pathogenic, while the other two were classified as VUS.

XPR1 variants

Five distinct *XPR1* variants were found in six unrelated probands (3.4%, Table 4). Two of these variants had already been associated with PFBC. One of our unrelated French patients carried the same p.(Leu145Pro) variant reported in the original *XPR1* paper [22]. The other variant, p.(Leu87Pro), was found in a case already reported [23]. These two variants were not found in gnomAD and can be classified as pathogenic based on published functional evidence [22, 23]. Three additional predicted-damaging missense variants were found (Table 4). While p.(Thr233Ser) was found in two unrelated PFBC individuals, it was also found in two individuals within the gnomAD database (MAF = 8.1e−06). On the other hand, both p.(Arg459Cys) and p.(Asn619Asp) were not found in gnomAD. Furthermore, for p.(Arg459Cys), the unaffected proband's mother did not carry this variant and had a normal brain CT scan. Both p.(Thr233Ser) and p.(Arg459Cys) variants were therefore classified as likely pathogenic, while there was not sufficient evidence for p.(Asn619Asp), hence classified here as VUS.

Clinical presentation

Herein, we reported a total of 56 PFBC patients (32F; 24M), including the 45 probands that were found to carry VUS or

Table 3 Details on *PDGFRB* variants and phenotype of variant carriers

Family number	Case ID	Study	Novel variant or ref.	ACMG class	Variant type	cDNA	Protein	Domain (missense) or predicted protein consequences	gnomAD	Mutation Taster	Polyphen2	SIFT	Ethnicity	Sex	Clinical summary	AAO	Family History	CT scan
37	IT-PFBC-9[a]	Italy	Novel	3	Missense	c.676C>T	p.(Arg226Cys)	Extracellular, Ig-like C2-type 3	Absent	DC (0.99)	PD (1)	D (0.01)	Caucasian	M	Paroxysmal kinesigenic dyskinesia[a], CBZ responsive	11	Negative	Pa, Pu, Ca, T, D
38	IT-PFBC-10	Italy	Novel	3	Missense	c.1787C>T	p.(Pro596Leu)	Outside of Protein Kinase domain, Cytoplasmic	8.147e −6 (3.254e −5, South Asian; 8.988e −6, NFE)	DC (1)	PD (1)	D (0)	Caucasian	F	Asymptomatic (migraine)	NA	Negative	Pa, Pu
39	Proband	USA	Novel	5	Missense	c.2531A>G	p.(Asp844Gly)	Cytoplasmic, protein kinase	Absent	DC (0.99)	PD (0.998)	D (0.01)	Caucasian	F	Sleepwalking	Childhood	Positive	Pa, Pu, WM, D
	Paternal aunt													F	NA	NA		NA

ACMG class: 5—pathogenic, 4—likely pathogenic, 3—variant of unknown significance. Novel variant refers to variants that have not been previously reported in PFBC patients. gnomAD frequency, in parentheses is the maximal subpopulation frequency for South Asians and Non-Finnish Europeans (NFE). Family history was considered positive if at least one first-degree relative exhibited at least one neuropsychiatric symptom by interview

Variants were submitted to the https://coppolalab.ucla.edu/lovd_pfbc/genes/PDGFRB database. Reference sequence: NM_002609.3

AAO age at onset, *Pa* pallidum, *Pu* putamen, *Ca* caudate nuclei, *T* thalamus, *D* dentate nuclei, *Co* cerebral cortex, *WM* subcortical white matter, *CBZ* carbamazepine, *NA* not available, *DC* disease causing, *PossD* possibly damaging, *PD* probably damaging, *T* tolerated, *D* deleterious

[a]The *PRRT2* and *PNKD* genes were sequenced in this patient and no change was detected

Table 4 Details on *XPR1* variants and phenotype of variant carriers

Family number	Case ID	Study	Novel variant or ref.	ACMG class	Variant type	cDNA	Protein	Domain (missense) or predicted protein consequences	gnomAD	Mutation Taster	Polyphen2	SIFT	Ethnicity	Sex	Clinical summary	AAO	Family History	CT scan
40	EXT 1003 001	France	23 [same patient]	5	Missense	c.260T>C	p.(Leu87Pro)	SPX domain	Absent	DC (1)	PD (1)	D (0)	Caucasian	M	Dysarthria with parkinsonian and cerebellar features, concentration deficit, mild executive dysfunction, micrography, parkinsonism, anxiety	37	Positive	Pa, Pu, Ca, T, D, Ve, WM, Co
41	EXT 1187 001	France	22	5	Missense	c.434T>C	p.(Leu145Pro)	SPX domain	Absent	DC (1)	PD (1)	D (0.01)	Caucasian	M	Extrapyramidal syndrome, cognitive impairment, dysarthria, behavioral disturbances	29	Positive	Pa, Pu, Ca, T, D, Co (MRI)
	EXT 1187 002													F	Bradykinesia, psychomotor slowing	38		Pa, Pu, Ca, T, D, Co
42	IT-PFBC-11	Italy	Novel	4	Missense	c.697A>T	p.(Thr233Ser)	Outside from SPX domain	8.133e−6 (1.795e−5, NFE)	DC (0.99)	PossD (0.885)	D (0.03)	Caucasian	F	Mild Cognitive Impairment	81	Negative	Pu, Pa
43	IT-PFBC-12	Italy	Novel	4	Missense	c.697A>T	p.(Thr233Ser)	Outside from SPX domain	8.133e−6 (1.795e−5, NFE)	DC (0.99)	PossD (0.885)	D (0.03)	Caucasian	F	Vertigo	50	Negative	Pa, Pu
44	ROU 5059 001	France	Novel	4	Missense	c.1375C>T	p.(Arg459Cys)	Outside from SPX domain	Absent	DC (1)	PD (1)	D (0)	Caucasian	M	L-Dopa-responsive extrapyramidal syndrome, mild intellectual disability	55	Negative	Pa, Pu, Ca, D
45	EXT 1219 001	France	Novel	3	Missense	c.1855A>G	p.(Asn619Asp)	Outside from SPX domain	Absent	DC (1)	PD (1)	D (0)	Caucasian	M	Sudden deafness, mild cerebellar syndrome	69	Positive	Pa, Pu, Ca, D, T

ACMG class: 5—pathogenic, 4—likely pathogenic, 3—variant of unknown significance. Novel variant refers to variants that have not been previously reported in PFBC patients. gnomAD frequency, in parentheses is the maximal subpopulation frequency for Non-Finnish Europeans (NFE). Family history was considered positive if at least one first-degree relative exhibited at least one neuropsychiatric symptom by interview

Variants were submitted to the https://coppolalab.ucla.edu/lovd_pfbc/genes/XPR1 database. Reference sequence: NM_004736.3

Associated references: [22, 23]

AAO age at onset, *Pa* pallidum, *Pu* putamen, *Ca* caudate nuclei, *T* thalamus, *D* dentate nuclei, *Co* cerebral cortex, *WM* subcortical white matter, *NA* not available, *DC* disease causing, *PossD* possibly damaging, *PD* probably damaging, *T* tolerated, *D* deleterious

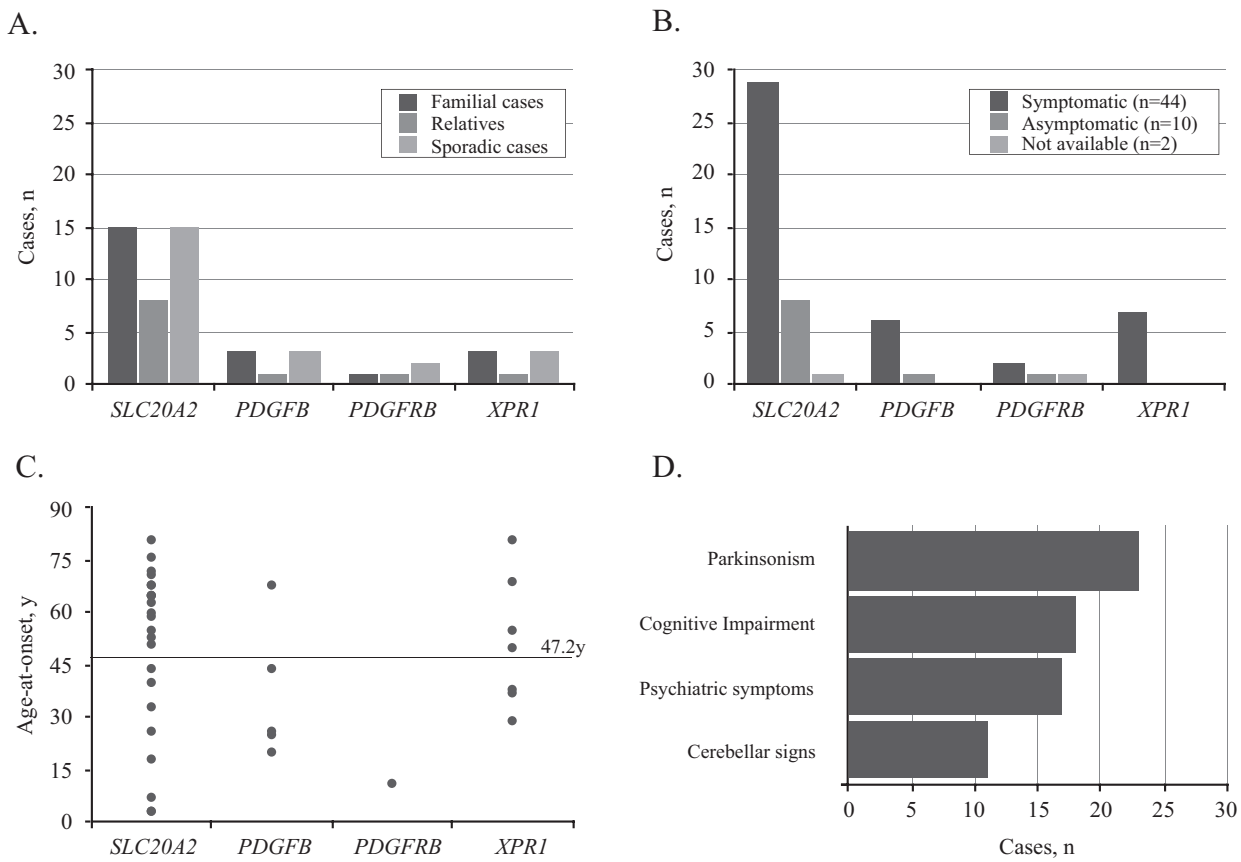

Fig. 1 Clinical presentation of 56 variant carriers. **a** Number of familial (including relatives) and sporadic cases, and **b** number of symptomatic and asymptomatic individuals per gene. **c** Distribution of age-at-onset (years) per gene (horizontal line represents the average age of onset across all 37 cases with known age-at-onset). **d** Frequency of main symptoms among the 44 symptomatic variant carriers.

(likely) pathogenic variants, and 11 relatives that had brain calcifications and the same variant as the proband (Fig. 1a). Detailed clinical and radiological data were available in 54/56 patients (Tables 1–4), and at the time of genetic testing, 44 (81.5%) of these were symptomatic (Fig. 1b). Mean age at clinical onset was 47.2 years (Fig. 1c) (median = 52 y, range: 3–81 y, age at onset was unknown for eight cases, including one with onset in childhood) and mean age at last examination was 57.4 years in symptomatic patients and 47.5 in asymptomatic patients. Parkinsonism (alone or combined with other clinical manifestations) was the most frequent finding, present in 23/44 (52.3%) of symptomatic patients, mostly with an akinetic–rigid presentation (Fig. 1d). Cognitive impairment was documented in 18/44 (40.9%) symptomatic cases, psychiatric disturbances (depression, psychosis, anxiety) in 17/44 (38.6%), while 11/44 (25.0%) patients had cerebellar signs. In addition, migraine was reported by 10/54 patients (18.5%); in five of these patients neurological examination was unremarkable and therefore they were considered asymptomatic.

Discussion

We screened the four known PFBC-causative genes in a series of 177 PFBC patients and identified 41 distinct variants, in a total of 45 unrelated probands. Taking into account only likely pathogenic and pathogenic variants, for which evidence is sufficient to propose genetic counseling, 34 out of the 177 (19.2%) unrelated probands carried such variants. However, the overall variant detection rate can increase up to 25.4% (45/177), if future studies provide new evidence to reclassify the VUS we found as causal. As expected, *SLC20A2* showed the highest contribution with variants identified in 16.9% (30/177) of the probands, followed by *XPR1* and *PDGFB*, each with 3.4% (6/177), and then *PDGFRB* with 1.7% (3/177). These rates are consistent with those reported in other French series that, similar to ours, had patients with and without known family history [33], in contrast to previous reports that showed high mutation rates in patients with a positive family history [34]. Even though we screened novel unrelated probands, we detected new but also previously reported PFBC variants, sometimes in patients originating from the same

country as the original carrier. It should be noted that, based on available family information, none of the patients in our series seem to be related to any of the PFBC carriers already published in the literature.

SLC20A2 was the first PFBC-causative gene to be identified, linking cerebral inorganic phosphate metabolism to PFBC's pathophysiology [3]. Evidence that *SLC20A2* haploinsufficiency causes PFBC is strong as both PTV and total/partial deletions have been identified [3, 26, 29]. This hypothesis has been confirmed in mouse models [5, 35, 36], and by in vitro assessment of some of the missense variants [3]. In our series, including patients with positive family history and apparently sporadic cases, we confirmed *SLC20A2* as the major causative gene, accounting for at least 13.0% of the cases (adding up to 16.9% when including VUS).

XPR1 was the most recent PFBC gene to be identified [22], and in our series, variants within this gene are as frequent as *PDGFB* variants. Pathogenicity of *XPR1* variants reported to date has been ascertained based on: strong segregation [22], recurrence among unrelated patients, and/or functional data showing a defect in inorganic phosphate transport [22, 23]. Interestingly, all known pathogenic variants are located in the SPX domain of XPR1, the function of which remains uncertain. We identified three novel missense variants, all predicted damaging, but located outside the SPX domain. Functional analyses are needed to further clarify their role.

The identification of protein-truncating *PDGFB* variants following the identification of missense *PDGFRB* variants, provided the first evidence that decreased PDGFB–PDGFRβ signaling was causative of PFBC. Loss of function and missense variants, as well as a partial *PDGFB* deletion have been identified to date [1, 9, 12, 37], supporting haploinsufficiency as causal mechanism. Here, we report four novel variants, including one PTV, one stop loss and two missense variants, of which one could be classified as likely pathogenic.

Since the original paper identifying *PDGFRB* as a PFBC causal gene, only four established pathogenic *PDGFRB* variants have been reported in the literature. These showed strong segregation evidence [6] and/or functional evidence of a loss of protein function [8, 19, 20]. Another missense variant, p.(Glu1071Val), originally considered as VUS has since been reclassified as likely benign based on functional studies [7, 19, 20]. More recently, 2 novel variants were identified in Chinese PFBC cases: a c.3G>A variant leading to a loss of the start codon, and a missense p.(Asp737Asn) variant [38]. Although the latter variant was considered a VUS, the start loss variant could be classified as pathogenic if considered truncating, however its functional effect remains unclear as an alternative in-frame ATG codon could theoretically be used. Herein, we report 3 additional missense variants, though only one of them could be classified as pathogenic based on segregation and functional data.

The PFBC phenotypic spectrum is wide and diverse, with intra and interfamilial heterogeneity. Although some of the variants found in this study are recurrent, their low frequency precluded any genotype-phenotype correlations, and therefore we focused on all carriers. We found that 81.5% of those with clinical information available were considered symptomatic, with severity ranging from minor signs on clinical examination to severe disability. In previous reports, including a prior independant data freeze of the French PFBC series and a meta-analysis study, the proportion of symptomatic patients was indeed lower, 58 and 64%, respectively [1, 39]. Here, the relatively high proportion of symptomatic carriers is likely due to an inclusion bias, as symptomatic probands are more likely to be offered genetic screening than asymptomatic individuals and few relatives could be included in the present report (11/56 versus 35/57 in [1]). Age of onset was comparable to previous screens, with a wide range from 3 to 81 years. Consistent with previously published series, the most frequent symptoms in our series were parkinsonism (52.3% of symptomatic individuals), cognitive impairment (40.9%), and psychiatric signs (38.6%). Interestingly, 18.5% of the 54 patients with available clinical data reported migraine without atypical features, which is in the same range as the general population [40], suggesting that migraine in patients with brain calcifications may be coincidental. This ratio is consistent with those reported in an independent series and a literature review study [1, 39], while there are also reports that showed lack of segregation between brain calcification and migraine [41].

In summary, by screening the known PFBC genes in four cohorts from America and Europe, including sporadic and familial cases, we identified variants interpreted as VUS, likely pathogenic, or pathogenic in 25.4% of the 177 probands. While variants from the latter two classes can be used for genetic counseling, segregation and/or functional studies of the VUS are necessary to help clarify their role in PFBC, and therefore no presymptomatic testing can be recommended given the current level of evidence. The novel variants reported here will help with interpretation of future genetic screens of unrelated PFBC patients and provide a list of candidates for functional studies. Finally, further prospective follow-up studies in patients carrying pathogenic variants in PFBC-related genes are needed to widen our knowledge about disease course, genetic and/or environmental factors which could influence disease penetrance and progression.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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