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%). 81.5% of carriers were symptomatic and the most recurrent symptoms were parkinsonism (54.5% of symptomatic patients), cognitive impairment and psychiatric disturbances (43.2% each), 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.

Keywords: primary familial brain calcification; PDGFB; PDGFRB; SLC20A2; XPR1
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), and include a wide spectrum of movement disorders (dystonia, parkinsonism, tremor, chorea), neuropsychiatric symptoms (behavioral disturbances, psychosis, mood disorder, cognitive impairment), cerebellar signs and other symptoms, while up to 42% of the patients remain asymptomatic. 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 absence of other known causes of brain calcification. PFBC is typically inherited as an autosomal dominant trait, and to date four causative genes have been identified.

SLC20A2 (solute carrier family 20, member 2) was the first gene to be linked to PFBC. Since its discovery, many protein-truncating and deleterious missense variants have been identified, accounting for up to 40% of the familial cases. 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.

Variants in the PDGFRB gene, encoding the platelet-derived growth factor receptor β (PDGF-Rβ), and in the PDGFB gene (PDGF-Rβ’s main ligand), have been reported in more than 20 unrelated probands so far. PDGFB-PDGFRβ signaling mediates survival, differentiation and migration of mesenchymal cells, including the vascular smooth muscle cells affected by calcifications in PFBC. While increased signaling is associated with cancers, overgrowth and progeria syndromes, 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.

MATERIAL AND METHODS

Patients. We included patients with brain calcification that 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 (CNV).** Quantitative multiplex PCR of short fluorescent fragments (QMPSF) was used to assess the presence of 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 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.\textsuperscript{27}

**Variant assessment.** Variant classification was conducted following ACMG-AMP
recommendations.\textsuperscript{24} Briefly, these criteria included: 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,\textsuperscript{25}
http://gnomad.broadinstitute.org/), computational and predictive data (Polyphen 2, 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 5 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
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 the 4 series**

By screening the four known PFBC causative genes in 177 unrelated probands from 4
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 two out of the 177

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unrelated probands were previously reported. 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, including 6 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 the one case already reported in the literature. The remaining 18 SLC20A2 variants were novel, of which 9 were protein-truncating variants (PTV) and were therefore classified as pathogenic.

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, supporting evidence for pathogenicity.

Among the additional 7 novel VUS identified, 2 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 (MaxEntScan 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 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 6 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 2 novel missense variants, both absent from gnomAD and predicted damaging by \textit{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.

\textbf{PDGFRB variants}

Three distinct \textit{PDGFRB} variants were found in 3 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 2 individuals in gnomAD (MAF=8.1e-06). Segregation data was only available for the family carrying the p.(Asp884Gly) 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.

\textbf{XPR1 variants}

Five distinct \textit{XPR1} variants were found in 6 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 \textit{XPR1} paper.\textsuperscript{22} The other variant, p.(Leu87Pro), was found in a case already reported.\textsuperscript{23} These two variants were not found in gnomAD and can be classified as pathogenic based on published functional evidence.\textsuperscript{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 evidences for p.(Asn619Asp), hence classified here as VUS.

**Clinical presentation**

Herein, we reported a total of 56 PFBC patients (32 F; 24 M), including the 45 probands that were found to carry VUS or (likely) pathogenic variants, and 11 relatives that had brain calcifications and the same variant as the proband (**Figure 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 (**Figure 1B**). Mean age at clinical onset was 47.2 years (**Figure 1C**) (median = 52y, range: 3-81y, age at onset was unknown for 8 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 24/44 (54.5%) of symptomatic patients, mostly with an akinetic-rigid presentation (**Figure 1D**). Cognitive impairment was documented in 19/44 (43.2%) symptomatic cases, as were psychiatric disturbances (depression, psychosis, anxiety), while 13/44 (29.5%) patients had cerebellar signs. In addition, migraine was reported by 10/54 patients (18.5%); in 5 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 find new evidence to reclassify the VUS we found as causal. As expected, \textit{SLC20A2} showed the highest contribution with variants identified in 16.9% (30/177) of the probands, followed by \textit{XPR1} and \textit{PDGFB}, each with 3.4% (6/177), and then \textit{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,\textsuperscript{33} in contrast to previous reports that showed high mutation rates in patients with a positive family history\textsuperscript{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.

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

\textit{XPR1} was the most recent PFBC gene to be identified,\textsuperscript{22} and in our series, variants within these gene are as frequent as \textit{PDGFB} variants. Pathogenicity of \textit{XPR1} variants reported to date has been ascertained based on: strong segregation\textsuperscript{22}, recurrence among unrelated patients and/or functional data showing a defect in inorganic phosphate transport\textsuperscript{22,23}. Interestingly, all known pathogenic variants are located in the SPX domain of XPR1, the function of which
remains uncertain. We identified 3 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-PDGFR\( \beta \) 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 4 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 4 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 another French PFBC series and a meta-analysis
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study, the proportion of symptomatic patients was indeed lower, 58% and 64%, respectively.\textsuperscript{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 \textsuperscript{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 (54.5% of symptomatic individuals), cognitive impairment, and psychiatric signs (43.2% each). 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,\textsuperscript{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,\textsuperscript{1,39} while there are also reports that showed lack of segregation between brain calcification and migraine.\textsuperscript{41}

In summary, by screening the known PFBC genes in 4 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. Lastly, 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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Table Legends:

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

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. 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/SLC20A2 database. Reference sequences: NG_032161.1 and NM_006749.4.

Associated references: 4, 3, 6, 7, 28,29, 30, 31, 32

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

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. 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. Variants were submitted to the https://coppolalab.ucla.edu/lovd_pfbc/genes/PDGFB database. Reference sequences: NG_012111.1 and NM_002608.2.
Table 3. Details on PDGFRB variants and phenotype of variant carriers.

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. 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.

* The PRRT2 and PNKD genes were sequenced in this patient and no change was detected. Variants were submitted to the https://coppolalab.ucla.edu/lovd_pfbc/genes/PDGFRB database. Reference sequence: NM_002609.3.

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

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. 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

Figure Legends:

**Figure 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 variant carrier. C. Distribution of age-at-onset (years) per gene carrier (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.
REFERENCES


Figure 1
<table>
<thead>
<tr>
<th>Family number</th>
<th>Case ID</th>
<th>Study</th>
<th>Novel variant or ref.</th>
<th>ACMG class</th>
<th>Variant type</th>
<th>cDNA</th>
<th>Protein</th>
<th>Domain (missense) or predicted protein consequences</th>
<th>gnomAD</th>
<th>Mutation Taster</th>
<th>Polyphen2</th>
<th>SIFT</th>
<th>Ethnicity</th>
<th>Sex</th>
<th>Clinical summary</th>
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<th>Family History</th>
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<td>p.?</td>
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<td>PD (1)</td>
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<td>Progressive involuntary movements, neuropathic pain, chronic headache</td>
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<td>Absent</td>
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<td>B (0.155)</td>
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<td>Comorbid Conditions</td>
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**Phenotype Details:**
- **Premature stop codon** indicates an early stop codon in the gene sequence.
- **Evidence of Nonsense mediated decay** suggests that the premature stop codon is a result of nonsense-mediated decay, a process that may occur when a codon is recognized as a stop codon, even if it is not a normal stop codon.
- **Akinetic-rigid syndrome** refers to a condition characterized by muscle stiffness and difficulty in initiating voluntary movements.
- **Bipolar disorder** is a type of mood disorder characterized by episodes of mania and depression.
- **Postural/kinetic tremor** refers to tremors that occur during movement (kinetic) or when at rest (postural).
- **Subjective memory impairment** indicates that a person reports a decline in their memory with no objective evidence of cognitive impairment.
- **Migraine** is a type of headache characterized by pulsating pain, often associated with nausea and sensitivity to light and sound.
- **Down syndrome** is a genetic disorder caused by an extra copy of chromosome 21, characterized by intellectual disability and specific physical features.

**Diagnosis:**
- **Asymptomatic (migraine)** indicates that the individual does not report any symptoms.
- **Positive** or **Negative** refers to the result of genetic testing or clinical evaluation.
- **Pa, Pu, D, Co** indicates presence or absence of specific symptoms or conditions.
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<th>Gender</th>
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<th>Pathogenicity</th>
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<td>c.1524-2A&gt;G p.?.</td>
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<td>Dysarthria, gait disorder, akinetic-rigid syndrome, memory impairment and dysexecutive signs</td>
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<td>Missense / Splicing</td>
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<td>F</td>
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<td>M</td>
<td>Mild to moderate intellectual disability, bipolar disorder, mild akinetic-rigid syndrome signs, ataxia, mild postural and intention tremor</td>
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</tbody>
</table>
|   | 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
|---|-----------|--------|--------|---|-----------|-----------|-------------|----------------------|-------|------------|------------|-----------|-----------|---|-----------|----|----------|---
| 28 | Proband   | USA    | Novel  | 4 | Inframe deletion (27bp) | c.1822_1848del | p.Ile608_Trp 616del | Phosphate transporter |Absent | NA         | NA         | NA        | Caucasian | M | ADHD     | NA | Positive | Pa, Pa, Ca, T, Co
| 29 | Father     | USA    | 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, Pa, D, WM
| 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, Pa, D, WM

Table 1. Details on SLC20A2 variants and phenotype of variant carriers. 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. 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/SLC20A2](https://coppolalab.ucla.edu/lovd_pfbc/genes/SLC20A2) database. Reference sequences: NG_032161.1 and NM_006749.4.
<table>
<thead>
<tr>
<th>Family number</th>
<th>Case ID</th>
<th>Study</th>
<th>Novel variant or ref.</th>
<th>ACMG class</th>
<th>Variant type</th>
<th>cDNA</th>
<th>Protein</th>
<th>Domain (missense) or predicted protein consequences</th>
<th>gnomAD</th>
<th>Mutation Taster</th>
<th>Polyphen 2</th>
<th>SIFT</th>
<th>Ethnicity</th>
<th>Sex</th>
<th>Clinical summary</th>
<th>AA O</th>
<th>Family History</th>
<th>CT scan</th>
</tr>
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<tbody>
<tr>
<td>31</td>
<td>EXT 929 001</td>
<td>France</td>
<td>Novel</td>
<td>4</td>
<td>Missense</td>
<td>c.394G&gt;C p.(Gly132Arg)</td>
<td>PDGF domain</td>
<td>Absent</td>
<td>DC (1)</td>
<td>PD (1)</td>
<td>D (0)</td>
<td>Caucasian</td>
<td>F</td>
<td>Asymptomatic</td>
<td>NA</td>
<td>Negatивe</td>
<td>Pa, Pu, D</td>
<td></td>
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<tr>
<td>32</td>
<td>ROU 1184 001</td>
<td>France</td>
<td>Novel</td>
<td>3</td>
<td>Missense</td>
<td>c.425G&gt;A p.(Arg142His)</td>
<td>PDGF domain</td>
<td>Absent</td>
<td>DC (0.97)</td>
<td>PD (1)</td>
<td>T (0.33)</td>
<td>Caucasian</td>
<td>F</td>
<td>Personality disorder, depressive episodes with memory impairment. Progressive cognitive decline, Akinetic-rigid syndrome, pyramidal signs, gait disorder, frontal behavioral disorder</td>
<td>68</td>
<td>Negatивe</td>
<td>Pa, Pu, D</td>
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<tr>
<td>33</td>
<td>EXT 1196 001</td>
<td>France</td>
<td>9</td>
<td>5</td>
<td>Nonsense</td>
<td>c.445C&gt;T p.(Arg149Ter)</td>
<td>Premature stop codon</td>
<td>Absent</td>
<td>DC (1)</td>
<td>NA</td>
<td>NA</td>
<td>Caucasian</td>
<td>M</td>
<td>Dysexecutive syndrome with memory impairment, anxiety, depression, akinetic-rigid syndrome, tremor</td>
<td>44</td>
<td>Positive</td>
<td>Pa, Pu, Ca, D, T (MR)</td>
<td></td>
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<tr>
<td>34</td>
<td>USA Novel 5</td>
<td>USA</td>
<td>Novel</td>
<td>5</td>
<td>Splicing</td>
<td>c.456+1G&gt;A p.?</td>
<td>Predicted skipping of exon 4 introducing a frameshift</td>
<td>Absent</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Caucasian</td>
<td>F</td>
<td>Severe migraine, history of depression</td>
<td>20</td>
<td>Positive</td>
<td>Pa, Pu, Ca, WM, D, Co</td>
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<tr>
<td>35</td>
<td>EXT 1251 001</td>
<td>France</td>
<td>Novel</td>
<td>5</td>
<td>Stop loss</td>
<td>c.724T&gt;C p.(Ter242Gln ExtTer89)</td>
<td>Extended protein, loss of function</td>
<td>Absent</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Caucasian</td>
<td>F</td>
<td>Seizures, migraine, depression, cognitive impairment (memory, executive dysfunction)</td>
<td>25</td>
<td>Negatивe</td>
<td>Pa, Pu, Ca, D (MRI)</td>
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<tr>
<td>36</td>
<td>ROU 5019 001</td>
<td>France</td>
<td>9</td>
<td>5</td>
<td>Stop loss</td>
<td>c.726G&gt;C p.(Ter242Tyr ExtTer89)</td>
<td>Extended protein, loss of function</td>
<td>Absent</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Caucasian</td>
<td>M</td>
<td>Orofacial dyskinesia, oral tics, pyramidal signs, alcohol abuse, comorbid aneurysm of the right medial cerebral artery</td>
<td>NA</td>
<td>Positive</td>
<td>Pa, Pu, Ca, D, T, Co, WM</td>
</tr>
</tbody>
</table>

Table 2. Details on PDGFB variants and phenotype of variant carriers. 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. 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 following database: https://coppolalab.ucla.edu/lovd_pfbc/genes/PDGFB. Reference sequences: NG_012111.1 and NM_002608.2.
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<thead>
<tr>
<th>Family number</th>
<th>Case ID</th>
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<th>Novel variant or ref.</th>
<th>ACMG class</th>
<th>Variant type</th>
<th>cDNA</th>
<th>Protein Domain (missense) or predicted protein consequence</th>
<th>gnomAD</th>
<th>Mutation Taster</th>
<th>Polyphen2</th>
<th>SIFT</th>
<th>Ethnicity</th>
<th>Sex</th>
<th>Clinical summary</th>
<th>AAO</th>
<th>Family History</th>
<th>CT scan</th>
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</thead>
<tbody>
<tr>
<td>37</td>
<td>IT-PFBC-9*</td>
<td>Italy</td>
<td>Novel</td>
<td>3</td>
<td>Missense</td>
<td>c.676C&gt;T</td>
<td>p.(Arg226Cys)</td>
<td>Extracellular, Ig-like C2-type 3</td>
<td>Absent</td>
<td>DC (0.99)</td>
<td>PD (1)</td>
<td>D (0.01)</td>
<td>Caucasian</td>
<td>M</td>
<td>Paroxysmal kinesigenic dyskinesia*, CBZ responsive</td>
<td>11</td>
<td>Negative</td>
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<tr>
<td>38</td>
<td>IT-PFBC-10</td>
<td>Italy</td>
<td>Novel</td>
<td>3</td>
<td>Missense</td>
<td>c.1787C&gt;T</td>
<td>p.(Pro596Leu)</td>
<td>Outside of Protein Kinase domain, Cytoplasmic</td>
<td>DC</td>
<td>PD (1)</td>
<td>D (0)</td>
<td>CA</td>
<td>Caucasian</td>
<td>F</td>
<td>Asymptomatic (migraine)</td>
<td>NA</td>
<td>Negative</td>
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<tr>
<td>39</td>
<td>Proband</td>
<td>USA</td>
<td>Novel</td>
<td>5</td>
<td>Missense</td>
<td>c.2531A&gt;G</td>
<td>p.(Asp844Gly)</td>
<td>Cytoplasmic, protein kinase</td>
<td>Absent</td>
<td>DC (0.99)</td>
<td>PD (0.998)</td>
<td>D (0.01)</td>
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<td>F</td>
<td>Sleepwalking</td>
<td>Positive</td>
<td>Pa, Pu, WM, D</td>
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<td>39</td>
<td>Paternal aunt</td>
<td>USA</td>
<td>Novel</td>
<td>5</td>
<td>Missense</td>
<td>c.2531A&gt;G</td>
<td>p.(Asp844Gly)</td>
<td>Cytoplasmic, protein kinase</td>
<td>Absent</td>
<td>DC (0.99)</td>
<td>PD (0.998)</td>
<td>D (0.01)</td>
<td>Caucasian</td>
<td>F</td>
<td>Sleepwalking</td>
<td>Positive</td>
<td>Pa, Pu, WM, D</td>
</tr>
</tbody>
</table>

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

* The PRRT2 and PNKD genes were sequenced in this patient and no change was detected.

Variants were submitted to the following database: https://coppolalab.ucla.edu/lovd_pfbc/genes/PDGFRB database. Reference sequence: NM_002609.3.
<table>
<thead>
<tr>
<th>Family number</th>
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<th>Variant type</th>
<th>cDNA</th>
<th>Protein</th>
<th>Domain (missense) or predicted protein consequences</th>
<th>gnomAD</th>
<th>Mutatio n Taster</th>
<th>Polyphene n2</th>
<th>SIFT</th>
<th>Ethnicity</th>
<th>Sex</th>
<th>Clinical summary</th>
<th>AAO</th>
<th>Family History</th>
<th>CT scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>EXT 1003 001</td>
<td>France</td>
<td>23 [same patient]</td>
<td>5</td>
<td>Missense</td>
<td>c.260T&gt;C</td>
<td>p.(Leu87Pro )</td>
<td>SPX domain Absent</td>
<td>DC (1)</td>
<td>PD (1)</td>
<td>D (0)</td>
<td>Caucasian</td>
<td>M</td>
<td>Dysarthria with parkinsonian and cerebellar features, concentration deficit, mild executive dysfunction, micrography, parkinsonism, anxiety</td>
<td>37</td>
<td>Positive</td>
<td>Pa, Pu, Ca, T, D, Ve, WM, Co</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>EXT 1187 001</td>
<td>France</td>
<td>22</td>
<td>5</td>
<td>Missense</td>
<td>c.434T&gt;C</td>
<td>p.(Leu145Pro )</td>
<td>SPX domain Absent</td>
<td>DC (1)</td>
<td>PD (1)</td>
<td>D (0.01)</td>
<td>Caucasian</td>
<td>M</td>
<td>Extrapyramidal syndrome, cognitive impairment, dysarthria, behavioral disturbances</td>
<td>29</td>
<td>Positive</td>
<td>Pa, Pu, Ca, T, D, Co (MRI)</td>
<td></td>
</tr>
<tr>
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<td>EXT 1187 002</td>
<td>France</td>
<td>22</td>
<td>5</td>
<td>Missense</td>
<td>c.434T&gt;C</td>
<td>p.(Leu145Pro )</td>
<td>SPX domain Absent</td>
<td>DC (1)</td>
<td>PD (1)</td>
<td>D (0.01)</td>
<td>Caucasian</td>
<td>M</td>
<td>Bradykinesia, psychomotor slowing</td>
<td>38</td>
<td></td>
<td>Pa, Pu, Ca, T, D, Co</td>
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<tr>
<td>42</td>
<td>IT-PFBC-11</td>
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<td>Novel</td>
<td>4</td>
<td>Missense</td>
<td>c.697A&gt;T</td>
<td>p.(Thr233Se )</td>
<td>outside from SPX domain</td>
<td>8.133e-6 (1.795e-5, NFE)</td>
<td>DC (0.99)</td>
<td>PossD (0.885)</td>
<td>D (0.03)</td>
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<td>F</td>
<td>Mild Cognitive Impairment</td>
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<td>Negative</td>
<td>Pa, Pa</td>
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<td>43</td>
<td>IT-PFBC-12</td>
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<td>Novel</td>
<td>4</td>
<td>Missense</td>
<td>c.697A&gt;T</td>
<td>p.(Thr233Se )</td>
<td>outside from SPX domain</td>
<td>8.133e-6 (1.795e-5, NFE)</td>
<td>DC (0.99)</td>
<td>PossD (0.885)</td>
<td>D (0.03)</td>
<td>Caucasian</td>
<td>F</td>
<td>Vertigo</td>
<td>50</td>
<td>Negative</td>
<td>Pa, Pa</td>
</tr>
<tr>
<td>44</td>
<td>ROU 5059 001</td>
<td>France</td>
<td>Novel</td>
<td>4</td>
<td>Missense</td>
<td>c.1375C&gt;T</td>
<td>p.(Arg459Cy s)</td>
<td>outside from SPX domain</td>
<td>Absent</td>
<td>DC (1)</td>
<td>PD (1)</td>
<td>Caucasian</td>
<td>M</td>
<td>L-Dopa-responsive extrapyramidal syndrome, mild intellectual disability</td>
<td>55</td>
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<td>Pa, Pu, Ca, D</td>
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<td>45</td>
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<td>3</td>
<td>Missense</td>
<td>c.1855A&gt;G</td>
<td>p.(Asn619Asp)</td>
<td>outside from SPX domain</td>
<td>Absent</td>
<td>DC (1)</td>
<td>PD (1)</td>
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<td>M</td>
<td>Sudden deafness, mild cerebellar syndrome</td>
<td>69</td>
<td>Positive</td>
<td>Pa, Pu, Ca, D, T</td>
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</table>

Table 4. Details on XPR1 variants and phenotype of variant carriers. 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. 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 following database: https://coppolalab.ucla.edu/lovd_pfbc/genes/XPR1 database. Reference sequence: NM_004736.3.