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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–PDGFR-B 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–PDGFR-B signaling [8, 19, 20]. Although PDGFB–PDGFR-B 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 lentilcular nuclei, beyond the age-specific severity threshold [7], a normal phosho-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>
<thead>
<tr>
<th>Family number</th>
<th>Case ID</th>
<th>Study</th>
<th>Study 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 Taster</th>
<th>Polyphen2 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>1</td>
<td>EXT 1291 001</td>
<td>France</td>
<td>Novel</td>
<td>5</td>
<td>Nonsense</td>
<td>c.149T&gt;G</td>
<td>p.(Leu50Ter)</td>
<td>Premature stop codon</td>
<td>Absent</td>
<td>NA</td>
<td>NA</td>
<td>Caucasian</td>
<td>F</td>
<td>Psychosis and extrapyramidal syndrome</td>
<td>63</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>IT-PFBC- 7</td>
<td>Italy</td>
<td>32</td>
<td>4</td>
<td>Missense</td>
<td>c.212G&gt;A</td>
<td>p.(Arg71His)</td>
<td>Phosphate transporter</td>
<td>Absent</td>
<td>DC (0.9)</td>
<td>PD (1)</td>
<td>D (0)</td>
<td>Caucasian</td>
<td>F</td>
<td>Asymptomatic</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>EXT 878 001</td>
<td>France</td>
<td>Novel</td>
<td>3</td>
<td>Predicted splicing</td>
<td>c.289+5G&gt;A</td>
<td>p.?</td>
<td>Predicted loss of 5' splicing donor site</td>
<td>Absent</td>
<td>NA</td>
<td>NA</td>
<td>Caucasian</td>
<td>F</td>
<td>Pain, akinetic-rigid syndrome with tremor, gait disorder, and hypophonia</td>
<td>72</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>IT-PFBC- 1</td>
<td>Italy</td>
<td>Novel</td>
<td>3</td>
<td>Predicted splicing</td>
<td>c.290-8A&gt;G</td>
<td>p.?</td>
<td>Predicted loss of 3' splicing acceptor site</td>
<td>Absent</td>
<td>DC (1)</td>
<td>PD (1)</td>
<td>D (0.02)</td>
<td>Polynesian</td>
<td>M</td>
<td>Akinetic-rigid Parkinsonism, LD responsive</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>EXT 1132 001</td>
<td>France</td>
<td>Novel</td>
<td>3</td>
<td>Missense/ splicing</td>
<td>c.290G&gt;A</td>
<td>p.(Gly97Asp)</td>
<td>First base of exon 3; however, splicing tools predict a minor effect on splicing (MaxEntScan score change: -7%)</td>
<td>Absent</td>
<td>DC (1)</td>
<td>PD (1)</td>
<td>D (0.02)</td>
<td>Polynesian</td>
<td>M</td>
<td>Anxiety, depression, apathy, somatoform signs, and attention deficit</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>Proband</td>
<td>USA</td>
<td>28,29</td>
<td>5</td>
<td>Nonsense</td>
<td>c.338C&gt;G</td>
<td>p.(Ser113Ter)</td>
<td>Premature stop codon</td>
<td>Absent</td>
<td>DC (1)</td>
<td>NA</td>
<td>NA</td>
<td>F</td>
<td>NA</td>
<td>NA</td>
<td>Positive</td>
</tr>
<tr>
<td>7</td>
<td>IT-PFBC- 6</td>
<td>Italy</td>
<td>28 [same patient]</td>
<td>5</td>
<td>Nonsense</td>
<td>c.338C&gt;G</td>
<td>p.(Ser113Ter)</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>Focal unilateral chorea (hand)</td>
<td>53</td>
</tr>
<tr>
<td>8</td>
<td>EXT 945 001</td>
<td>France</td>
<td>Novel</td>
<td>5</td>
<td>Frameshift</td>
<td>c.382del</td>
<td>p.(Val128SerfsTer43)</td>
<td>Premature stop codon</td>
<td>Absent</td>
<td>NA</td>
<td>NA</td>
<td>Caucasian</td>
<td>M</td>
<td>Cerebellar ataxia, dysarthria, memory impairment with dysexecutive signs, and depression</td>
<td>76</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>Proband</td>
<td>USA</td>
<td>Novel</td>
<td>4</td>
<td>Missense</td>
<td>c.541C&gt;T</td>
<td>p.(Arg181Trp)</td>
<td>Phosphate transporter domain</td>
<td>4.08e-6 (4.51e-5, NFE)</td>
<td>DC (0.99)</td>
<td>PD (0.995)</td>
<td>T (0.06)</td>
<td>Caucasian</td>
<td>M</td>
<td>Progressive involuntary movements, neuropathic pain, and chronic headache</td>
<td>60</td>
</tr>
<tr>
<td>10</td>
<td>ROU 375 004 (mother)</td>
<td>France</td>
<td>6</td>
<td>5</td>
<td>Missense</td>
<td>c.551C&gt;T</td>
<td>p.(Pro184Leu)</td>
<td>Cyttoplasmic</td>
<td>Absent</td>
<td>DC (1)</td>
<td>PD (0.98)</td>
<td>D (0.05)</td>
<td>Caucasian</td>
<td>F</td>
<td>Restless leg syndrome</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>ROU 375 003 (sister)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ROU 375 002 (sister)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Table 1 Details on SLC20A2 variants and phenotype of variant carriers
<table>
<thead>
<tr>
<th>Family number</th>
<th>Case ID</th>
<th>Study</th>
<th>Novel variant or ref.</th>
<th>ACMG class type</th>
<th>cDNA</th>
<th>Protein</th>
<th>Domain (missense) or predicted protein consequences</th>
<th>gnomAD 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>
</tr>
</thead>
<tbody>
<tr>
<td>ROU 375 001</td>
<td>EXT 1146 001</td>
<td>France 7</td>
<td>Missense</td>
<td>c.581A&gt;G</td>
<td>p.(Asn194Ser)</td>
<td>Transmembrane</td>
<td>1.446e−5</td>
<td>DC (0.99)</td>
<td>B (0.155)</td>
<td>T</td>
<td>NA</td>
<td>M</td>
<td>Akinetico-rigid syndrome, tremor, cerebellar ataxia, dysexecutive signs, and memory impairment</td>
<td>68</td>
<td>Positive</td>
<td>Pa, Pu, Ca, T, D, Co, WM, Ver</td>
</tr>
<tr>
<td>12</td>
<td>EXT 1180 001</td>
<td>France</td>
<td>Novel</td>
<td>c.730+1G&gt;T</td>
<td>p.?</td>
<td>Predicted skipping of exon 6 (in-frame) or use of alternative splice site</td>
<td>Absent</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Caucasian</td>
<td>F</td>
<td>Bradykinesia, tremor, and dysexecutive signs</td>
<td>40</td>
<td>Negative</td>
<td>Pa, Pu, T, D, Co</td>
</tr>
<tr>
<td>13</td>
<td>IT-PFBC-  5b (first cousin)</td>
<td>Italy</td>
<td>Novel</td>
<td>c.739C&gt;T</td>
<td>p.(Gln247Ter)</td>
<td>Premature stop codon</td>
<td>Absent</td>
<td>DC (1)</td>
<td>NA</td>
<td>NA</td>
<td>Caucasian</td>
<td>F</td>
<td>Asymptomatic</td>
<td>NA</td>
<td>Positive</td>
<td>Pa, Pu, Ca, T, D, Co, WM</td>
</tr>
<tr>
<td>14</td>
<td>ROU 5028 001</td>
<td>France 30</td>
<td>Novel</td>
<td>c.1158C&gt;A</td>
<td>p.(Tyr386Ter)</td>
<td>Premature stop codon with evidence of nonsense-mediated decay</td>
<td>Absent</td>
<td>DC (1)</td>
<td>NA</td>
<td>NA</td>
<td>Caucasian</td>
<td>F</td>
<td>Asymptomatic</td>
<td>NA</td>
<td>Positive</td>
<td>Pa, Pu, T, D, Co</td>
</tr>
<tr>
<td>15</td>
<td>EXT 1083 001</td>
<td>France 30</td>
<td>Novel</td>
<td>c.1207C&gt;T</td>
<td>p.(Arg403Ter)</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>Akinetico-rigid syndrome, oro-facial dyskenesia and dystonia (induced by L-dopa), pyramidal signs, gait disorder, and hallucinations (induced by L-dopa)</td>
<td>51</td>
<td>Negative</td>
<td>Pa, Pu, Ca, D, T, Co, WM, Ver</td>
</tr>
<tr>
<td>16</td>
<td>IB02BR</td>
<td>Brazil</td>
<td>Novel</td>
<td>c.1187dup</td>
<td>p.(Pro397AlafsTer18)</td>
<td>Premature stop codon</td>
<td>Absent</td>
<td>DC (1)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>F</td>
<td>Parkinsonism</td>
<td>NA</td>
<td>Positive</td>
<td>NA</td>
</tr>
<tr>
<td>17</td>
<td>EXT 1063 001</td>
<td>France 30</td>
<td>Novel</td>
<td>c.1301C&gt;G</td>
<td>p.(Ser434Ter)</td>
<td>Premature stop codon with evidence of nonsense-mediated decay</td>
<td>Absent</td>
<td>DC (1)</td>
<td>NA</td>
<td>NA</td>
<td>Caucasian</td>
<td>M</td>
<td>Akinetico-rigid syndrome</td>
<td>65</td>
<td>Negative</td>
<td>Pa, Pu, Ca, D, T, Co, WM, Ver</td>
</tr>
<tr>
<td>18</td>
<td>IT-PFBC- 2</td>
<td>Italy 31</td>
<td>Missense</td>
<td>c.1426G&gt;T</td>
<td>p.(Ser476Ter)</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>Akinetico-rigid syndrome, bipolar disorder, Mild cerebellar ataxia</td>
<td>44</td>
<td>Positive</td>
<td>Pa, Pu, Ca, D, T, Co, WM, Ver</td>
</tr>
<tr>
<td>Family number</td>
<td>Case ID</td>
<td>Study</td>
<td>Novel variant or ref.</td>
<td>ACMG class Variant type</td>
<td>cDNA</td>
<td>Protein</td>
<td>Domain (missense) or predicted protein consequences</td>
<td>gnomAD Taster</td>
<td>Mutation Taster</td>
<td>Polyphen2 SIFT</td>
<td>SIFT</td>
<td>Ethnicity</td>
<td>Sex</td>
<td>Clinical summary</td>
<td>AAO</td>
<td>Family history</td>
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<td>20</td>
<td>IT-PFBC-3</td>
<td>Italy</td>
<td>Novel</td>
<td>3</td>
<td>Missense</td>
<td>c.1463A&gt;G</td>
<td>p.(His488Arg)</td>
<td>Phosphate transporter</td>
<td>Absent</td>
<td>DC (0.99)</td>
<td>B (0.005)</td>
<td>T (0.83)</td>
<td>Caucasian</td>
<td>F</td>
<td>Subjective memory impairment, normal psychometry</td>
<td>59</td>
</tr>
<tr>
<td>21</td>
<td>Proband</td>
<td>USA</td>
<td>3</td>
<td>5</td>
<td>Missense</td>
<td>c.1492G&gt;A</td>
<td>p.(Gly498Arg)</td>
<td>Phosphate transporter</td>
<td>Absent</td>
<td>DC (0.99)</td>
<td>PD (0.994)</td>
<td>D (0.00)</td>
<td>Caucasian</td>
<td>M</td>
<td>L-dopa-responsive parkinsonism, increased muscle tone and pain</td>
<td>NA</td>
</tr>
<tr>
<td>22</td>
<td>IT-PFBC-8a (proband)</td>
<td>Italy</td>
<td>3</td>
<td>5</td>
<td>Missense</td>
<td>c.1492G&gt;A</td>
<td>p.(Gly498Arg)</td>
<td>Phosphate transporter</td>
<td>Absent</td>
<td>DC (0.99)</td>
<td>PD (0.994)</td>
<td>D (0.00)</td>
<td>Caucasian</td>
<td>M</td>
<td>Akinetic–rigid parkinsonism, dystarthritis</td>
<td>68</td>
</tr>
<tr>
<td>23</td>
<td>EXT 1136 001</td>
<td>France</td>
<td>Novel</td>
<td>5</td>
<td>Splicing</td>
<td>c.1524-2A&gt;G</td>
<td>p.?</td>
<td>Predicted skipping of exon 9 (in-frame) or use of alternative splice site</td>
<td>Absent</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Caucasian</td>
<td>F</td>
<td>Dysarthria, gait disorder, akinetic–rigid syndrome, memory impairment, and dysexecutive signs</td>
<td>71</td>
</tr>
<tr>
<td>24</td>
<td>EXT 1318 001</td>
<td>France</td>
<td>Novel</td>
<td>3</td>
<td>Missense \ splicing</td>
<td>c.1523G&gt;A</td>
<td>p.(Ser508Asn)</td>
<td>Last base of exon 8; splicing tools predict a major effect on splicing (MaxEntScan score change:-59.5%)</td>
<td>Absent</td>
<td>DC (1)</td>
<td>PB (0.999)</td>
<td>D (0.01)</td>
<td>Caribbean</td>
<td>F</td>
<td>Right upper-limb dystonia, intention tremor, bradykinesia, mood disorder, and migraine</td>
<td>33</td>
</tr>
<tr>
<td>25</td>
<td>Proband</td>
<td>USA</td>
<td>Novel</td>
<td>5</td>
<td>Frameshift</td>
<td>c.1637_1638delCA</td>
<td>p.(Thr546ArgfsTer52)</td>
<td>Premature stop codon</td>
<td>Absent</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Caucasian</td>
<td>F</td>
<td>Migraine, vestibular signs</td>
<td>NA</td>
</tr>
<tr>
<td>26</td>
<td>EXT 1235 001</td>
<td>France</td>
<td>4</td>
<td>4</td>
<td>Missense</td>
<td>c.1753G&gt;A</td>
<td>p.(Ala58Thr)</td>
<td>Phosphate transporter</td>
<td>Absent</td>
<td>DC (1)</td>
<td>PD (0.999)</td>
<td>T (0.09)</td>
<td>African</td>
<td>F</td>
<td>Dementia and parkinsonism</td>
<td>NA</td>
</tr>
<tr>
<td>27</td>
<td>EXT 1138 001</td>
<td>France</td>
<td>4</td>
<td>5</td>
<td>Frameshift</td>
<td>c.1755_1766del</td>
<td>p.(Asn587SerfsTer7)</td>
<td>Premature stop codon</td>
<td>Absent</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Caucasian</td>
<td>M</td>
<td>Mild-to-moderate intellectual disability, bipolar disorder, mild akinetic–rigid syndrome signs, ataxia, mild postural and intention tremor</td>
<td>3</td>
</tr>
<tr>
<td>28</td>
<td>IT-PFBC-4</td>
<td>Italy</td>
<td>Novel</td>
<td>3</td>
<td>Missense</td>
<td>c.1765G&gt;A</td>
<td>p.(Gly589Arg)</td>
<td>Phosphate transporter</td>
<td>Absent</td>
<td>DC (0.99)</td>
<td>PD (0.99)</td>
<td>D (0.01)</td>
<td>Caucasian</td>
<td>F</td>
<td>Dementia</td>
<td>81</td>
</tr>
<tr>
<td>29</td>
<td>Proband</td>
<td>USA</td>
<td>Novel</td>
<td>4</td>
<td>In-frame deletion (27bp)</td>
<td>c.1822_1848del</td>
<td>p.(Ile608_Trp616del)</td>
<td>Phosphate transporter</td>
<td>Absent</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Caucasian</td>
<td>M</td>
<td>Anxiety, dystonia</td>
<td>NA</td>
</tr>
</tbody>
</table>

Other than the above, there are additional cases with specific clinical summaries and ethnicities, indicating a variety of neurological and psychiatric conditions, but the details beyond the initial 29 cases are not provided in the table.
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 relatives, 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>
<thead>
<tr>
<th>Family number</th>
<th>Case ID</th>
<th>Study 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 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>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>929 001</td>
<td>France</td>
<td>Novel 4</td>
<td>Missense</td>
<td>c.394G&gt;C</td>
<td>p.(Gly132Arg)</td>
<td>PDGF domain Absent DC (1) PD (1) D (0) Caucasian F</td>
<td>Asymptomatic</td>
<td>NA</td>
<td>Negative Pa, Pu, D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>1184 001</td>
<td>France</td>
<td>Novel 3</td>
<td>Missense</td>
<td>c.425G&gt;A</td>
<td>p.(Arg142His)</td>
<td>PDGF domain Absent DC (0.97) PD (1) T (0.33) Caucasian 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>Negative Pa, Pu, D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>1196 001</td>
<td>France</td>
<td>9</td>
<td>Nonsense</td>
<td>c.445C&gt;T</td>
<td>p.(Arg149Ter)</td>
<td>Premature stop codon Absent DC (1) NA NA Caucasian M</td>
<td>Dysexecutive syndrome with memory impairment, anxiety, depression, akinetic–rigid syndrome, tremor</td>
<td>44</td>
<td>Positive Pa, Pu, Ca, D, T (MRI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>USA</td>
<td>Proband</td>
<td>Novel 5</td>
<td>Splicing</td>
<td>c.456+1G&gt;A</td>
<td>p.?</td>
<td>Predicted skipping of exon 4 introducing a frameshift Absent NA NA NA NA F</td>
<td>Severe migraine, history of depression</td>
<td>20</td>
<td>Positive Pa, Pu, Ca, WM, D, Co</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mother</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F Migraine, history of depression</td>
<td>26</td>
<td>Pa, Pu, Ca, T, D, WM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2 (continued)

<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 Mutation Taster</th>
<th>Polyphen2 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>35</td>
<td>EXT 1251 001</td>
<td>France</td>
<td>Novel 5</td>
<td>Stop loss</td>
<td>c.724T&gt;C</td>
<td>p.(Ter242GlnExtTer89)</td>
<td>Absent</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>Negative Pa, Pu, Ca, D (MRI)</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>ROU 5019 001</td>
<td>France</td>
<td>9</td>
<td>Stop loss</td>
<td>c.726G&gt;C</td>
<td>p.(Ter242TyrExtTer89)</td>
<td>Absent</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 Pa, Pu, Ca, D, T, Co, WM</td>
<td></td>
</tr>
</tbody>
</table>

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/PDGFβ database](https://coppolalab.ucla.edu/lovd_pfbc/genes/PDGFβ 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 (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 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.

**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

<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>
<th>AAO</th>
<th>Family History</th>
<th>CT scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>IT-PFBC-9a</td>
<td>Italy</td>
<td>Novel 3</td>
<td></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&lt;sup&gt;a&lt;/sup&gt;, CBZ responsive</td>
<td>11</td>
<td>Negative</td>
<td>Pa, Pu, Ca, T, D</td>
</tr>
<tr>
<td>38</td>
<td>IT-PFBC-10</td>
<td>Italy</td>
<td>Novel 3</td>
<td></td>
<td>Missense</td>
<td>c.1787C&gt;T</td>
<td>p.(Pro596Leu)</td>
<td>Outside of Protein Kinase domain, Cytoplasmic</td>
<td>DC (1)</td>
<td>PD (1)</td>
<td>D (0)</td>
<td>F</td>
<td>Caucasian</td>
<td>F</td>
<td>Asymptomatic (migraine)</td>
<td>NA</td>
<td>Negative</td>
<td>Pa, Pu</td>
</tr>
<tr>
<td>39</td>
<td>Proband</td>
<td>USA</td>
<td>Novel 5</td>
<td></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>Childhood</td>
<td>Positive</td>
<td>Pa, Pu, WM, D</td>
</tr>
</tbody>
</table>

**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**

**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](https://c 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**

<sup>a</sup>The PRRT2 and PNKD genes were sequenced in this patient and no change was detected.
<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 Taster</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>
</tr>
</thead>
<tbody>
<tr>
<td>EXT 1003</td>
<td>001</td>
<td>France</td>
<td>23</td>
<td>Missense</td>
<td>c.260T&gt;C</td>
<td>p.(Leu87Pro)</td>
<td>SPX domain</td>
<td>Absent</td>
<td>DC (1)</td>
<td>PD (1)</td>
<td>D (0)</td>
<td></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>
</tr>
<tr>
<td>EXT 1187</td>
<td>001</td>
<td>France</td>
<td>22</td>
<td>Missense</td>
<td>c.434T&gt;C</td>
<td>p.(Leu145Pro)</td>
<td>SPX domain</td>
<td>Absent</td>
<td>DC (1)</td>
<td>PD (1)</td>
<td>D (0.01)</td>
<td></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>
</tr>
<tr>
<td>EXT 1187</td>
<td>002</td>
<td>France</td>
<td></td>
<td>Missense</td>
<td>c.697A&gt;T</td>
<td>p.(Thr233Ser)</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></td>
<td>Caucasian</td>
<td>F</td>
<td>Mild Cognitive Impairment</td>
<td>81</td>
<td>Negative</td>
<td>Pa, Pu, Pa</td>
</tr>
<tr>
<td>IT-PFBC-11</td>
<td></td>
<td>Italy</td>
<td>Novel</td>
<td>Missense</td>
<td>c.697A&gt;T</td>
<td>p.(Thr233Ser)</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></td>
<td>Caucasian</td>
<td>F</td>
<td>Vertigo</td>
<td>50</td>
<td>Negative</td>
<td>Pa, Pu</td>
</tr>
<tr>
<td>IT-PFBC-12</td>
<td></td>
<td>Italy</td>
<td>Novel</td>
<td>Missense</td>
<td>c.1375C&gt;T</td>
<td>p.(Arg459Cys)</td>
<td>Outside from SPX domain</td>
<td>Absent</td>
<td>DC (1)</td>
<td>PD (1)</td>
<td>D (0)</td>
<td>Caucasian</td>
<td>M</td>
<td>t-Dopa-responsive extrapyramidal syndrome, mild intellectual disability</td>
<td>55</td>
<td>Negative</td>
<td>Pa, Pu, Ca, D</td>
<td></td>
</tr>
<tr>
<td>ROU 5059</td>
<td>001</td>
<td>France</td>
<td>Novel</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>
<td>D (0)</td>
<td>Caucasian</td>
<td>M</td>
<td>Sudden deafness, mild cerebellar syndrome</td>
<td>69</td>
<td>Positive</td>
<td>Pa, Pu, Ca, D, T</td>
<td></td>
</tr>
</tbody>
</table>

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

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 (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. Parkinsonism (alone or combined with other clinical manifestations) was the most frequent finding, present in 23/44 (52.3%) of symptomatic patients. Parkinsonism (alone or combined with other clinical manifestations) was the most frequent finding, present in 23/44 (52.3%) of symptomatic patients. Parkinsonism (alone or combined with other clinical manifestations) was the most frequent finding, present in 23/44 (52.3%) of symptomatic patients. Parkinsonism (alone or combined with other clinical manifestations) was the most frequent finding, present in 23/44 (52.3%) of symptomatic patients.
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 independent 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.

**References**


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