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## Mutations in *SLC20A2* are a major cause of familial idiopathic basal ganglia calcification

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### Abstract

Familial idiopathic basal ganglia calcification (IBGC) or Fahr's disease is a rare neurodegenerative disorder characterized by calcium deposits in the basal ganglia and other brain regions, which is associated with neuropsychiatric and motor symptoms. Familial IBGC is genetically heterogeneous and typically transmitted in an autosomal dominant fashion. We performed a mutational analysis of *SLC20A2*, the first gene found to cause IBGC, to assess its genetic contribution to familial IBGC. We recruited 218 subjects from 29 IBGC-affected families of varied ancestry and collected medical history, neurological exam, and head CT scans to characterize each patient's disease status. We screened our patient cohort for mutations in *SLC20A2*. Twelve novel (nonsense, deletions, missense, and splice site) potentially pathogenic variants, one synonymous variant, and one previously reported mutation were identified in 13 families. Variants predicted to be deleterious cosegregated with disease in five families. Three families showed nonsegregation with clinical disease of such variants, but retrospective review of clinical and neuroimaging data strongly suggested previous misclassification. Overall, mutations in *SLC20A2* account for as many as 41 % of our familial IBGC cases. Our screen in a large series expands the catalog of *SLC20A2* mutations identified to date and demonstrates that mutations in *SLC20A2* are a major cause of familial IBGC. Non-perfect segregation patterns of predicted deleterious variants highlight the challenges of phenotypic assessment in this condition with highly variable clinical presentation.

### Keywords

Basal ganglia calcification; Fahr's; Genetics; Sequencing; Mutations

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## Introduction

Familial idiopathic basal ganglia calcification (IBGC) or Fahr's disease is an enigmatic neurodegenerative disorder characterized by calcium deposits in the basal ganglia and other brain regions in the absence of metabolic abnormalities or other causes of secondary calcification, such as infectious disease. Approximately 0.5–1.0 % of CT scans in patients over age 50 exhibit incidental sporadic calcification of the basal ganglia. In contrast, familial IBGC is typically transmitted in an autosomal dominant fashion and is genetically heterogeneous. More than 30 families with Mendelian forms of IBGC have been reported in the literature. However, its true prevalence remains unknown [1]. Clinical features include a variable combination of neuropsychiatric and motor symptoms including dystonia, parkinsonism, ataxia, psychosis, dementia, chorea, and frontal–subcortical cognitive dysfunction [2, 3]. The variability in clinical presentation and penetrance, as well as the presence of phenocopies and relatively high prevalence of other causes of secondary calcifications, have been significant confounding factors in elucidating the genetic basis of familial IBGC [4–6]. Efforts to ascertain a genetic location responsible for IBGC have resulted in the identification of three genetic loci through linkage analysis: IBGC1 on chromosome 14 (14q13), IBGC2 on chromosome 2 (2q37), and IBGC3 on chromosome 8 (8p21.1–8q11.23) [7–9]. Recently, Wang et al. reported the first causative gene linked to IBGC by identifying seven IBGC families with mutations in *SLC20A2*, a gene located in the IBGC3 region that encodes for type III sodium-dependent phosphate transporter 2 (PiT2) [10].

Here, we present a mutational analysis of *SLC20A2* in 218 patients from 29 IBGC families of varied ancestry to further examine the genetic contribution of *SLC20A2* mutations in a large cohort of IBGC families. We identified 12 novel sequence variants predicted to be deleterious, one rare synonymous variant, and one previously known mutation in 13 of these families. Our findings show that mutations in *SLC20A2* are a major cause of familial IBGC and expand the catalog of *SLC20A2* mutations identified to date.

## Patients and methods

### Patient recruitment and assessment

We identified 218 individuals belonging to 29 IBGC-affected families collected through the UCLA Medical Center and from a number of collaborating institutions. Some of these families were included in previous clinical or genetic studies (Table 1). Informed consent was obtained, and the investigation was approved by the UCLA Institutional Review Board. Medical history and neurological examinations were performed in all probands and additional family members for most families. Serum calcium and parathormone levels were assayed in at least one proband from most families to exclude calcium dysregulation and other metabolic disorders that would cause brain calcifications unrelated to familial IBGC.

### Neuroimaging

Head CT scans were performed as part of the diagnostic workup or reviewed for the presence of calcifications or other brain abnormalities. Subjects with CT scans positive for

calcification were given an affected disease status, while CT-negative patients >50 years who remained asymptomatic until their death were assigned an unaffected disease status. Subjects whose CT scans were negative but were under the age of 50, or whose CT scan results were not available, were classified as unknown.

### Molecular genetics and analytical methods

Blood samples were obtained from the participants, and genomic DNA was extracted using standard methods. Using published primer pairs [10], we amplified all of the exonic and flanking intronic regions of *SLC20A2* by PCR in two CT-positive affected patients from each family. The PCR solution and touchdown PCR cycling conditions were prepared and optimized using standard procedures. The final purified amplicons were sequenced in both forward and reverse directions by Sanger sequencing on the ABI 3730 platform (Applied Biosystems) to produce chromatogram traces that were analyzed using the CodonCode software (CodonCode Corporation). When variants were identified, all available family members in each family were screened using variant-specific primer pairs following the protocol described above. Online databases of human genetic variation were used to assess the novelty of the variants identified: the National Heart, Lung, and Blood Institute (NHLBI) Exome Variant Server (<http://evs.gs.washington.edu/EVS/>, accessed July 2012), dbSNP135 as reported in the UCSC Genome Browser (<http://genome.ucsc.edu/>), and the 1000 Genomes Project (<http://www.1000genomes.org>, 20100804 release 12 May 2012). The pathogenic potential of the identified variants was predicted using Sorting Intolerant from Tolerant (SIFT; <http://sift.bii.a-star.edu.sg/>), PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), and the Human Splicing Finder software (<http://www.umd.be/HSF/HSF.html>, May 2009 release) [11–13].

### Role of the funding source

The sponsors had no role in the study design, data collection, analysis, or interpretation.

### Results

A total of 218 subjects from 29 families of various ancestries were included in the study. Major clinical features and CT findings of our IBGC family cohort are summarized in Table 1. At least one affected subject from each family exhibited movement, psychiatric, and/or cognitive symptoms typical of familial IBGC. In five families, several asymptomatic individuals were also classified as affected because of significant bilateral basal ganglia calcifications identified on CT scans. Sequence analysis of the 29 probands identified 1 previously published mutation, 1 rare synonymous variant, and 12 novel variants in *SLC20A2* in 13 families, including three nonsense variants, three deletions, three splice site variants, and three missense variants (Figs. 1 and 2, Table 2). None of the 12 novel variants had been reported in dbSNP135, the 1000 Genomes database, the NHLBI Exome Sequencing Project, or in the previous study from Wang et al. [10]. We did not identify *SLC20A2* variants or mutations in 16 of the 29 IBGC families screened. There was no clear correlation between age of symptom onset, severity of disease symptoms, or any particular clinical phenotype in IBGC families with *SLC20A2* mutations compared to IBGC families without *SLC20A2* mutations.

To further explore pathogenicity, we studied the 12 novel variants for segregation and predicted deleteriousness. Three nonsense variants were discovered: (1) c.514A>T (family F22), introducing a stop codon in exon 4 (p.Lys172\*); (2) c.760C>T (family F15), introducing a stop codon in exon 7 (p.Arg254\*); and (3) c.1652G>A (family F20) introducing a stop codon in exon 9 (p.Trp551\*). Three deletions altering the protein reading frame were identified: (1) c.508delT (family F1) leading to a premature stop codon in exon 4 (p.Leu170\*); (2) c.583 584delGT (family F5), predicted to substitute a leucine for a valine followed by a frame shift terminating after 61 aberrant amino acids (p.Val195Leufs\*61); and (3) c.1828 1831delTCCC (family F2) which substitutes an alanine for a serine followed by a frameshift and premature termination after 17 amino acids (p.Ser610Alafs\*17). Three of the identified variants were located at natural 5' donor splice sites: (1) c.1523+1G>A (family F7) was located one base pair immediately flanking the 3' end of exon 8; (2) c.1794+1G>A (family F9); and (3) c.1794+1G>C (family F29), were both located at the same position, one base pair immediately flanking the 3' end of exon 10. The substitution of a guanine for an adenine or cytosine one nucleotide adjacent to the exon changes the highly critical GU dinucleotide essential for splicing and would most likely result in skipping of the affected exon equating to a large deletion in the final protein product. For the F7 family, the splice site variant would likely result in the loss of exon 8, the largest exon in *SLC20A2*, while in the F9 and F29 families, the splice site variant would likely result in the loss of exon 10. In both cases, exon exclusion is predicted to introduce an early stop codon (p.Gly312-Valfs\*8 and p.Ser570Argfs\*30). In family F7, the splicing variant (c.1523+1G>A) was in linkage disequilibrium with a missense variant in the coding region of exon 8 (c.1145G>A) substituting an arginine for a glutamine (p.Arg382Gln) predicted as probably damaging by Polyphen-2 and tolerated by SIFT (Table 2). Two additional novel missense variants were identified: (1) c.1506C>A (family F24) substituting a glutamine for a histidine at residue 502 (p.His502Gln), predicted to be a critical region for the transport function [14], and (2) c.1703C>T (family F19) causing the change of a leucine to a proline at codon 568 (p.Pro568Leu). We also identified a previously known single base pair mutation (c.1802C>T) resulting in the amino acid substitution of a leucine for a serine at residue 601 (p.Ser601Leu) in family F23 [10]. Currently, this is the only *SLC20A2* mutation that has been reported in more than one IBGC-affected family. It is not clear at this point if this mutation arose independently in both families or if the families share a common founder ancestor.

Finally, we identified a rare synonymous sequence variant with an allele frequency of 0.0009 in the 1000 Genomes Project database (c.1101C>G, in the F18 family, p.Pro367Pro) of unknown pathogenic significance. The pathogenic potential of the nonsynonymous coding variants was analyzed using both PolyPhen-2 and SIFT, all were predicted to be damaging to the protein product in at least one out of the two prediction software packages, and several variants were predicted to be damaging by both (Table 2). In particular, the variants p.Leu170\* and p.Val195Leufs\*61 were predicted to induce nonsense-mediated decay, a surveillance mechanism that would result in a degradation of the aberrant RNA product analogous to a complete deletion of one copy of the *SLC20A2* gene [11]. Cosegregation analysis was performed in the families for which DNA was available for more than one affected subject (8 of 13). Five families (F7, F19, F9, F15, and F20)

demonstrated perfect segregation with disease status (Table 3). In contrast, two putatively affected subjects in F1, two affected subjects in F5, and one affected subject in the F2 family did not carry the *SLC20A2* variant found in all other respective affected family members suggesting that they were phenocopies. Review of CT scans for family F1 (Fig. 3) revealed a strong contrast between the subjects who tested positive for mutations, who presented with clearly abundant and symmetrical calcification typical of IBGC-affected individuals, and those mutation negative, who presented only minimal calcifications, consistent with a phenocopy. For the remaining five families, only one affected subject was available in four families (F22, F23, F24, and F29), and we were not able to ascertain a segregation pattern, whereas in the family with the synonymous change (F18), one affected and one subject of unknown status shared the mutation. Excluding the three families with non-perfect segregation, 5 out of 23 families (22 %) where a segregation analysis was possible have segregating deleterious mutations in *SLC20A2*. Overall, considering the likelihood of phenocopies in families F2 and F5 and the predicted pathogenicity of the other variants, *SLC20A2* variants and mutations may account for as many as 41 % (12 out of 29) of IBGC-affected families in our patient population.

## Discussion

The recent identification of loss-of-function *SLC20A2* mutations in familial IBGC-affected patients finally advances the understanding of the molecular etiology of IBGC by establishing the first genetic location responsible for this disease [10]. Our systematic screen of 29 IBGC families identified 1 previously reported mutation, 1 rare synonymous variant, and 12 novel *SLC20A2* variants predicted to be deleterious, with at least five showing full segregation with disease status, indicating that mutations in *SLC20A2* are a major cause of familial IBGC. Furthermore, we identified *SLC20A2* variants in IBGC families of multiple ancestries, across different countries, supporting the conclusion that *SLC20A2* mutations are linked to IBGC worldwide. Nine out of the 14 mutations we identified are predicted to introduce a stop codon, pointing to haploinsufficiency as a causal mechanism for IBGC due to mutations in *SLC20A2*. Additionally, previous studies identifying the histidine at residue 502 [14], a position found to harbor a variant in family F24 (p.His502Gln), as critical for transport functionality in PiT2 highlights the loss of phosphate transport capacity as a major factor in the molecular etiology of IBGC. Also notable is that three of the four missense variants identified in our IBGC cohort are located within the ProDom domain (PD001131) shared by all PiT transporters (Fig. 1).

Defining disease status in IBGC is complicated by several factors that have likely hampered identification of clear genetic linkage signals: (1) the broad variability of symptom manifestations, ranging from migraine and minor psychiatric symptoms to severe movement and cognitive disorders; (2) the number of additional neurologic and systemic diseases that may cause secondary brain calcifications; and (3) the common occurrence of age-related, idiopathic calcium deposits in the basal ganglia. While some IBGC family members with basal ganglia calcification are asymptomatic, others reporting neuropsychiatric or motor symptoms are CT negative for calcifications. This poses the question as to whether the onset age of basal ganglia calcifications is variable in these patients or whether their symptoms have a different etiology. The minimum age at which absence of calcifications on a CT scan

excludes the disease remains unknown, contributing to ambiguity in identifying patients that harbor a pathogenic mutation but are asymptomatic and CT negative at the time of data collection. Although CT status may not completely reflect disease status, both because it can be normal in younger family members and because nonspecific calcifications are often present in older individuals, it is currently the most reliable test for diagnosing IBGC.

We found cosegregation of mutations with disease in five out of the eight families where a cosegregation analysis was possible (Table 3), consistent with the families reported by Wang et al. [10]. We did not identify variant carriers who were not affected, suggesting 100 % sensitivity of the clinical/CT evaluation. In contrast, 2 out of 11 affected family members from the F1 family, 2 out of 10 affected from the F5 family, and 1 out of the 10 affected from the F2 family had received affected disease status based upon clinical examination and/or CT scan, but did not carry predicted–deleterious *SLC20A2* sequence variants. Possible reasons for this finding include (1) incorrect clinical evaluation or CT scan analysis and therefore suboptimal specificity of the clinical/CT-based diagnosis. Consistent with this is the observation that CT calcifications in some of these patients are minor (Fig. 3) and are compatible with an incidental finding that appears in 0.5–1.0 % of routine CT scans and that is unrelated to familial IBGC [4–6], as well as the observation that both of the F5 individuals who did not harbor the variant identified in the F5 family were asymptomatic and had been classified as affected based solely on CT scan; (2) noncausality of the identified sequence variants, which is unlikely given the predicted deleteriousness of the sequence variants (all microdeletions leading to frameshift) and the cosegregation with disease in the vast majority of other family members; and (3) possible technical factors, including false-negative mutation detection (nonamplification or degradation of the mutated allele), which is unlikely since the mutation is detected in other family members, or sample identification errors.

Importantly, the discovery of a novel and predicted deleterious *SLC20A2* variant in the F1 family, which we previously reported to have significant linkage to disease at the 14q13 locus (designated IBGC1), suggests that the genetic mutation responsible for IBGC in this family was not on chromosome 14 but rather on chromosome 8 within *SLC20A2*. Notably, both individuals with discordant disease and genetic status contributing to the non-perfect segregation pattern observed in this family were also included in the initial cohort of 11 patients enrolled in the linkage mapping previously performed in this family. It is likely that the discordant disease status of these two individuals is due to clinical ascertainment and/or phenocopy, highlighting once again the importance of accurate phenotypic assessment when performing linkage studies, as linkage analysis relies heavily upon correct identification of affected individuals. The exclusion of IBGC1 from linkage analysis studies in larger cohorts of distinct IBGC families has also demonstrated that IBGC1 is not a major genetic locus for this disease [15]. The discovery of deleterious mutations in *SLC20A2* as a cause of familial IBGC greatly advances our understanding of this complex disease and will be crucial in the development of future treatments for IBGC patients as well as other conditions associated with brain calcification. Our assessment of the genetic contribution of *SLC20A2* mutations in our cohort of 218 familial IBGC patients demonstrates that as many as 41 % (or 12 out of 29) of the families studied have predicted deleterious sequence variants or mutations in *SLC20A2*. This finding strongly suggests causality and establishes *SLC20A2* as a key gene

for familial IBGC. Furthermore, the identification of 12 novel variants—all predicted to be highly disruptive to protein function—broadens the spectrum of known *SLC20A2* mutations and adds to the genetic knowledge of this relatively unknown disease-causing gene. More work is still needed to explain the variability in penetrance and expressivity within families. Identifying additional causal genes for IBGC will provide valuable insight for understanding the molecular etiology responsible for the clinical heterogeneity observed in patients with this disease and will ultimately contribute to the identification of therapeutic targets.

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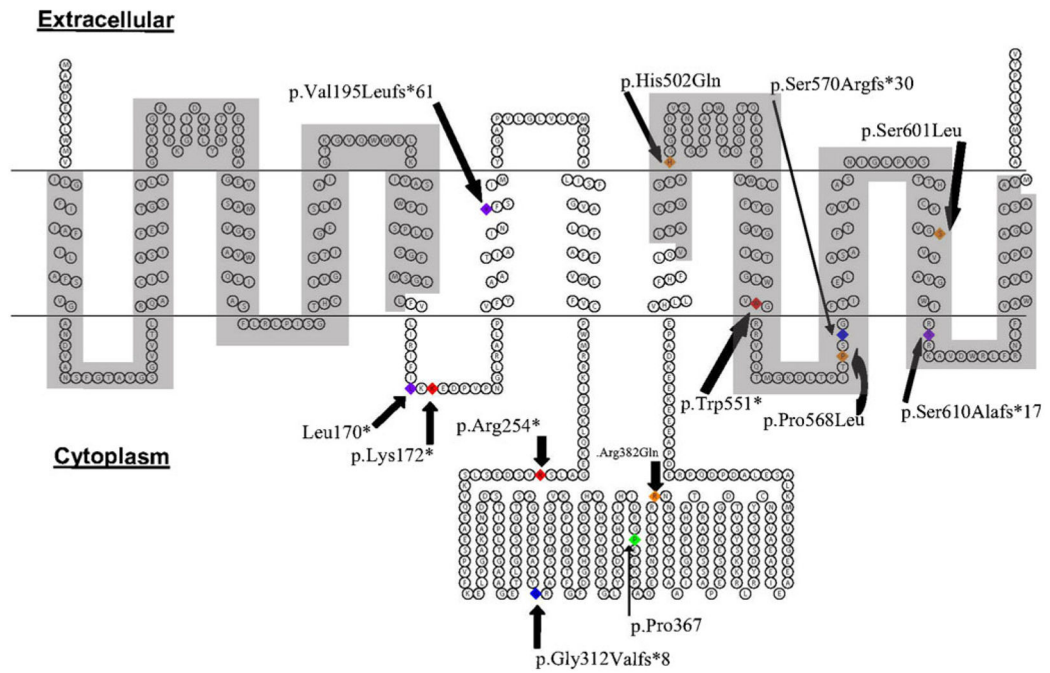
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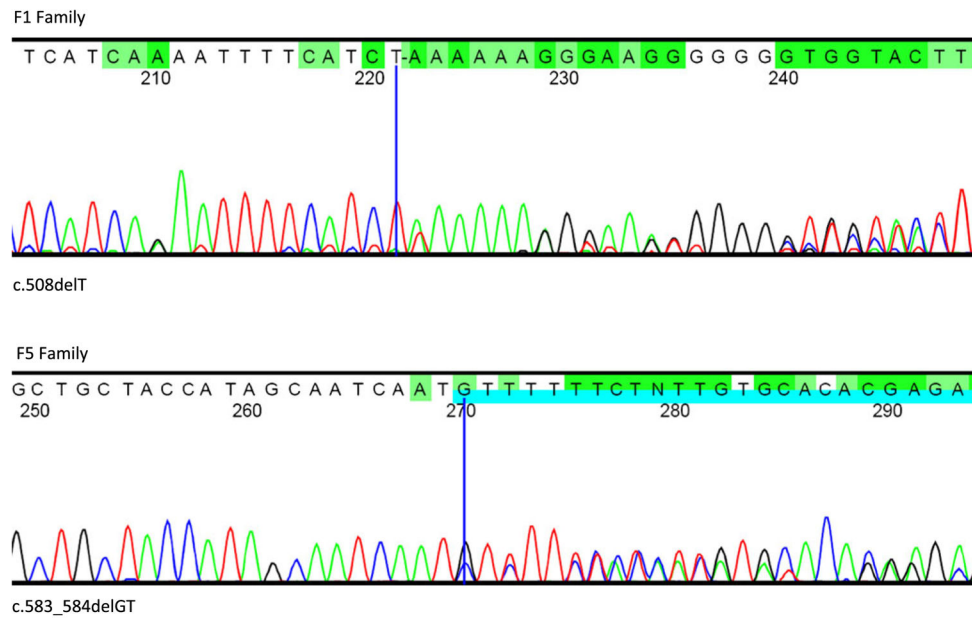
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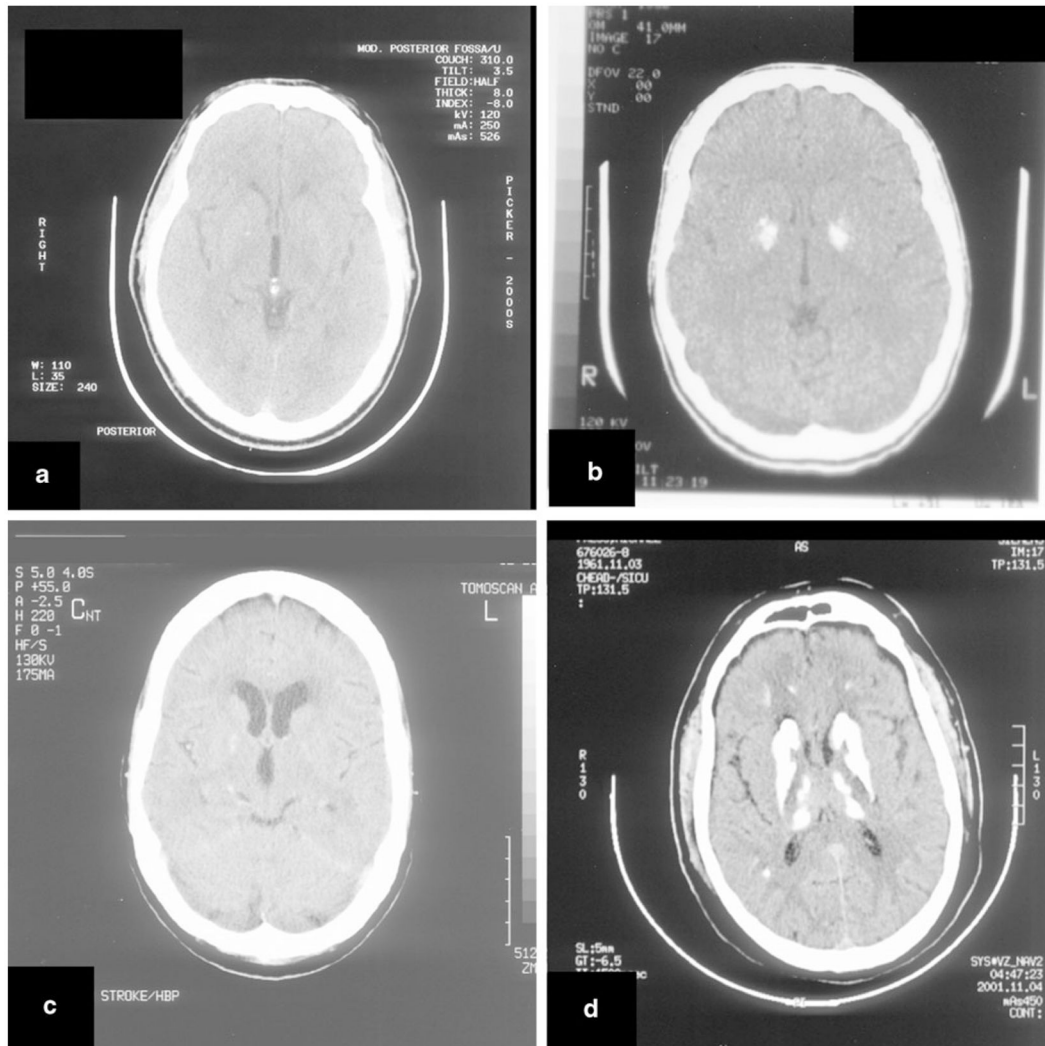


**Fig. 1.** Structure model of PiT2 protein with the variant locations. *Red residues* denote nonsense variants, *orange residues* denote missense variants, *blue residues* denote splice site variants, *purple residues* denote insertions/deletions, and *green residues* denote synonymous variants. ProDom domains (I11–L161 and V492–V640) are highlighted in gray



**Fig. 2.** DNA sequence chromatograms of the *SLC20A2* variants identified in IBGC-affected families. Representative partial sequence chromatograms for family F1 (mutation: c. 508delT) and F5 (p.Val195Leufs\*61). The dark blue cursor denotes the position of the indicated mutation and the subsequent frameshift is shown with the blue and green highlighted bases





**Fig. 3.** Brain CT images of nonsegregating individuals and positive controls. **a** CT classified as positive for a F1 family member but tested negative for the variant; **b** CT-positive F1 family member with the variant showing abundant calcifications; **c** CT classified as positive for F5 family member at age 75 but tested negative for the variant; **d** CT-positive F5 family member with the variant showing abundant calcifications

Table 1

## Main clinical and neuroimaging features of IBGC families

IBGC family	Geographic origin/descent	N. of available members	Affected	Unaffected	Unknown	Clinical symptoms	Diagnostic workup	Calcifications	Reference
F1	North American	31	11	7	13	Parkinsonism, focal dystonia, tremor, and dysphagia	Serum calcium and PTH	+++ (BBG, globus pallidus, thalamus, white matter)	[7]
F2	European	30	10	6	14	8 individuals normal neurological, cognitive, psychiatric features; 2 individuals dementia, parkinsonism, bipolar I		Dense calcification in at least one area	[3]
F3		30	7	5	18	Parkinsonism, dizziness, slurred speech, and balance disorder	Serum calcium and PTH		NP
F4	North American/Swedish	17	10	3	4	Dementia, chorea, slurred speech, palilalia, gait disturbance, 5 asymptomatic	Serum calcium and PTH	+++ (BBG, white matter, cerebellum)	[15]
F5	North American/Irish	16	10	2	4	Parkinsonism and dystonia, 3 asymptomatic	Serum calcium and PTH	+++ (BBG, thalamus, white matter)	[15]
F6	Serbian	14	6	8	0	Parkinsonism, severe gait disturbances with freezing of gait, and dyskinesia; 2 asymptomatic			[16]
F7		12	6	0	6	Cramps and headaches	Serum calcium and PTH		NP
F8	German	10	6	1	3	Dizziness, epilepsy, headaches	Serum calcium and PTH	+++ (BBG)	[15]
F9	North American/Chinese	9	6	0	3	Dizziness, dementia, muscle spasms, and cramps; 2 asymptomatic	Serum calcium and PTH	+++ (BBG, cerebellum)	
F10		6	3	0	3		Serum calcium and PTH		NP
F11	German/Irish	5	2	0	3	Dizziness and syncope, speech delay, cognitive delay, tremor, global developmental delay, family member with bipolar disorder	Serum calcium and PTH	+++ (BBG, thalamus, white matter)	NP
F12	Spanish	5	2	2	1	No cognitive or movement disorder, neurosensory hearing loss, myopia, astigmatism, migraine headache, scoliosis, pectus		+++ (BBG, thalamus, cerebellum, brainstem, cortico-subcortical)	

IBGC family	Geographic origin/descent	N. of available members	Affected	Unaffected	Unknown	Clinical symptoms	Diagnostic workup	Calcifications	Reference
F13	Finnish/Swedish	4	4	0	0	Migraine, speech difficulties, essential tremor, and neuropsychiatric symptoms	Serum calcium and PTH	+++ (BBG, thalamus, white matter, and cerebellum)	NP
F14		4	3	0	1	Dysarthria, micrographia, balance disorder	Serum calcium and PTH		NP
F15		3	2	0	1	Depression and muscle spasms	Serum calcium and PTH		NP
F16	Portuguese	3	3	0	0	Migraine, vertigo, anxiety, depression, personality and behavioral problems, intellectual and language delay		++ (BBG, dentate nucleus calcification by fifth decade), + (earlier decades)	NP
F17	Thai	3	2	0	1	Parkinsonism, dystonia, ataxia, cognitive impairment, psychosis, paraparesis	Serum calcium and PTH	+++ (BBG, subcortical)	NP
F18	Libyan	2	1	0	1		Serum calcium and PTH		NP
F19		2	1	1	0	Clawed hand and slurred speech, dopa-responsive parkinsonism	Serum calcium and PTH	+++ (BBG, cerebellum, subcortical white matter, bilateral temporal lobes)	NP
F20	English/Irish, German	2	2	0	0	Fainting episodes, complaint of headache, no cognitive impairment	Serum calcium and PTH		NP
F21	Spanish	2	2	0	0	Dopa-responsive parkinsonism, dysarthria, subcortical cognitive impairment, stroke		+++ (BBG, cerebellum)	NP
F22		1	1	0	0	Dystonia, cramps, depression, insomnia, headaches, and muscle spasms	Serum calcium and PTH	+++ (BBG, dentate nucleus)	NP
F23	Ashkenazi Jewish/Russian	1	1	0	0	Asymptomatic (1), tremor, psychosis, behavioral problems (1), parkinsonism (1)	Serum calcium and PTH		NP
F24	French	1	1	0	0	Severe migraine	Serum calcium and PTH	++ (white matter, thalamus)	NP
F25	(Asian) Indian	1	1	0	0	Headaches, movement disorders	Serum calcium and PTH		NP
F26	Scottish	1	1	0	0		Serum calcium and PTH		NP
F27	Spanish	1	1	0	0	Dementia, psychiatric disorder, parkinsonism, facial palsy, leukemia		+++ (BBG, cerebellum)	NP

IBGC family	Geographic origin/descent	N. of available members	Affected	Unaffected	Unknown	Clinical symptoms	Diagnostic workup	Calcifications	Reference
F28	Spanish	1	1	0	0	Mild cognitive impairment, ataxia		+++ (BBG, subcortical, cerebellum)	NP
F29	European (Irish, English, Hungarian)	1	1	0	0	Segmental dystonia (cervical and torso); mild frontal and subcortical findings on neuropsychological testing (slowed processing speed, difficulty with learning and retrieval); family history of dementia and multiple sclerosis	Serum calcium and PTH	+++ (BBGC, dentate)	NP

BBG bilateral basal ganglia, NP unpublished, PTH parathormone

Table 2

*SLC20A2* variants and mutations identified in 13 IBGC families

N.	cDNA <sup>a</sup>	Amino acid <sup>b</sup>	Location	IBGC family	Mutation type	Polyphen-2 prediction	SIFT prediction	Human Splicing Finder
1	c.508delT	p.Leu170*	Exon 4	F1	Nonsense	Not available	Damaging subject to nonsense decay	
2	c.514A>T	p.Lys172*	Exon 4	F22	Nonsense	Not available	Damaging	
3	c.583_584delGT	p.Val195Leufs*61	Exon 5	F5	Frameshift	Not available	Damaging and subject to nonsense-mediated decay	
4	c.760C>T	p.Arg254*	Exon 7	F15	Nonsense	Not available	Damaging due to stop	
5 <sup>c</sup>	c.1101C>G	p.Pro367Pro	Exon 8	F18	Synonymous	Not available	Not available	
6	c.1145G>A	p.Arg382Gln	Exon 8	F7	Missense	Probably damaging	Tolerated	
7	c.1506C>A	p.His502Gln	Exon 8	F24	Missense	Probably damaging	Damaging	
8	c.1523+1G>A	p.Gly312Valfs*8	IVS 8	F7	Splice site			Natural 5' donor site
9	c.1652G>A	p.Trp551*	Exon 9	F20	Nonsense	Not available	Damaging	
10	c.1703C>T	p.Pro568Leu	Exon 9	F19	Missense	Probably damaging	Damaging	
11	c.1794+1G>A	p.Ser570Argfs*30	IVS 10	F9	Splice site			Natural 5' donor site
12	c.1794+1G>C	p.Ser570Argfs*30	IVS 10	F29	Splice site			Natural 5' donor site
13 <sup>d</sup>	c.1802C>T	p.Ser601Leu	Exon 11	F23	Missense	Probably damaging	Damaging	
14	c.1828_1831delTCC	p.Ser610Alafs*17	Exon 11	F2	Frameshift	Not available	Damaging (nonsense-mediated decay not predicted)	

<sup>a</sup>Numbering according to GenBank reference build NM\_006749.3 starting at the translation initiation codon

<sup>b</sup>Numbering according to GenPept reference build NP\_006740.1

<sup>c</sup>Rare variant with allele frequency of 0.0009 in the 1000 Genomes Project database

<sup>d</sup>Previously reported by Wang et al. [10]

Table 3

Cosegregation analysis of variants in *SLC20A2*

IBGC Family	Proband Variant	Family members with variant			Family members without variant		
		Affected	Unaffected	Unknown	Affected	Unaffected	Unknown
F1	c.508delT	9		2	2	8	10
F2	c.1828_1831delTCCC	9		1	1	6	13
F5	c.583_584delGT	8			2	2	4
F7	c.1523+1G > A c.1145G > A	6		1			5
F9	c.1794+1G > A	6		1			2
F15	c.760C > T	2					1
F19	c.1703C > T	1				1	
F18	c.1101C > G	1		1			
F20	c.1652G > A	2					
F22	c.514A > T	1					
F23	c.1802C > T	1					
F24	c.1506C > A	1					
F29	c.1794+1G > C	1					

Numbers of subjects in each category are reported by *SLC20A2* variant status. Shaded cells highlight possible cosegregation mismatches