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THREE NOVEL MISSENSE MUTATIONS IN *SLC20A2* ASSOCIATED WITH IDIOPATHIC BASAL GANGLIA CALCIFICATION

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Dear Editors,

Primary Familial Brain Calcification (PFBC, MIM#213600), also known as Fahr's syndrome, is a rare condition characterized by primary symmetric and bilateral brain calcifications, predominantly affecting the basal ganglia, thalamus, and cerebellum. Diagnosis of PFBC is corroborated by typical Computed Tomography (CT) findings, and eventually confirmed by the identification of a causative mutation in one of the four known autosomal dominant IBGC genes (*SLC20A2*, *PDGFB*, *PDGFRB* and *XPR1*; OMIM#158378, 190040, 173410, 605237). *SLC20A2* accounts for up to 50% of PFBC cases [1]. Fifty different *SLC20A2* mutations have been described, all supporting haploinsufficiency as pathogenic mechanism. Geographic and ethnic distribution of *SLC20A2* variants does not support the idea of founder effects [2].

The mean age at onset is 31.7 ± 27.0 years (mean \pm SD) [3]. Clinical features are variable, with the three most frequently observed categories being psychiatric signs (70-80% of cases), movement disorders (40-77% of cases), and cognitive impairment (40-65% of cases) [4].

Three independent patients with clinical and neuroradiologic features compatible with PFBC were referred to our Genetics Unit. Informed consent was obtained from all participants and the study was approved by the internal Ethics Committee of the Department of Medical Sciences, University of Torino, Italy.

Case 1 was a 48-year old Italian female patient with a 3-year history of headaches. She reported three-four episodes per month that lasted for 24-48 hours. Headaches were characterized by unilateral pulsating pain of moderate-to-severe intensity, accompanied by nausea, vomiting, and photo/phonophobia. Clinical symptoms fulfilled the criteria for the diagnosis of episodic migraine without aura (ICHD-III beta version, code 1.1). Neurological examination showed hyperreflexia only at the lower limbs. A CT-scan showed severe calcifications in the bilateral globus pallidus, caudate nuclei, thalamus and dentate nuclei (Figure³ 1A). Laboratory tests excluded any parathyroid dysfunction, but showed a mild Vitamin D deficiency (19.8 ng/ml, normal values >30 ng/ml). Neuropsychological examination was normal with an increased level of anxiety (STAIx-1= 54 and

STAIx-2= 57). Patient's mother and son suffer from migraines, but they were not available for genetic testing.

Case 2 was a 72-year old Italian male with a diagnosis of bipolar disorder (BD) type 2. A positive family history was reported (deceased mother and one sister). Since age 41-yrs he showed episodes of elated and expansive mood. At 68 yrs., he started presenting cognitive decline. The neuropsychological examination revealed impairment of the frontal functions and visuospatial abilities (Montreal Cognitive Assessment, MOCA: 20/30). Neurological examination showed frontal release signs, such as the bilateral palmomental reflex. Laboratory tests were normal. Brain CT-scan and MRI showed large calcifications in the basal ganglia and cerebellar dentate nuclei (Figure 1B). Interestingly, also his sister, who denied the CT-scan, exhibited basal ganglia calcifications at transcranial sonography.

Case 3 was a 41-year-old Albanian female who presented with a long history of migraines without aura of moderate-to-severe intensity, and characterized by pulsating pain, accompanied by nausea, and photo/phonophobia, lasting up to 24 hours. Neurological examination was normal as well as laboratory investigations. A CT-scan at 37 yrs. showed calcifications at the bilateral putamen and small deposits at the bilateral globus pallidus and dentate nuclei (Figure 1C). Interestingly, 4 years later, the CT-scan showed an increase of ~30% in calcification volume (Figure 1D).

Sanger sequencing of *SLC20A2* (NM_006749) identified three novel missense variants: i) case 1, c.188G>A in exon 2 (p.Gly63Asp; Figure 1E); ii) case 2, c.187G>A in exon 2 (p.Gly63Ser; Figure 1F). Notably, the mutation segregates in the patient's sister affected by BD type 2 and cognitive impairment; iii) case 3, c.1196A>C in exon 8 (p.His399Pro; Figure 1G). All three changes were not reported in genetic databases (ExAc, dbSNPs, HapMap, 1000 Genomes, Exome Variant Server); the mutations affected highly conserved amino acids (Figure 1H), and were predicted as pathogenic by bioinformatics (Supplemental Table 1). Based on ACMG recommendations, the three variants are classified as "class 4-likely pathogenic".

Our study further support a lack of genotype/phenotype correlation for *SLC20A2* mutations [4]. In two cases, glycine 63 was affected; the proband with p.Gly63Asp had migraines at onset, whereas the p.Gly63Ser substitution (case 2 and his sister) was associated with BD type 2, followed by cognitive impairment involving frontal functions (dysexecutive syndrome and visuospatial deficits). BD has been described in few PFBC patients [1, 5, 6]. However, only two cases of BD are reported in patients carrying *SLC20A2* mutations: one with BD type 1 and motor tics, and one unspecified BD [1, 6]. To our knowledge, case 2 represents the first *SLC20A2*-mutated patient presenting with BD type 2.

Notably, MRI showed globus pallidus alteration also in case 2 with BD. This finding correlates with recent observations in BD, suggesting an involvement of this nucleus in manic episodes [7].

The relationship between basal ganglia calcifications (BGC) and neuropsychiatric symptoms is controversial. The basal ganglia are interconnected with frontal areas in parallel cortico-striato-thalamo-cortical circuits, organized in five distinct functional loops. Three circuits play a major role in executive functions (prefrontal loop), regulation of social behavior and mood (orbito-frontal loop) and motivation (anterior cingulate circuit). Disruption of these fronto-subcortical loops has been proposed to occur in BGC [8].

Cases 1 and 3 further support migraines without aura as the sole initial presentation of *SLC20A2*-related IBGC [1, 9]; as this clinical feature is frequent in the general population, it is possible that it can co-occur with IBGC by chance.

Finally, we demonstrated a progression of the calcifications at the basal ganglia within just 4 years of the disease (case 3), suggesting that, following an initial trigger, the growth of the calcifications progresses rapidly. This observation could lead to a read-out for potential therapies, including the recently suggested vitamin D administration, which should reduce brain calcifications by upregulating *SLC20A2* gene expression [10]. Regarding this, we found hypovitaminosis D in case 1, which may have modified the clinical progression.

In conclusion, we added BD type 2 as a novel psychiatric phenotype associated with *SLC20A2* mutations, suggesting that BD patients, as well as those with adult onset migraines, should be investigated for the presence of basal ganglia calcifications.

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FIGURE LEGEND

Figure 1. *CT-scans and genetic findings.*

A) and B) Severe calcifications in the bilateral globus pallidus, caudate nuclei, thalamus and dentate nuclei were present in the CT-scans of cases 1 (48 yrs.) and 2 (68 yrs.). C) Bilateral small calcifications in the putamen, globus pallidus and dentate nucleus in case 3 (37 yrs.). D) CT-scan of case 3 performed 4 years later (41 yrs.) than in panel C. A mild increase of the calcifications in the bilateral dentate nuclei is visible at epiphyseal level (Osirix software (www.osirix-viewer.com): 480,086 pix² in panel C vs 628,266 pix² in panel D).

E, F, G) Genetic analyses showing the c.188G>A(p.Gly63Asp), c.187G>A(p.Gly63Ser) and c.1196A>C(p.His399Pro) missense changes. H) Evolutionary conservation of the glycine 63 and the histidine at position 399 using the “Multiz Alignment of 100 Vertebrates” track of the UCSC genome browser (www.genome.ucsc.edu).

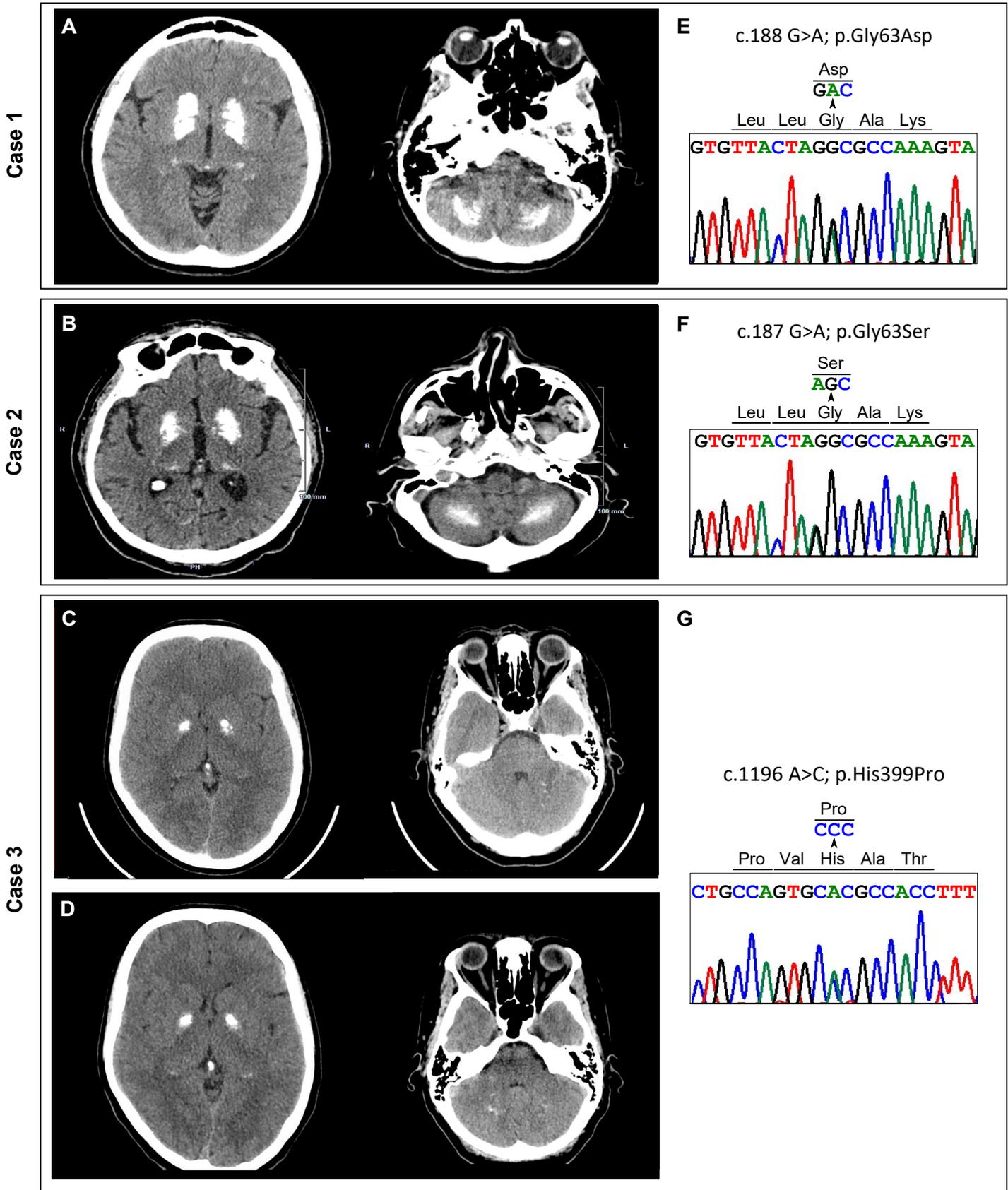
Supplemental Table 1. *In silico* pathogenicity prediction of *SLC20A2* variants.

Software	Case 1		Case 2		Case 3	
	p.Gly63Asp		p.Gly63Ser		p.His399Pro	
	Description	Output value	Description	Output value	Description	Output value
PolyPhen-2 ²¹	Probably damaging	1	Probably damaging	1	Benign	0.002
I-Mutant 2.0 ²²	Decrease in Stability	6	Decrease in Stability	6	Increase in Stability	3
Mutation Tasting ²³	Disease causing	0.99	Disease causing	0.99	Disease causing	0.99
PMUT ²⁴	Pathogenic	0.8	Neutral	0.3	Pathogenic	0.9
PHD-SNP ²⁵	Disease	n.a	Disease	n.a.	Disease	n.a
ExAC	0		0		0	
dbSNP147	0		0		0	

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Figure 1



H

	63	399		63	399
	↓	↓		↓	↓
Human	VLLGAKV	LPVHATF	Elephant	VLLGAKV	LPVHATF
Rhesus	VLLGAKV	LPVHATF	Opossum	VLLGAKV	LPVHATF
Mouse	VLLGAKV	LPVHATF	Chicken	VLLGAKV	LPVHATF
Rabbit	MLLGAKV	LPVHATF	X-tropicalis	VLLGAKV	LPVHATF
Cow	VLLGAKV	LPV L ATF	Zebrafish	MLLGAKV	LPVQATF