

A whole exome sequencing study of a Korean proband with idiopathic basal ganglia calcification and its daughter

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Abstract

Idiopathic basal ganglia calcification (IBGC) is characterized by brain calcification and a wide variety of neurological and psychiatric symptoms. In families displaying an autosomal dominant inheritance pattern, three causative genes have been identified: SLC20A2, PDGFRB, and very recently, PDGFB. While in clinical practice sporadic presentation of IBGC is frequent, well-documented reports of true sporadic occurrences are rare. We report a case of a 61-year-old woman who presented with depressive and dystonic symptoms revealing IBGC. Her 41-year-old daughter was healthy. In the proband, we identified 4 mutations in PDGFB, and 1 exonic mutation in SLC20A2, all of which were absent in the daughter. These mutations may result in a loss-of-function of PDGF-B or SLC20A2, which has been shown to cause IBGC in humans and disrupts the blood-brain barrier in mice resulting in brain calcification. Herein, we present the occurrence of a sporadic patient of IBGC and its causative mutations.

Abbreviations: IBGC = idiopathic basal ganglia calcification, PDGFRb = platelet-derived growth factor receptor b, SLC20A2 = SoLute Carrier family 20 member A2.

Keywords: basal ganglia, calcification, exome, sequencing

1. Introduction

Idiopathic basal ganglia calcification (IBGC) is characterized by the presence of brain calcification affecting at least the basal ganglia, and it is diagnosed after excluding other potential causes of calcification. There is a wide intra- and interfamilial diversity of symptoms and ages of onset. The most common signs are cognitive impairment, psychiatric signs, movement disorders, gait disorders, dysarthria, cerebellar syndrome, pyramidal signs, and seizures.^[1] Most familial cases show autosomal dominant inheritance. Three causative genes have recently been discovered. Loss-of-function mutations were first found in SLC20A2, which may impact inorganic phosphate metabolism in the brain, causing approximately 40% of IBGC cases.^[2,3] Next, causative mutations were identified in PDGFRB, which encodes the transmembrane receptor platelet derived growth factor receptor

b (PDGFRb).^[4] Finally, loss of function of PDGF-B (encoded by the PDGFB gene), the main ligand of PDGFRb, was recently shown to cause IBGC in humans; it also causes brain calcification by disrupting the integrity of the blood-brain barrier in mice.^[5] This study aims to present a proband of a 61-year-old woman presenting with psychiatric symptoms that revealed a diagnosis of IBGC due to mutations within PDGFB and SLC20A2.

2. Materials and methods

The proband and her daughter gave informed, written consent in accordance with the principles of the Declaration of Helsinki. The study was approved by our institutional review board. For whole exome sequencing analysis, 50 ng of gDNA were used for target amplification according to the manufacturer's instructions (Ion AmpliSeq Exome Kit, LifeTechnologies). The quantity and the quality of the captured libraries were assessed using a Library Quantification Kit (LifeTechnologies) and an Agilent High Sensitivity DNA Kit (Agilent Technologies), respectively. Emulsion PCR was performed using a OneTouch 2 instrument (Life Technologies) with an Ion PI Template OT2 200 Kit v2, following the manufacturer's instructions. The enrichment of template-positive Ion Sphere Particles (ISP) in the Ion PI chip was achieved using the Ion OneTouch ES enrichment system (Life Technologies). The Ion PI chip was prepared and loaded according to the manufacturer's recommendations. The Ion Torrent platform-specific pipeline software (Torrent Suite v4.0) was used to separate the barcoded reads, generate a sequence alignment with the hg19 human genome reference, perform a target-region coverage analysis, and filter and remove poor signal reads. The alignment file from Torrent Suite was transferred to Ion Reporter (Ion Reporter v4.0) for variant file generation using default parameters. Variants were annotated using exome

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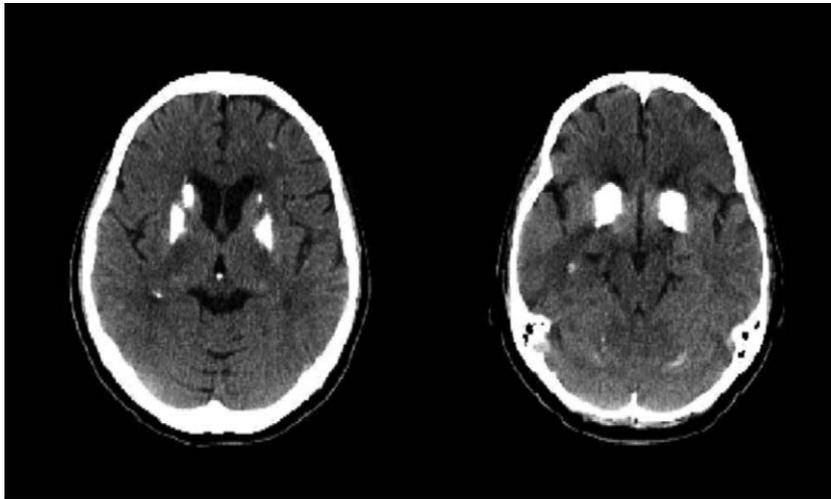


Figure 1. Brain imaging. Brain CT scans on axial view show focal high density at left frontal lobe and diffuse calcification at both basal ganglia and cerebellum.

sequencing project (ESP) and exome aggregation consortium (ExAC) population databases, mutation types, in silico predictions (PolyPhen, SIFT, SnpEff), etc. Variants were excluded from the analysis if they had a coverage of <10X. Variants were called as pathogenic based on the ClinVar database.

Sequencing data will be deposited in the NCBI Sequence Read Archive (SRA) (<http://www.ncbi.nlm.nih.gov/sra>), with accession numbers.

3. Results

3.1. Clinical presentation

A 61-year-old woman was hospitalized for depression, agitation, anxiety, anorexia, feelings of guilt, ideas of reference, and persecutory delusions. She provided no family medical history, and her personal medical history was marked by migraines without aura, leading to the identification of faint calcification of both lenticular nuclei in a CT scan performed after hospitalization.

Neurological examination, CT scan, and electromyography were all normal. No locoregional cause was found. The cerebral CT scan showed calcification of both lenticular and caudate nuclei (Fig. 1). An extensive etiological assessment of brain calcification (according to Nicolas et al^[4]) was negative. We made a diagnosis of IBGC.

3.2. Genetics

No potentially pathogenic variants were identified in PDGFRB in the proband. We found four heterozygous single nucleotide substitutions in PDGFRB (c.-51T>C, c.63+2978T>C, c.-53T>C,

and c.63+2976T>C). These mutations were not found in her healthy daughter. As no SNPs were present in the PDGFRB-coding sequence, we could not use a more sensitive approach, such as the SNaPshot technique. In addition, a mutation in SLC20A2 (c.1606C>T; p.Leu536Leu) was found as a result of sequencing its entire coding region and exon–intron boundaries (Table 1).

Two variants which were detected only in the daughter in PDGFRB (c.934+77T>C, c.40+91G>A) are suggested to have a possibly protective function against IBGC (Table 2).

Variants that were detected in both the mother and daughter are also presented for reference in Table 3.

4. Discussion

The patient presented with agitation, anxiety, anorexia, feelings of guilt, ideas of reference, and persecutory delusion, which are defined as neuropsychiatric symptoms responsible for depression.^[6] Severe depression may be responsible for suicide. Depression is a rare manifestation of IBGC,^[7] and such a severe and acute indication of IBGC has never been reported to our knowledge.

IBGC is usually inherited according to an autosomal dominant pattern. As clinical manifestations of brain calcification are not constant, clinically sporadic presentations of IBGC may be due to mutations inherited from an asymptomatic parent, and then passed on to offspring in some cases.

We demonstrate here that a true sporadic occurrence of IBGC, possibly due to mutations, can occur.

The mutations occurred at four positions (c.-51T>C, c.63+2978T>C, c.-53T>C, and c.63+2976T>C) in PDGFRB. This

Table 1

Mutations detected in only mother.

Chromosome: position	Gene	cDNA change	Amino acid change	dbSNP	SnpEff
chr22:39636928	PDGFRB	c.-51T>C	NA	rs5757575	MODIFIER
chr22:39636928	PDGFRB	c.63+2978T>C	NA	rs5757575	MODIFIER
chr22:39636930	PDGFRB	c.-53T>C	NA	rs5757576	MODIFIER
chr22:39636930	PDGFRB	c.63+2976T>C	NA	rs5757576	MODIFIER
chr8:NA	SLC20A2	c.1606C>T	p.Leu536Leu	NA	LOW

NA = not applicable, PDGFRb = platelet-derived growth factor receptor b, SLC20A2 = Solute Carrier family 20 member A2.

Table 2**Mutations detected in only daughter.**

Chromosome: position	Gene	cDNA change	Amino acid change	dbSNP	SnPEff
chr5:149513072	PDGFRB	c.934+77T>C	NA	rs2240780	MODIFIER
chr5:149516480	PDGFRB	c.40+91G>A	NA	rs2240781	MODIFIER

NA=not applicable, PDGFRb=platelet-derived growth factor receptor b.

Table 3**Mutations detected in both mother and daughter.**

Chromosome: position	Gene	cDNA change	Amino acid change	dbSNP	SnPEff
chr5:149495253	PDGFRB	c.*73A>G	NA	rs2229562	MODIFIER
chr5:149495395	PDGFRB	c.3252A>G	p.Pro1084Pro	rs246388	LOW
chr5:149497177	PDGFRB	c.3137+4A>G	NA	rs246391	LOW
chr5:149499672	PDGFRB	c.2601A>G	p.Leu867Leu	rs246395	LOW
chr5:149503670	PDGFRB	c.2023+143C>T	NA	rs2304061	MODIFIER
chr5:149509270	PDGFRB	c.1579+50T>C	NA	rs1864972	MODIFIER
chr8:42295022	SLC20A2	c.1008C>T	p.His336His	rs111553899	LOW

NA=not applicable, PDGFRb=platelet-derived growth factor receptor b, SLC20A2=Solute Carrier family 20 member A2.

suggests a clustering of mutations in the PDGFB gene. Such a phenomenon has been studied using pangenomic data from concordant monozygotic twins with autism, and a mutability index (MI) was calculated for each exon as an estimate of the relative mutation rate at single nucleotide resolution.^[8]

Interestingly, the c.1606C>T (p.Leu536Leu) variant in SLC20A2 was previously found as a de novo mutation in another patient, however, to our knowledge, paternity was not verified.^[5]

We did not find further mutations in PDGFB and SLC20A2, including the previously reported one,^[5] in this patient or her daughter. Occurrence of a PDGFB mutation is therefore probably quite rare in IBGC cases. However, the mutation in PDGFB is clearly detrimental, as it is predicted to result in a shortened protein with loss of critical functional domains, including interface-contributing residues of the PDGF-B:PDGFRb complex.^[9]

To conclude, we demonstrate a true sporadic occurrence of IBGC due to a synonymous mutation in SLC20A2. Our results further support the involvement of the loss of function of PDGF-B and SLC20A2 in IBGC and suggest a possible hotspot for single nucleotide substitutions in these two genes.

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