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SHORT COMMUNICATION

Primary familial brain calcification caused by a novel homozygous *MYORG* mutation in a consanguineous Italian family

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Abstract

Primary familial brain calcification (PFBC) is a rare disorder mostly characterized by calcium deposits in the basal ganglia and a wide spectrum of neurologic and psychiatric symptoms, typically inherited as an autosomal dominant trait. Recently, *MYORG* was reported as the first autosomal recessive causal gene in PFBC patients of Chinese and Middle Eastern origin. Herein, we describe the first PFBC patient of European descent found to carry a novel homozygous *MYORG* mutation (p.N511Tfs*243). Interestingly, the patient's father, a heterozygous carrier of the same mutation, showed diffuse bilateral cerebral calcifications with no symptoms other than very mild postural tremor.

Keywords Primary familial brain calcification · Recessive · *MYORG* · Homozygous

Introduction

Primary familial brain calcification (PFBC) is a rare inherited disorder characterized by calcium deposits in the basal ganglia and other brain regions, such as thalami, cerebellum, and subcortical white matter. Clinically, PFBC is associated with a wide spectrum of neurologic and psychiatric features [1], which can start at any age (onset range 3–81 years) [2]. Patients can develop parkinsonism, tremor, dystonia, ataxia, dysphagia, seizures, chronic headache, cognitive impairment, and psychosis [3]. The amount of calcifications correlates with the symptomatic status, but their localization does not account for the whole clinical spectrum [4].

PFBC is typically inherited as an autosomal dominant trait, with four causative genes identified so far: *SLC20A2* [5] and *XPR1* [6], which encode inorganic phosphate transmembrane transporters, and *PDGFRB* and *PDGFB* [7, 8], encoding the platelet-derived growth factor receptor β and its main ligand that are involved in blood-brain barrier integrity. Complete or partial genomic deletions (*SLC20A2* and *PDGFB*) [9–11] have also been found in a small subset of patients. A comprehensive review of the literature suggested a correlation between specific gene mutations and some neurological features; mutations in *SLC20A2* were more frequently associated with parkinsonism, *PDGFB* with headache, *PDGFRB* with psychiatric symptoms, and *XPR1* with cognitive impairment [12]. However, further studies are needed to confirm these retrospective findings.

More recently, the first autosomal recessive causal gene for PFBC was reported in six Chinese unrelated families found to carry compound heterozygous or homozygous mutations in the *MYORG* gene [13]. *MYORG* encodes a glycosyl hydrolase involved in myogenesis and expressed throughout the brain, particularly in the cerebellum, but its role in the pathogenesis of PFBC is yet unclear.

Eliana Marisa Ramos, Alessandro Roca, Giuseppe De Michele and Giovanni Coppola contributed equally to this work.

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Material and methods

Subjects The proband and his family members (Fig. 1A) were clinically evaluated at the Federico II University of Naples, Italy, through family history assessment, neurologic and neuropsychological examination, and neurophysiology and neuroimaging studies. Informed consent was obtained from all individual participants included in the study.

Whole exome sequencing Genomic DNA was extracted from peripheral blood of all participating family members. Whole exome regions were captured using the SeqCap EZ Human Exome Kit v3.0 (Roche NimbleGen, Madison, WI) and sequenced on an Illumina HiSeq4000 at the UCLA Neuroscience Genomics Core (UNGC, www.semel.ucla.edu/ungc). Sequence reads were mapped to the GRCh37/hg19 reference genome and variants were joint-called following GATK's best practices recommendations [14]. Ingenuity Variant Analysis (QIAGEN, Redwood City, CA) software was used for variant annotation and filtering.

Results

Clinical summary A 39-year-old Italian male (II-2) was referred to our department with a 4-year history of dysarthria and gait disturbance. He had no relevant medical history and there were no reports of neurological illnesses in his family. His parents were consanguineous; the father was reportedly healthy, while his mother died at age 50 from a lung carcinoma. On neurological examination he had gait ataxia, mild bilateral dysmetria and moderate dysarthria, mild upper limb

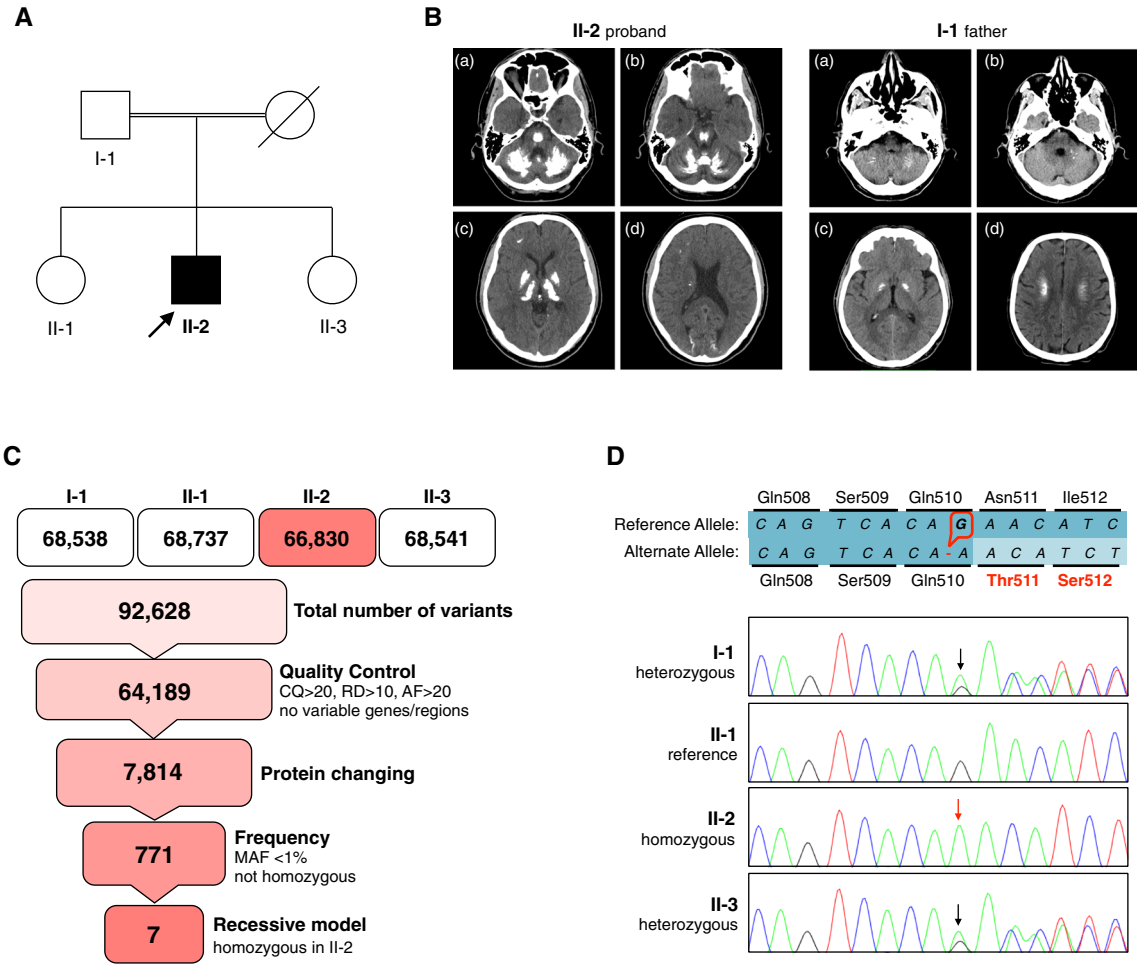

Fig. 1 Familial, neuroimaging, and genetic findings. (A) Pedigree of the family described in this case report. (B) Brain CT scan at 4 different axial levels showing: multiple calcifications in the proband (II-2), localized in both cerebellar hemispheres (massively) and in the pons (a), in the cerebellar vermis and in the midbrain (b), bilaterally in the thalamus and globus pallidus (c), and in the occipital cortex, right corona radiata, and right frontal gray/white matter junction (d); scattered calcified foci in the father (I-1), evident in the deep cerebellar hemispheres (a), in the left middle cerebellar peduncle (b) bilaterally in the globus pallidus (c), and along the fibers of the coronae radiatae (d). (C) Filtering of variants identified within four targeted exomes. (D) Trace view of the homozygous frameshift *MYORG* variant p.N511Tfs*243 (red) identified in the proband (II-2). Two of the three relatives screened (I-1 and II-3) were heterozygous carriers of the deletion. Reference sequence: NM_020702.4

bradykinesia, and brisk tendon reflexes. Unenhanced brain Computed Tomography (CT) scan revealed extensive calcifications of the basal ganglia, thalami, pons, cerebellum, occipital, and frontal lobes (Fig. 1B). Blood tests, including parathyroid hormone, serum calcium and serum phosphate, were normal. Brain Magnetic Resonance imaging confirmed the localization of the calcifications appearing as hypointense foci on T2-weighted images. The neuropsychological assessment showed impairment of long-term memory, visuospatial abilities, and verbal fluency. Nerve conduction studies and electromyography were normal. On examination, the 68-year-old proband's father (I-1) had a very mild postural tremor. CT scan showed diffuse bilateral calcification of the basal ganglia, cerebellum, and periventricular white matter (Fig. 1B). The two sisters (II-1 and II-3) were reportedly healthy and their neurological examination was normal.

Genetic screening The proband was first screened for the four dominant PFBC genes, where no pathogenic variants were found. Since the proband's parents were consanguineous and were both reportedly healthy (no signs of neurological diseases), we assumed a recessive mode of inheritance in this family. From the 92,628 total variants identified among the four exomes sequenced, we filtered out variants with low-quality, that are not predicted to cause a protein-change, and with a minor allele frequency (MAF) > 1% or homozygotes reported in the ExAC and gnomAD databases (Fig. 1C). As both siblings were also reportedly healthy, we then focused on homozygous variants in the proband that were not completely shared by the unaffected siblings, resulting in a total of seven candidate variants (Supplementary Table 1). Among these variants, we identified a novel (absent from the gnomAD database) frameshift variant (c.1530delG:p.N511Tfs*243) in *MYORG*, the newest, and first recessive, PFBC causal gene [13]. This variant was homozygous in the proband (II-2) while one of the unaffected sisters (II-3) and the father (I-1) were heterozygous carriers (Fig. 1D).

Discussion

A recent study identified *MYORG* as a common recessive causal gene for PFBC in Chinese families, and showed that *Myorg* homozygous knockout mice developed calcium phosphate deposits in the brain, recapitulating the disease phenotype observed in PFBC patients [13]. Additional PFBC recessive cases, including new Chinese families and patients of Middle Eastern origin, were since found to carry *MYORG* mutations (https://coppolalab.ucla.edu/lovd_pfbc/genes/MYORG) [15–18]. Based on these findings, and family history being consistent with a recessive mode of inheritance, it seems likely that the novel *MYORG* homozygous frameshift variant identified herein is pathogenic.

The *MYORG* gene (previously known as *KIAA1161* or *NET37*) encodes a 714 amino-acid protein from the glycosyl hydrolase 31 family, with recent findings indicating that it may regulate protein glycosylation in the endoplasmic reticulum of astrocytes in the brain [13]. The specific frameshift variant identified in this family, p.N511Tfs*243, is predicted to alter the C-terminal of the protein and disrupt its glycosidase domain.

We also highlight that dysarthria, a prominent feature in our patient, has already been reported as the earliest symptom in the Middle Eastern *MYORG* homozygous patients [15, 16] and it was also very frequent in the Chinese patients [17, 18]. On the contrary, dysarthria is not common in PFBC associated with *SLC20A2*, *PDGFB*, and *PDGFRB* mutations (6.7%, 2.3%, 0%, respectively) and is present in 2/6 cases associated with *XPR1* mutations, according to a recent comprehensive review [12]. Therefore, we hypothesize that, as the four dominant genes have already been correlated to specific neurological features [12], *MYORG* pathogenic variants could be similarly more frequently related to dysarthria.

In the original study [13], heterozygous carriers were reported to have negative CT scans, whereas three Middle Eastern heterozygous carriers revealed punctuated calcifications limited to the basal ganglia, a finding present in about 20% of CT scans in the elderly and not considered clinically meaningful [15]. Instead, in the family described herein, the heterozygous carrier I-1 (father) showed significant and diffuse cerebral calcifications, moreover in locations usually spared by age-related calcium deposits. Further studies would clarify if this finding is related to the advanced age of the carrier or other genetic or environmental factors.

In conclusion, we report herein a novel *MYORG* homozygous mutation in a PFBC patient of European descent and show that brain calcifications can be found in heterozygous carriers. Currently, it is not clear if postural tremor in the proband's father could be considered a clinical manifestation of the heterozygous state. With the increasing number of PFBC cases associated with recessive mutations in *MYORG*, this gene should be added to genetic panels for PFBC, especially in patients where a recessive mode of inheritance is suspected.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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