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LETTER TO THE EDITOR

A biallelic mutation links *MYORG* to autosomal-recessive primary familial brain calcification

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Sir,

Primary familial brain calcification (PFBC) is an adult-onset hereditary disease marked by calcium deposits in multiple parts of the brain and progressive symptoms that can include cerebellar and extrapyramidal syndromes, dysarthria and neuropsychiatric alterations (Fahr's syndrome) (Fahr, 1931; Nicolas *et al.*, 2013*a, b*). Heterozygous mutations in *PDGFB*, *PDGFRB*, *XPR1* and *SCL20A2* can cause autosomal-dominantly inherited PFBC (Wang *et al.*, 2012; Keller *et al.*, 2013; Nicolas *et al.*, 2013*a, b*; Legati *et al.*, 2015), while autosomal-recessively inherited cases remained genetically unexplained. Most recently, however, Yao *et al.* (2018) have suggested that biallelic mutations in the gene *KIAA1161/MYORG* are a recessive cause for PFBC, based on the analysis of six Chinese families. However, an independent confirmation is currently missing and no *MYORG*-linked PFBC case has been reported from outside China. Here, we show complementary data indicating that *MYORG* is a novel PFBC disease gene and provide an in-depth phenotype characterization.

The index patient (Fig 1; Patient IV.1) is a currently 43-year-old male of Turkish origin who presented to our department with progressive cerebellar dysarthria, and subsequently also gait ataxia, dysphagia and forgetfulness starting at the age of 39 years. Moreover, family members reported personality changes with emotional instability, depressive episodes, and intermittent aggressive behaviour. On neurological exam, mild gait disturbance, a dysmetric finger-nose-test and brisk deep tendon reflexes on the right side were noted. Logopedic exam confirmed the severe disturbance predominantly of the pharyngeal phase of deglutition. Neuropsychological examination consisting of Corsi-Block Span, Digit Span, Paired Associate Word Learning, Doors-Test, Symbol Digit Modalities Test, and WAIS Picture Arrangement revealed a mild cognitive impairment with reduced verbal and non-verbal episodic memory, verbal primary memory and attention capacity. Cranial CT and cranial MRI including susceptibility-weighted imaging revealed symmetric calcifications (hyperdense or hypointense, respectively) in the basal ganglia

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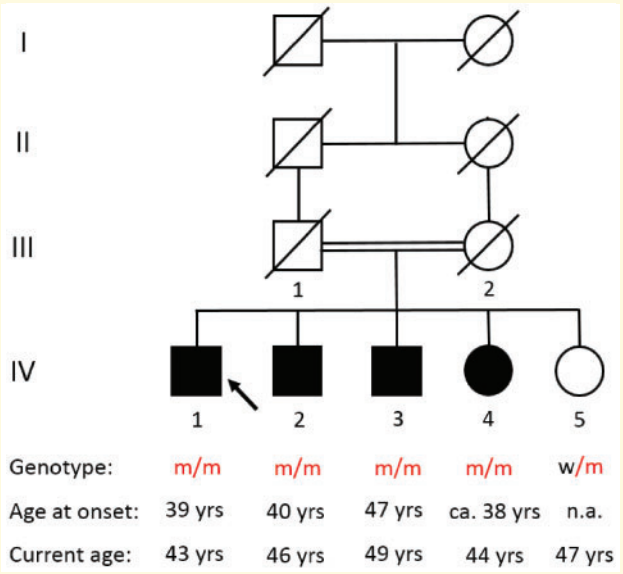

Figure 1 Pedigree of the PFBC family. The PFBC phenotype is linked to the homozygous c.1964A>G/p.Ile655Thr mutation in *MYORG*. The index patient is marked with an arrow; filled symbols = PFBC phenotype; m/m = homozygous mutation; w/m = heterozygous mutation.

(pallidum), red nucleus, posterior thalamus, dentate nucleus extending into the cerebellar hemispheres, and periventricular white matter regions (Fig. 2; Patient IV.1). As observed before in PFBC patients (Nicolas *et al.*, 2013*a, b*), the patient also presented with occipital cortical calcifications [Fig 2A(4 and 8)]. Hyperintense corticospinal tracts and periventricular microangiopathy were also observed. Video-oculography documented abnormal oculomotor dysfunctions including hypermetric saccadic eye movements which are in agreement with cerebellar dysfunction. Electroencephalography was completely unremarkable. Hypoparathyroidism, gangliosidoses (GM1-2), Krabbe's disease, Gaucher's disease, metachromatic leukodystrophy, sialidosis and aminoaciduria were excluded. Furthermore, HIV testing, interferon-gamma release assay and immunofixation were negative. CSF analysis showed CSF-specific oligoclonal IgG bands but a normal leucocyte count, protein values and lactate concentration. Ferritin concentrations were normal both in CSF and serum.

Consequently, the diagnosis of idiopathic Fahr's syndrome was initially made, but further work-up of the family history suggested a genetic cause: mother and father of the index patient, who are first-degree cousins (Fig. 1, Subjects III.1 and 2), died at the age of 67 and 77 years from ischaemic stroke and esophageal cancer, respectively, without signs of PFBC. However, at the time of his first presentation in our clinic, both brothers of the index patient were already known to have brain calcifications resembling those observed in the index patient (Fig. 1 and 2; Patients IV.2 and 3).

The index patient's 46-year-old brother (Fig. 1; Patient IV.2) started to develop progressive cerebellar dysarthria and dysphagia 6 years ago. A cranial MRI study revealed widespread symmetric calcifications with an identical pattern as the index patient [Fig. 2B(1–4)]. At the same time, family members reported emotional instability, and 'schizophrenia' was diagnosed according to his brothers. However, despite his consent to perform a genetic analysis, the patient refused further clinical work-up.

The second, 49-year-old brother, Patient IV.3 (Fig. 1) developed subtle neurological deficits in the past 2 years. He complains about mild memory problems and slightly slurred speech. On clinical examination mild cerebellar dysarthria, slight dysmetria of the upper extremities and elevated deep tendon reflexes in the lower extremities were observed. Cerebellar saccadic eye movement deficits were confirmed by video-oculography. Formal neuropsychological testing revealed a slightly reduced processing speed. MRI of the brain showed symmetric calcifications resembling the findings in his brothers [Fig. 2C(1–4); Patient IV.3]. However, in agreement with the milder clinical manifestation compared to his brothers, the calcifications were less pronounced and spared the occipital cortex [Fig. 2C(4)]. Routine laboratory testing was unremarkable.

Taking together consanguinity of the parents, disease manifestation in at least three siblings but absence of a PFBC syndrome in other generations, an autosomal-recessive inheritance was most likely. In line with this assumption, targeted testing for known autosomal-dominant PFBC genes (*PDGFB*, *PDGFRB*, *XPR1* and *SCL20A2*) remained negative. Consequently, we performed whole exome sequencing (WES) of the three brothers to elucidate the cause of disease in this family. Upon informed consent, sequencing, read mapping and variant calling was performed on HiSeq2000/2500 systems (Illumina) as described (Freischmidt *et al.*, 2015). Variant filtering for rare (minor allele frequency < 1:100) non-synonymous variants present homozygously in all three affected brothers left only one candidate variant, c.1964A>G (p.Ile655Thr) in *MYORG* (also known as *KIAA1161*). As a heterozygous mutation can misleadingly appear homozygous due to a deletion on the other chromosome, we used the program ExomeDepth for the identification of copy number variants. The single coding exon of *MYORG* was sequenced to an average coverage of ~300 reads. ExomeDepth revealed a copy number of ~2 in all three whole exome sequenced patients, and thus did not provide evidence for a deletion at the position of the candidate variant in *MYORG*. This variant has a low allele frequency of 2.5×10^{-5} (7/279 962 alleles; only heterozygote individuals detected) according to GnomAD (http://gnomad.broadinstitute.org/). Also high-throughput sequencing of additional 1190 Turkish control individuals without PFBC revealed only 1 of 2380 alleles with the c.1964A>G/p.Ile655Thr variant (frequency 4.2×10^{-4}; 773 whole genome and 417 whole exome sequencing datasets; mean coverage at this position = 23 reads).

To confirm co-segregation of this mutation with disease phenotype, we approached the two sisters of the three affected male patients. Sanger sequencing showed that they

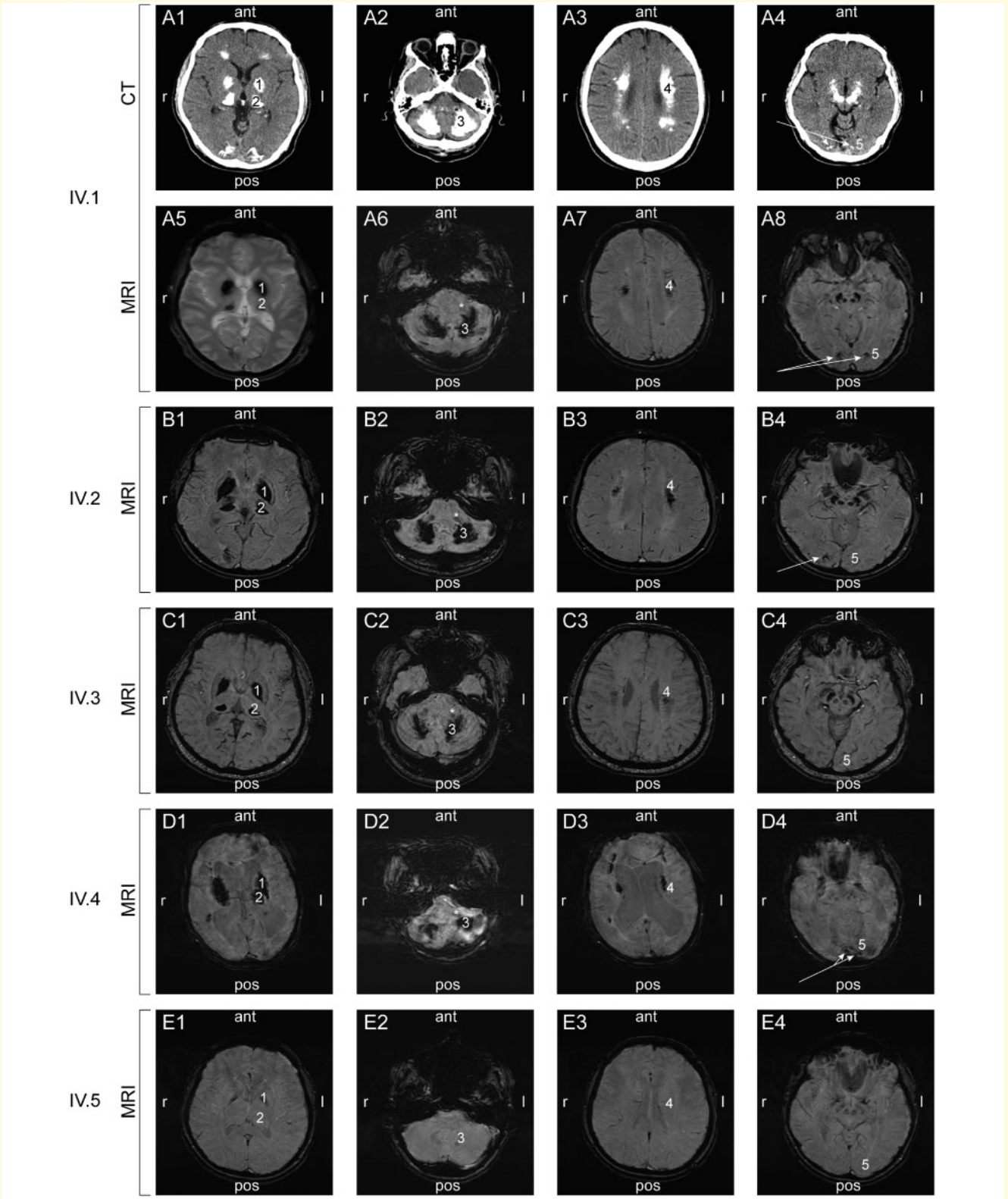

Figure 2 Imaging results. Cranial CT scan (**A1**–**A4**) and SWI (susceptibility weighted MRI imaging) sequences (**A5** to **E4**) of siblings IV.1 to IV.5 (referring to the pedigree in Fig. 1). In individuals IV.1–IV.4 (**A1** to **D4**) with a homozygous c.1964A>G/p.Ile655Thr mutation in *MYORG* symmetric calcifications are observed in the basal ganglia (marked by '1'), the posterior thalamus (2), the dentate nucleus (3) extending into the cerebellar peduncles, and the periventricular white matter (4). Moreover, cortical calcifications are observed mainly in the occipital lobe (5) in Patients IV.1, IV.2 and IV.4 (white arrows). Pictures of Subject IV.4 additionally show some motion artefacts due to agitation of the subject, as well as a meningioma in the left frontal hemisphere (**D3**). MRI of Subject IV.5 (heterozygous c.1964A>G/p.Ile655Thr *MYORG* mutation) are normal and lack any signals indicating calcium deposits. ant = anterior; l = left; pos = posterior; r = right.

carried the p.Ile655Thr *MYORG* mutation in a homozygous (Fig. 1; Patient IV.4) or heterozygous (Patient IV.5) state.

Clinical judgement of the 44-year-old sister Patient IV.4 (homozygous mutation) was complicated by the fact that she sustained a severe car accident with brain trauma and several weeks of coma at the age of 13 years. Moreover, she has a meningioma in the left frontal lobe (Patient IV.4 in Fig. 2; visible in D3), and symptomatic epilepsy for 20 years. Notably, in the years after the car accident, the patient had regained walking abilities and speech until about 6 years ago, when she started to continuously deteriorate. Currently, she is conscious but tetraplegic, mutistic, aphagic and fed via a percutaneous gastrostomy. Cranial MRI showed the known meningioma and a slight midline shift, as well as a global brain atrophy, enlarged ventricles and left fronto-parietal and temporal cortical defects as sequelae of the brain trauma in childhood. However, distinct basal ganglia, brain stem, white matter, occipital cortex and cerebellum calcifications were also noted, with a symmetrical pattern highly reminiscent of the findings in her three brothers (Fig. 2, Patient IV.4). The cranial imaging result is therefore fully compatible with her homozygous *MYORG* mutation, and suggests that development of PFBC has contributed to her clinical worsening starting at the age of ~38 years.

The second 47-year-old sister carries the *MYORG* mutation only in a heterozygous state (Fig. 1; Patient IV.5). As predicted based on an autosomal-recessive mode of inheritance, she had a completely normal brain MRI without any calcium deposits (Fig. 2; Patient IV.5). Apart from a congenital strabismus, she had no neurological or neuropsychological signs or symptoms.

The PFBC phenotype in this family is therefore perfectly co-segregating with the homozygous c.1964A>G/p.Ile655Thr *MYORG* mutation. In synopsis with the most recent findings by Yao *et al.* (2018), the results demonstrate that *MYORG* mutations are the so far only known recessively inherited cause for typical PFBC. Furthermore, we describe the first *MYORG*-associated PFBC patients outside China. The point mutation identified in this Turkish family has not been found in any of the Chinese patients, and is the currently most C-terminal *MYORG* mutation. It is located in a putative glucosidase domain of the MYORG protein, and could lead to a loss of enzymatic activity. Currently, however, direct experimental proof for enzymatic activity of MYORG is missing and respective tests are not established yet. Moreover, we provide a detailed phenotyping of *MYORG*-associated PFBC including cranial CT, cranial MRI, neuropsychological testing, video-oculography and CSF analysis. The clinical and imaging phenotypes of the patients described here resemble the cases presented by Yao *et al.* and are within the known general spectrum of PFBC/Fahr's syndrome, which typically comprises symptoms related to basal ganglia dysfunction, cerebellar deficits as well as neuropsychological and psychiatric manifestations. PFBC should thus be recognized as a possible organic differential diagnosis for psychoses. Whether the CSF-specific antibody production observed in the index patient is an incidental finding or reflects an intrathecal immune reaction related to PFBC remains speculative. In this context, it is interesting to note that most PFBC genes are highly expressed in cells that establish the neuro-vascular unit (astrocytes in the case of *MYORG*), and respective mutations could impair the blood–brain barrier with subsequent CNS immune alteration. Finally, considering the autosomal-recessive inheritance, we recommend testing for *MYORG* mutations in PFBC/Fahr's syndrome patients without an autosomal-dominant inheritance or with a negative family history.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Competing interests

The authors report no competing interests.

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