Neurological Sciences (2019) 40:1283–1285
https://doi.org/10.1007/s10072-018-3684-8

LETTER TO THE EDITOR

A novel SLC20A2 gene mutation causing primary familial brain calcification in an Ukrainian patient

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Received: 6 September 2018 / Accepted: 10 December 2018 / Published online: 3 January 2019

Dear Editor,

Primary familial brain calcification (PFBC) is a rare neurological disease characterized by symmetrical bilateral calcifications, which are mostly located in the basal ganglia but can also be detected in other areas of the brain [1]. The estimated prevalence of PFBC is < 1/1.000.000 [2], with a male:female ratio of about 2:1 [3]. Although symmetrical brain calcifications are frequently observed in routine CT scans [4], the disease usually begins in the fourth or fifth decade of life with a variable association of psychiatric symptoms, cognitive decline, and extrapyramidal syndrome. These cardinal aspects can also be accompanied by a broad constellation of other clinical features and, depending on the prevalent location of the calcium deposits, they include headache [5], seizures, ataxia and dysarthria [3], and transient ischemic attack or stroke [6]. On the other hand, a significant proportion of patients are asymptomatic at the time of diagnosis [4]. To date, four genes (*SLC20A2*, *XPR1*, *PDGFRB*, and *PDGFB*) have been identified as being responsible for the disease, the mutations of which are transmitted with an autosomal dominant pattern.

In this report, we describe clinical, neuroradiological and genetic aspects of a 49-year-old Ukrainian woman carrying a novel heterozygous nonsense mutation in the *SLC20A2* gene. She was admitted to our Division for a full clinical and instrumental assessment because of radiological finding of cerebral calcifications in the basal ganglia, centrum semiovale, and cerebellar hemispheres, incidentally discovered after a head trauma. (Fig. 1A–F). The patient complained mood depression in the last 2 years and reported two episodes of complex visual hallucinations with predominantly threatening content. Her past medical history was remarkable for hypothyroidism in actual pharmacological hormonal balance.

Extensive neuropsychological assessment revealed moderate depression along with subtle attentional and visuoconstructional deficits, compatible with a non-amnestic, multiple domain mild cognitive impairment (MCI) (Fig. 1O). Laboratory tests found that the serum calcium, phosphorus, magnesium, calcitonin, and parathormone were all within the normal range, excluding any secondary form of brain calcification. EEG was not contributive. Brain MRI confirmed the presence of multiple calcifications symmetrically involving the cerebellar white matter and dentate nucleus, hippocampus, lentiform nucleus and pulvinar, caudate nucleus, and frontoparietal white matter also with perivascular distribution following the medullary vessels. These structures appeared of various signal intensities on T1-weighted images due to the surface area effect of the calcium crystals (Fig. 1G–L).

The family history was contributive (Fig. 1M). The mother of the patient, who died at 70 years due to vascular complications of type II diabetes, suffered of memory disturbances and seizures since the age of 60 with radiological evidence of cerebral calcifications. Furthermore, an 80-year-old maternal aunt developed an unspecified tremorgenic syndrome and the maternal grandfather died at the age of 75 suffering from a psychiatric disorder.

Performed after written informed consent, genetic analysis (Fig. 1N) showed the heterozygous variation c.1375G> in the *SLC20A2* gene on the chromosome 8p11.21 (NM_006749), which resulted in a premature termination codon in position 459 (p.Glu459*). This mutation is not reported in the public accessible databases of human genetics (the Exome

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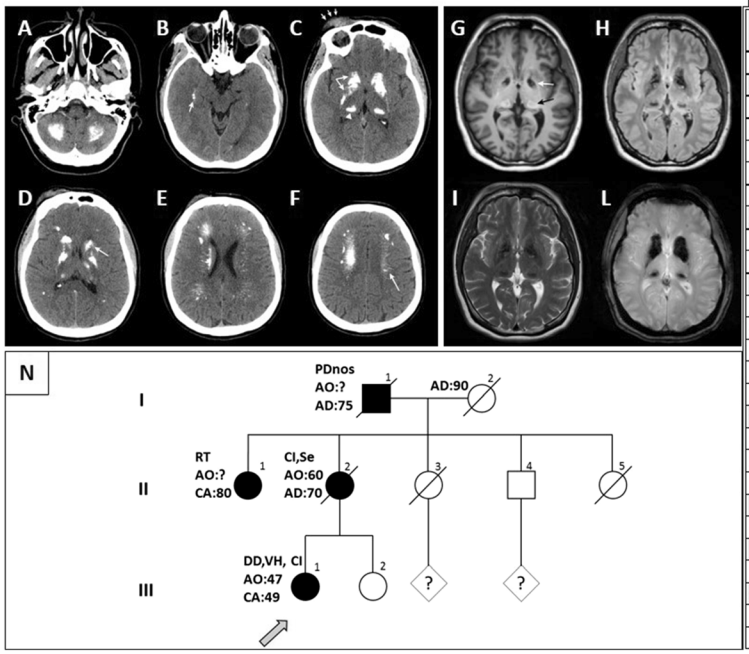

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GENERAL NEUROPSYCHOLOGICAL EXAMINATION	RAW SCORE	ADJUSTED SCORE	EQUIVALENT SCORE
Mini Mental State Examination (MMSE)*	30/30	-	≥ 23,8
ATTENTIONAL AND EXECUTIVE FUNCTIONS			
Frontal Assessment Battery (FAB)	17/18	16.7	4
Attentional Matrices	37/60	30.75	0
Phonological Verbal Fluency	23	24.1	2
Stroop Test: time	32.85″	31.10″	2
Stroop Test: interfered Color Naming	0	0	4
SHORT-TERM MEMORY			
Digit Span Forward	4	4.0	1
Visuo-Spatial Span	4	4.0	2
LONG-TERM MEMORY			
Story recall test: immediate recall	6.9/8	6.7	4
Story recall test: delayed recall	7.4/8	7.2	4
Rey's 15 word learning test: immediate recall	57/75	54.8	4
Rey's 15 word learning test: delayed recall	11/15	10.2	4
Rey–Osterrieth complex figure: delayed recall	12/36	11.25	2
CONSTRUCTIONAL PRAXIS ABILITIES			
Clock Drawing Test (CDT)*	10/10	-	≥ 8
Copying simple geometrical drawings	13/14	12	3
Rey–Osterrieth complex figure: direct copy	29/36	28	0
LANGUAGE ABILITIES			
Semantic Verbal Fluency	34	35	3
NON VERBAL REASONING ABILITIES			
Raven Matrices	25/36	24.8	2
CLINICAL SCALES			
Beck Depression Inventory BDI-II*	27	-	≤ 13
IADL	8/8	-	-

Fig. 1 (A–F) Non-enhanced brain computed tomography: extensive calcifications with symmetric involvement of the cerebellar white matter and dentate nucleus (A), hippocampus (B), lentiform nucleus and pulvinar (respectively labeled with arrows and arrowhead on C), caudate (arrow on D), frontoparietal white matter (e, f) also with perivascular distribution following the medullary vessels (arrow on F). Soft-tissue swelling of the right frontal region due to the recent trauma (little arrows on C). (G–L) Magnetic resonance imaging: it should be noted that calcifications appear with various signal intensities on T1-weighted images due to the surface area effect of the calcium crystals; in this case, globus pallidus was hypointense (white arrow) while pulvinar showed high signal intensity (black arrow); turbo field echo T1-weighted (G); fluid attenuated inversion recovery (H); turbo spin echo T2-weighted (I); and fast field echo T2*-weighted (L) images on the axial plane at the level of basal ganglia. (M) Neuropsychological evaluation. An equivalent score 0 means below the normal range, 1 means within normal limits, 2–4 mean normal range. *For these tests or scales, equivalent scores are not provided, but only cut-off scores for normal range. (N) Family pedigree. DD, depressive disorder; VH, visual hallucinations; CI, cognitive impairment; PDnos, psychiatric disorder not otherwise specified; RT, rest tremor; Se, seizures; AD, age of death; AO, age of onset; CA, current age

Aggregation Consortium, Exome Variant Server or 1000 Genome Project). It is located in an evolutionary conserved residue and computational analysis predicts a potentially damaging effect on the resulting protein (MutationTaster = 1.00, CADD-PHRED = 37, phyloP-Vertebrate = 3.97/6.42) (phyloP-Primate = 0.58/0.65). Accordingly, in line with the American College of Medical Genetics and Genomics guidelines [7], the variation p.Glu459* of the *SLC20A2* gene can be classified as likely pathogenic.

PFBC was first described in 1930 by the German neurologist Karl Theodor Fahr, who identified the disease in a patient with dementia and akinetic rigidity. Among the numerous nomenclatures reported in the literature, here the term "primary familial brain calcification" is preferred because it also underlines the familial etiology of the disease. Others have suggested the use of the term Fahr's syndrome to emphasize that the clinical and radiological features can be due to a multitude of other causes (i.e., endocrine, metabolic, neoplastic, infectious, malformative, and vascular).

In recent years, a lot of progress has been made towards understanding the pathogenic mechanisms of the disease. The four genes (*SLC20A2*, *XPR*, *PDGFRB*, and *PDGFB*) that cause the disease were identified. *SLC20A2* encodes for type III sodium-dependent inorganic phosphate-transporter-2 (PiT2), responsible for Pi uptake by cell [8], while *XPR1* (xenotropic and polytropic retrovirus receptor) gene encodes for a retroviral receptor involved in phosphate cell export [9]. *PDGFRB* and *PDGFB* genes encode for beta-type platelet-derived growth factor receptor and platelet-derived growth factor subunit B, respectively [10]. Moreover, PDGFRB/PDGFB interaction is crucial for maintaining blood-brain barrier integrity, and a putative role in the fine regulation of PiT2 and XPR1 receptors has also been proposed [10]. However, it should be emphasized that a pathogenic mutation of these genes has been found only in about 50% of individuals with a clinical diagnosis of PFBC.

SLC20A2 gene, with up to 67 mutations described (HGMD-professional), is the most frequent mutated gene in PFBC. Furthermore, all the mutations lead to haploinsufficiency [5] even if it has been speculated that a dominant negative mechanism may occur for some missense mutations [11]. The novel nonsense mutation *p.Glu459**, described here, determines a premature stop codon, making an

incomplete structure of PiT2. The consequent dysfunction of inorganic phosphate transport, originating mainly by the severe impairment of its uptake, likely causes a buildup of calcium phosphate and explains the accumulation of various metals in the brain inducing the calcifications characteristic of PFBC [8]. A recent review highlighted some characteristic aspects of *SLC20A2* mutations, such as the involvement of cerebellum, thalamus and subcortical white matter associated with a greater frequency of cognitive decline and parkinsonism [4]. In our patient, the neuroradiological findings along with the presence of a mild cognitive impairment (MCI) seem to be in line with this putative gene-specific lesional topography, but not the absence of extrapyramidal signs at the time of neurological examination. This latter aspect is however detectable in the family pedigree if we extend the observation to other family members, since a maternal aunt suffered from rest tremor. This suggests a remarkable phenotypic heterogeneity also within the same family. Moreover, the absence of a parkinsonism at the time of our examination does not exclude its possible appearance later in the disease course, as the full clinical spectrum in PFBC seems to be age-related, and psychiatric symptoms prevailing at onset in most of reported cases [12].

In conclusion, we described the clinical and neuroradiological features in a patient with PFBC carrying the novel Glu459*-*SLC20A2* mutation, which further expands the spectrum of *SLC20A2* mutations and of the PFBC clinical phenotypes.

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