PCYT2 mutations disrupting etherlipid biosynthesis : phenotypes converging on the CDP-ethanolamine pathway

LETTER TO THE EDITOR

PCYT2 mutations disrupting etherlipid biosynthesis: phenotypes converging on the CDP-ethanolamine pathway

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The study by Vaz *et al.* published in *Brian* in 2019 established a novel firm link between disrupted etherlipid biosynthesis and complex forms of hereditary spastic paraplegia (HSP) through the identification of recessive mutations in the *PCYT2* gene (Synofzik and Schule, 2017; Darios *et al.*, 2020; Rickman *et al.*, 2020). Before, genetic causes of HSP had been found in genes with diverse functional roles (Shribman *et al.*, 2019) including chaperone activity, axonal transport, mitochondrial function and many others. However, mutations in genes involved in complex lipid metabolism have increasingly been implicated in the pathology of HSP (*CYP7B1*, *CYP2U1*, *DDHD1*, *DDHD2*, *BSCL2*, *ERLIN2*, *FA2H* and *PNPLA6*/*NTE*, *SELENOI*/*EPT1*, *SERAC1*) (Windpassinger *et al.*, 2004; Rainier *et al.*, 2008; Goizet *et al.*, 2009; Alazami *et al.*, 2011; Schuurs-Hoeijmakers *et al.*, 2012; Tesson *et al.*, 2012; Cao *et al.*, 2013; Synofzik *et al.*, 2014; Ahmed *et al*., 2017; Roeben *et al.*, 2018), thus presenting a converging and possibly even treatable mechanistic theme in pathophysiology of manifold HSP and spastic ataxias (Synofzik and Schule, 2017; Darios *et al.*, 2020; Rickman *et al.*, 2020).

Phosphatidylethanolamine (PE) is a glycerophospholipid that, together with phosphatidylcholine, makes up more than 50% of the total phospholipids in eukaryotic cell membranes (McMaster, 2018). PE is particularly enriched in the brain where it constitutes ~45% of the phospholipid fraction. PE is the product of two major biochemical processes: the CDP-ethanolamine pathway (Kennedy pathway) and the decarboxylation of phosphatidylserine in mitochondria (Vance, 2015; McMaster, 2018). CTP:phosphoethanolamine cytidylyltransferase, encoded by *PCYT2*, performs a crucial role within the CDP-ethanolamine pathway. It catalyses the conversion of CTP and phosphoethanolamine into CDP-ethanolamine, an intermediate that is subsequently converted into PE by ethanolamine phosphotransferase (McMaster, 2018).

Here, we report an additional patient, in the first independent report on *PCYT2*, with a complex HSP caused by compound heterozygous mutations in *PCYT2*, highlighting a marked broadening of the phenotypical spectrum.

The patient under study was born from healthy non-consanguineous parents. All procedures were carried out as part of routine clinical care. Written informed consent was obtained from all participants for genetic studies. Whole-exome sequencing was performed within the PREPARE consortium (https://www.prepare-ataxia.com) on genomic DNA of the index patient and was performed using Illumina Nextera Rapid Capture Expanded exome kit for exome enrichment and Illumina HiSeq2000 platform for sequencing. Sequence reads were aligned to the UCSC Genome Browser hg19 reference sequence and the data were imported and annotated in the GENESIS (Gem.app) platform, a web-based tool for next generation sequencing data analysis (http://thegenesisprojectfoundation.org/) (Gonzalez *et al.*, 2015). We identified compound heterozygous mutations in *PCYT2* (NM_001184917.1): a missense

c.736G>A (p.Gly246Arg) and a frameshift c.524_527del (p.Asp175Valfs*109) (Fig. 1A). The *PCYT2* variants revealed by exome sequencing were validated by Sanger sequencing in the index patient and both parents, showing a trans orientation of the variants in the index patient (Fig. 1). No additional bi-allelic *PCYT2* variants were identified in 1700 HSP/ataxia samples of the PREPARE consortium, which likely makes *PCYT2*-related HSP/spastic ataxia a rare entity.

The currently 45-year-old male index patient has a history of progressive gait instability, appendicular ataxia, anosmia, visual and speech disturbances. Gait instability presented as the first symptom at the age of 20 months after initial acquisition of unassisted walking. Further slow regression of ambulation occurred until wheelchair dependency at 38 years of age. Clinical examination is in line with a spastic paraplegia in combination with cerebellar and posterior column ataxia. There was an absence of frontal release signs and no extrapyramidal symptoms were noted. Speech disturbances appeared at 22 months of age and progressed to moderately intelligible cerebellar dysarthric speech at present. In secondary school he was noted to have anosmia. Visual disturbances (night and colour blindness) commenced at age of 18 years and the patient was later diagnosed with rod-cone dystrophy as seen on fundoscopy and flash-electroretinography. Additional findings are scoliosis and a high arched palate. Normal intellectual development and absence of epilepsy are of particular interest. Brain MRI findings at the age of 29 showed markedly cerebellar but only discrete cerebral atrophy in the absence of other structural abnormalities or white matter lesions (Table 1 and Fig. 1B).

The CDP-ethanolamine pathway is a multistep process that uses ethanolamine to form PE and it has been directly implicated in HSP in two separate instances: *EPT1* and *PCYT2* (Ahmed *et al.*, 2017; Vaz *et al.*, 2019). Although the phenotypes associated with *EPT1* and *PCYT2* mutations fall within the broader spectrum of complex HSPs, there are some notable differences between the reported phenotypes. Before, intellectual disability was invariably present in *PCYT2* patients and ranged from mild to severe and was typically complicated by epilepsy. In *EPT1* patients, the intellectual disability is usually mild. Conversely, the patient in the current study was shown to have normal intellectual development and no history of epileptic seizures. Similar to the *EPT1* patients, our patient shows development of dysarthric speech and palate abnormalities.

Interestingly, recessive mutations in *PCYT1A* (also part of the CDP-ethanolamine pathway converting phosphocholine into CDP-choline) are causal in spondylometaphyseal dysplasia with cone-rod dystrophy and isolated retinal dystrophy (Hoover-Fong *et al.*, 2014; Testa *et al.*, 2017). Ophthalmic phenotypes seem to be a common feature of disruption in the Kennedy pathway; for instance, the rod-cone dystrophy in our patient, has been noted in several of the *EPT1* patients. In the previous report, poor visual acuity was noted in three of the reported *PCYT2* patients without a clear cause described. Further overlap can be observed when considering the skeletal abnormalities associated with *PCYT1A* mutations. Patients reported with *PCYT2* and *EPT1* mutations were all reported with short stature (standard deviation < -0.94). Moreover, while our patient is the only *PCYT2* patient with scoliosis, it has been reported for several *PCYT1A* patients (Yamamoto *et al.*, 2014).

The patient in the current study clearly demonstrates that phenotypes associated with *EPT1*, *PCYT1A* and *PCYT2* mutations overlap to a more substantial degree than previously reported. As such, dysregulation within the Kennedy pathway results in a broad phenotypic spectrum and can include variable combinations of spastic paraplegia, epilepsy, neurodevelopmental delay, ophthalmic and skeletal phenotypes (Fig. 1C).

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.

Figure legend

Figure 1 [JB1](**A**) Pedigree of affected patient and the healthy non-consanguineous parents. (**B**) MRI at age 29. *L*eft: Two FLAIR-sequence images showing no apparent abnormalities. *Right*: T_2-sequence showing on a sagittal plane cerebellar atrophy and mild global cortical atrophy. (**C**) Schematic representation of phenotypic correlations and severity of *PCYT2* (Vaz *et al*., 2019), *PCYT2* (this case description), *PCYT1* and *EPT1* associated disorders.

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