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LETTER TO THE EDITOR

Expanding the clinical and genetic spectrum of *PCYT2*-related disorders

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Recently, Vaz *et al.* reported four families with complex hereditary spastic paraplegia (cHSP) and biallelic variants in *PCYT2* encoding CTP: phosphoethanolamine cytidylyltransferase (ET), the rate-limiting enzyme for phosphatidylethanolamine biosynthesis. Patient-derived fibroblasts and plasma had significant abnormalities in both neutral etherlipid and etherphospholipid metabolism (Vaz *et al.*, 2019). We wish to broaden the phenotypic and genetic spectrum of *PCYT2*-related disorders with two additional patients. Clinical features are detailed in Table 1.

Case 1, a 46-year-old male, was born after a normal pregnancy from healthy first cousin consanguineous Spanish parents. He had an older sister who died at the age of 2 years due to a severe progressive muscle weakness of unknown aetiology. His development was considered as normal during childhood, until 12 years of age when he began to experience frequent falls and difficulties in running and climbing. Over the years, he reported weakness and lower limb stiffness. At age 49, neurological examination showed increased tone with proximal symmetrical weakness (MRC 4/5) of the lower limbs. Force and tone were normal in upper limbs. Deep tendon reflexes were globally brisk in all four limbs, with clonus and bilateral extensor plantar responses. He had hypopallesthesia of ankles with no other sensory deficits. No clinical signs of cerebellar involvement were present. He complained of urinary urge incontinence, was treated with baclofen, but improvement in leg spasticity was not clear and urinary urge worsened. Brain MRI at age 46 was strictly normal. EMG and nerve conduction studies were largely normal. Currently he is aged 59 years and walks with ankle-foot orthosis braces and needs a walking aid (cane) for longer distances. He finished primary school without difficulties, and obtained a job as a driver. Hitherto, there is no evidence of cognitive impairment.

Case 2 is a 7-years-old male born to healthy, non-consanguineous African American parents. He has two healthy younger sisters. The patient's mother had a positive group B strep test and she was treated with antibiotics prior to delivery. She reported occasional cannabis consumption during pregnancy. The patient was born at 38 weeks and 2 days

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Table 1 Clinical features of *PCYT2*-mutated individuals

	Case 1	Case 2	Vaz *et al.*, 2019 (Patients 1, 2, 3, 4, 5, respectively)
General information			
Age at last assessment, years	59	5	5.8, 20, 16.7, 9.9, 2.5
Gender	Male	Male	Male, Male, Male, Female, Male
Ethnicity	Spanish	African American	Hungarian, British, Turkish, US Caucasian, US Caucasian
Examination			
Dysmorphic features	No	High columella insertion with long appearing philtrum, broad nasal root, high arched palate	None of them
Spasticity	Yes	Yes, spastic quadriparesis	All patients
Hyperreflexia	Yes	Yes	All patients
Extensor Plantar Response	Yes	Yes	All patients
Other neurological features			
Developmental delay	No	Yes	All patients, ranging from mild to severe
Intellectual disability	No	Yes	All patients, ranging from mild to severe
Epileptic seizures	No	Multifocal epilepsy	All patients
Cerebellar ataxia	No	Yes	Patient 2
Hearing loss	No	No	Patient 3
Ophthalmological symptoms			
Cataracts	No	Yes, congenital cataracts	Not reported
Nystagmus	No	Yes, congenital nystagmus	Patients 2–5
Optic atrophy	No	Yes	Patient 3
Investigations			
Brain MRI	Normal	Abnormal increased T_2/FLAIR signal within the bilateral periventricular white matter	Patients 1–4: Progressive atrophy with subtle symmetric hyperintensities in the cerebral white matter (Patient 5 not performed)

via spontaneous vaginal delivery, weighting 3.92 kg (44% using CDC charts), with a length of 51 cm (25%), and an occipitofrontal circumference of 35 cm (13%). Apgar scores were 9 at 1 min and 9 at 5 min. He was noted to be tachypnoeic at 24 h of life, being admitted to the NICU. Tachypnoea resolved spontaneously and he was discharged home after 26 h. Ophthalmological assessment at birth showed congenital lateral gaze nystagmus, bilateral cataracts and bilateral optic atrophy. A global developmental delay was noted very early. He rolled over at 7 months and did not achieve the ability to sit unsupported; at 3 years he was able to hold a cup. Brain MRI at 7 months was normal, but follow-up MRI at the age of 2.2 years showed an abnormal increased T_2/FLAIR signal within the bilateral periventricular white matter, which was consistent with delayed myelination (Fig. 1E). Epilepsy with multifocal seizures began at 23 months of age. Failure to thrive was noted during infancy, and a gastrostomy tube was placed. The most recent examination at 7 years of age showed increased tone in all four extremities, globally brisk reflexes and upgoing plantar reflexes bilaterally. His nystagmus improved over time, and he is followed closely for his optic atrophy and cataracts (Supplementary Fig. 1). He continues with growth restriction, with his weight below 1% on the CDC growth charts with *Z*-score of –4.17. He has significant intellectual disability/cognitive impairment and attends school in a special education classroom.

Whole exome sequencing (WES) was performed in both patients (methods are provided in the Supplementary material). In Case 1 we identified a homozygous missense variant located at the last coding base pair of exon 11, within the donor splice site [NM_001184917.2:c.957G>C p.(Lys319Asn)] (Fig. 1A and B). This variant was not carried by his healthy sibling. Copy number variation (CNV) analysis in the patient's WES data excluded the presence of deletions (father's sample was unavailable) (Supplementary Fig. 3). This variant was absent from gnomAD, strongly conserved, and is located within the second cytidylyltransferase catalytic domain. *In silico* tools predicted an alteration of splicing (Human Splicing Finder and MaxEntScan: –42%). Concordantly, cDNA analysis showed two different transcripts: the first carried the missense variant, whereas the second used an alternative donor site in intron 11, resulting in the inclusion of 102 bp into the coding sequence, leading to the insertion of 34 amino acids [p.(Lys319Asn_Val320ins34)] (Fig. 1C). This insertion may alter PCYT2 activity by altering active site structure or global PCYT2 protein stability. In Case 2, we identified two compound heterozygous variants: a nonsense variant in exon 11 [NM_001184917.2:c.907delG p.(Val303Ter)] and a second nonsense variant in exon 14, resulting into premature truncation of the PCYT2 protein [already reported by Vaz *et al.* (2019) in four patients (NM_001184917.2:c.1129C>T p.(Arg377Ter)] (Supplementary Fig. 2).

To gain insight into the functional impact of the missense/splicing variant in Case 1, we used lipidomics profiling to quantify the phospholipid content of plasma and peripheral blood mononuclear cells (PBMC). Results showed a

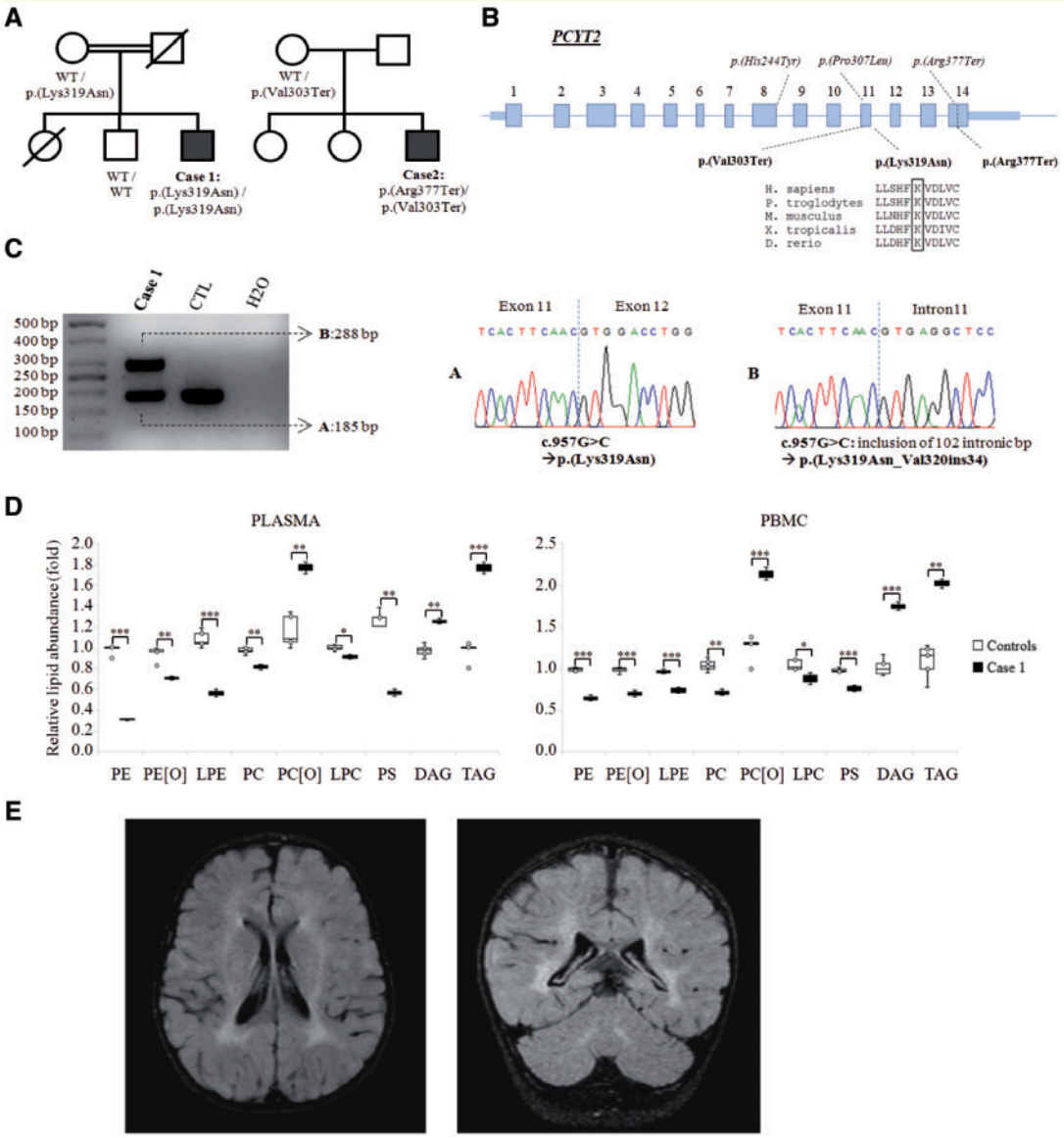

Figure 1 Family trees and *PCYT2* mutation features. (**A**) Family trees. Square = male; circle = female; filled symbols = affected individuals; open symbols = unaffected carriers. WT = wild-type allele. Double line indicates consanguinity. (**B**) Gene structure of *PCYT2* and reported mutations. Blue boxes represent *PCYT2* exons. Mutations in italics (*above*) represent mutations reported by Vaz *et al.* (2019), and mutations in bold (*below*) represent variants identified in this study. Amino acid sequence alignments of *PCYT2* across several species demonstrate conservation of the residues mutated by missense variants. (**C**) *PCYT2* cDNA analysis of Case 1. *Left*: Agarose gel electrophoresis showing the presence of two transcripts in Case 1. CTL = control individual; H2O = amplification negative control. *Right*: Sanger sequence analysis of isolated bands from agarose gels. (**D**) Lipid profile in human plasma and PBMC. Controls ($n = 5$) and Case 1 ($n = 1$). Data are presented as mean ± SD (relative pmol equivalent/ml in plasma and pmol equivalent/mg protein in PBMC) shown as fold increase of the patient compared to that of control individuals, who were sex and age-matched. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (2-tailed Student's *t*-test). DAG = diacylglycerol; LPC = lysophosphatidylcholine; LPE = lysophosphatidylethanolamine; PC = phosphatidylcholine; PC[O] = phosphatidylcholine etherphospholipid; PE = phosphatidylethanolamine; PE[O] = phosphatidylethanolamine etherphospholipid; PS = phosphatidylserine; TAG = triacylglycerol. (**E**) Brain MRI sequences of Case 2. Axial (*left*) and sagittal (*right*) FLAIR shows increased signal within the bilateral periventricular white matter, consistent with delayed myelination.

phospholipid and glycerolipid dysregulation consistent with previous data from Vaz *et al.* (Fig. 1D). While most phospholipid species were significantly decreased in Case 1 compared to controls, in particular phosphatidylethanolamine (PE), there was also a significant accumulation of PC[O], as well as the glycerolipids DAG and TAG (diacylglycerol, triacylglycerol). This profile is highly concordant with the lipidomics data reported in Vaz *et al.* (2019), thus providing evidence for an impaired activity of PCYT2 in this patient.

Deleterious variants in *PCYT2* have been proposed by Vaz *et al.* (2019) as a new cause of cHSP, defined by HSP combined with global developmental delay, intellectual disability, epilepsy and progressive cerebral atrophy. Our cases broaden the phenotypic and genotypic spectrum with one patient presenting with isolated mild pure HSP, in absence of all neurological signs of previously published cases, and a second patient with predominant visual impairment with cataracts, nystagmus, and optic atrophy.

Case 1 shows a pure spastic paraplegia phenotype without any additional signs or abnormal features in a recent brain MRI. The milder disease course may be explained by the phenotypic variability associated to *PCYT2* variants, already reported in Vaz *et al.* (2019). For instance, Patient 5, who developed a milder phenotype of cHSP with fine rotary nystagmus and mild intellectual disability, had the same homozygous nonsense variant as Patients 2, 3 and her brother Patient 4, who were more seriously affected. This illustrates the classical phenotypic variability in inborn errors of metabolism (Argmann *et al.*, 2016). A second explanation concerns the nature of the variant identified. Indeed, the homozygous variant c.957G>C found in Case 1 alters splicing and results into two different transcripts: one containing a missense variant p.(Lys319Asn), and a second containing in addition, an insertion that probably destabilizes PCYT2 protein. The coexistence of these two transcripts may possibly be linked to a less severe effect on PCYT2's activity; however, dysregulation of lipidic profiles appeared on the same range.

Although visual impairment was reported in Patients 2–5 of the cohort published by Vaz *et al.* (2019) who all harbour the p.(Arg377Ter) allele in homozygosis, only Patient 3 was described to have optic atrophy; thus our Case 2 argues for a higher penetrance of this sign than previously anticipated. Cataracts have only been observed in Case 2 reported in this study, who also harbours a p.(Arg377Ter) allele, and will need to be confirmed in future reports. Intriguingly, *PCYT2* was identified as a candidate gene linked to mouse retinal development through a systems biology approach, based in a pairwise correlation with a seed network of *Drosophila melanogaster* genes (Serb *et al.*, 2010). Moreover, mutations in other genes involved in CDP-choline/CDP-ethanolamine synthesis (Wortmann *et al.*, 2015) such as *DDHD1* (Bouslam *et al.*, 2005; Tesson *et al.*, 2012), *DDHD2* (Schuurs-Hoeijmakers *et al.*, 2012; Gonzalez *et al.*, 2013), *SELENOI* (Ahmed *et al.*, 2017) and *PNPLA6* (Synofzik *et al.*, 2014) cause both pure and complex HSP combined with eye abnormalities as a common denominator, highlighting the importance of this metabolic pathway in motor neuron and retinal cellular processes (Rickman *et al.*, 2019).

These cases illustrate the utility of WES to establish diagnosis of spastic paraplegias with broad clinical spectrum. This not only ends the diagnostic odyssey for a growing number of families, but may even open therapeutic options in the metabolic disorders; for example, choline substitution has already been successfully tested on a *Pcyt2*$^{+/-}$ mice with specifically reduced CDP-ethanolamine pathway and metabolic disease (Schenkel *et al.*, 2015). In that animal model choline supplementation improved the lipid profile, restoring fatty acid and triglycerides homeostasis, and therefore assessing its effects on neurological function in patients would be warranted.

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

Y.S. is an employee of GeneDx, Inc., a wholly owned subsidiary of OPKO Health, Inc. C.M. and S.S.T. are employees of Baylor Scott & White Health. The authors report that they have no competing interests related to the content of this article.

Supplementary material

Supplementary material is available at *Brain* online.

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