Defective phosphatidylethanolamine biosynthesis leads to a broad ataxia-spasticity spectrum

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With great interest, we read the article by Rickman et al. (2020), highlighting the link between defective phosphatidylethanolamine (PE) biosynthesis and hereditary spastic paraplegia (HSP). The cytidine diphosphate (CDP)-ethanolamine pathway is a three-step enzymatic cascade involved in PE biosynthesis. The rate-limiting enzyme in this pathway, ethanolamine-phosphate cytidylyltransferase (ET), is encoded by PCYT2 (OMIM 618770), and biallelic variants in this gene have been associated with a clinical spectrum of HSP (Vaz et al., 2019; Vélez-Santamaria et al., 2020). Selenoprotein 1 (SELENOI) (OMIM 607915, also known as EPT1), catalyses the final step in the biosynthesis of PE via the CDP-ethanolamine pathway (Horibata and Hirabayashi, 2007). Biallelic variants in SELENOI/EPT1 have also been linked to complex HSP in two families (Ahmed et al., 2017; Horibata et al., 2018). Here, we report two additional individuals harbouring biallelic variants of PCYT2 and SELENOI/EPT1.

Case 1 was a female, who was born as a second child to healthy, non-consanguineous German parents after an unremarkable pregnancy and birth. Her older sister died in adulthood of probable myocarditis. The affected individual...
failed to thrive, and at the age of 3 years she was noted to have severely reduced vision. At 5 years of age, early-onset severe retinal dystrophy (EOSRD) was diagnosed. In addition, bilateral cataracts were observed, and they were treated surgically at the age of 6 years. Epilepsy with mostly generalized tonic-clonic seizures was diagnosed at 13 years of age, and EEG showed occipital lobe epileptiform discharges. Progressive bilateral hearing loss was reported beginning at age 19 years and attributed to sensorineural damage. A muscle biopsy at age 14 years was normal (no signs of mitochondrial disease on immunohistochemistry). An intellectual impairment was not observed (total IQ at low normal range, with a selective lower value for ‘word fluid’) and the patient embarked on a university degree. At the age of 16 years, the patient complained of gait abnormalities and a physical examination showed signs of cerebellar ataxia with spontaneous nystagmus, ataxic gait and limb ataxia. Although hyperreflexia was noticeable, no spasticity was observed, and the Babinski sign was negative. No cerebellar atrophy with spontaneous nystagmus, ataxic gait and limb ataxia. Although hyperreflexia was noticeable, no spasticity was observed, and the Babinski sign was negative. No muscle weakness or dysmorphic features were detected. MRI of the brain demonstrated cerebellar atrophy (Fig. 1G) and that of the spine showed atrophy of the spinal cord predominantly at the thoracic level (Fig. 1G and Supplementary Fig. 2). The latency of the sensory evoked potential (P40) of the tibial nerve was delayed to 53 ms on the right side; no potential was present on the left side. At the age of 28 years, she was found dead in the bathtub, presumably after a seizure.

Exome sequencing on the proband’s DNA was performed as previously described (Wagner et al., 2019). Two heterozygous variants in PCYT2 (NM_001184917.2: c.1112 + 1G>A; and c.743T>A, p.(Val230Glu)) were identified. Sanger sequencing confirmed both variants in the index and carriership of one variant each in both parents (Fig. 1A and Supplementary Fig. 1). The canonical splice variant was absent from the Genome Aggregation Database (gnomAD), Queen Square Genome database of 16000 exomes and the Munich in-house database of 21000 exomes (Supplementary Table 1). In silico tools strongly predicted an alteration of splicing (Human Splicing Finder: –165%). Subsequent cDNA analysis conducted as previously described (Krenn et al., 2019) from fibroblast RNA revealed the presence of an additional longer splice product in comparison with a control sample on gel electrophoresis (Fig. 1I). It should be noted, however, that this experiment did not exclude the expression of correctly spliced RNA from the respective allele. Subsequent Sanger sequencing of the two bands revealed that the splice variant resulted in the retention of intron 12 and introduction of a premature stop codon after 17 amino acids (Fig. 1K).

The missense variant was absent from our in-house databases and seen only once in a heterozygous state in 25124 gnomAD alleles. In silico tools consistently predicted the missense variant to be deleterious (PolyPhen-2: 0.987, SIFT: 0, CADD: 32, M-CAP: 0.644) (Supplementary Table 1). This altered amino acid was highly conserved (Fig. 1E) and located within the second cytidylyltransferase (CTP) catalytic domain of PCYT2 (Fig. 1C) (Pavluc et al., 2014) immediately adjacent to the p.His226X227Gly228His229 catalytic motif. Molecular Operating Environment version 2019.0102 using the Amber10: EHT force field was used for molecular dynamic simulation to predict the effect of p.(Val230Glu) on PCYT2 structure and function. The ligand binding pocket of p.(Val230Glu) was less open than the wild-type and resulted in CTP being far less likely to dock in its normal binding site in the p.(Val230Glu) variant. In addition, there were alterations between preferred amino acid residue binding within the catalytic site for both CTP and phosphoethanolamine for the p.(Val230Glu) variant compared with the wild-type enzyme (Fig. 1N). The p.(Val230Glu) change was immediately adjacent to the PCYT2 catalytic motif and molecular dynamics modelling predicted deficient substrate binding for the p.(Val230Glu) variant.

Case 2 was a 5-year-old male, who was born full-term to consanguineous parents of Baluchi ethnicity from Iran. He was born after an unremarkable pregnancy with a below-average birth weight and occipitofrontal circumference (15th percentile). The disease manifested through remarkable tonic-clonic seizures and paroxysmal tonic upgaze. Phenobarbital significantly reduced the frequency of the seizures to one per week, and he has been seizure-free for the last 4 months. Because of the lack of growth and prominent feeding difficulties, his current body mass index is 11.2 (the 1st percentile). Additionally, he had severe microcephaly, scoliosis and congenital cataract. There are signs of facial dysmorphism including large, low-set protruding ears, hypertelorism and a wide nasal bridge (Fig. 1H).

His neurological examination revealed bilateral visual impairment, paroxysmal tonic upgaze and dysphagia together with central hypotonia, severe appendicular spasticity and distal and proximal flexion contractures in the upper limbs (Supplementary Video 1). In addition, there was hyperextension of the knees, external deviation of the feet, brisk tendon reflexes, reduced power, and wasting of muscles (Fig. 1H). While his metabolic screening was unrevealing, brain MRI showed cerebral hemispheric, pontine, medullary and cerebellar atrophy with extensive hypomyelination and ventriculomegaly (Fig. 1J). EEG showed bilateral posterior abnormalities in the context of artefactual activity. Electrophysiology studies and ophthalmological examination were not available.

To identify the genetic cause of the disease in the affected individual, exome sequencing on DNA extracted from the proband’s leucocytes and variant filtering were performed as previously described (Makrythanasis et al., 2018). A novel homozygous missense variant in exon 3 of SELENO/EPT1 (c.134C>T, p.(Pro45Leu) (NM_033505.3) residing within a 23.7 Mb region of homozygosity was identified. The p.(Pro45Leu) variant was located in a highly conserved SELENO/EPT1 protein in the N-terminal region of an...
Figure 1 Genetic and clinical summary of the investigated cases with functional characterization of SELENOI/EPT1 p.(Pro45Leu) deficiency and molecular modelling of the PCYT2 p.(Val230Glu) variant. (A) Family tree for Case 1. Square = male; circle = female; filled symbol = affected individual; open symbols = unaffected carriers; WT = wild-type allele. (B) Family tree for Case 2. Square = male; circle = female; filled symbol = affected individual. The double line indicates consanguinity. (C) Domain structure of PCYT2β with localization of the disease-associated variants. PCYT2β splicing results in the deletion of residues 180–197 from the central linked segment (Pavlovic et al., 2014).
endoplasmic reticulum luminal-facing domain (CADD score of 26.9 and high conservation GERP score of 5.96) (Fig. 1D and F). The variant was absent from gnomAD and predicted to be probably damaging (PolyPhen-2) or deleterious/damaging (PROVEAN) (Supplementary Table 1).

To determine the functional significance of the variant, we assessed the effect of p.(Pro45Leu) on SELENOI/EPT1 activity in a yeast strain (Supplementary material). Cells expressing SELENOI/EPT1 p.(Pro45Leu) displayed a significant increase in radiolabel associated with CDP-ethanolamine compared with wild-type SELENOI/EPT1, and a decrease in radiolabel associated with the downstream lipids PE and phosphatidylcholine (Fig. 1L). This was consistent with an impairment in CDP-ethanolamine pathway activity at the SELENOI/EPT1-catalysed step. Western blots in yeast showed SELENOI/EPT1 and the mutant version exhibited a projected molecular weight of 46 kDa and were detected at a similar level (Fig. 1M).

Subcellular lipidome imbalance has been shown to play an important role in the mechanisms underlying motor neuron diseases (Rickman et al., 2020). Numerous genes involved in lipid metabolism have already been linked to HSP (Ahmed et al., 2017). PCYT2 and SELENOI/EPT1 are among the recently characterized HSP-associated genes and are currently reported only in a few families. PCYT2 has been reported in six independent families presenting with a phenotypic spectrum ranging from pure to complex forms of HSP (Vaz et al., 2019; Vélez-Santamaría et al., 2020). This was consistent with an impairment in CDP-ethanolamine pathway activity at the SELENOI/EPT1-catalysed step. Western blots in yeast showed SELENOI/EPT1 and the mutant version exhibited a projected molecular weight of 46 kDa and were detected at a similar level (Fig. 1M).

Impaired vision with nystagmus and seizures were among the frequent symptoms in reports of PCYT2. Variable features have included hearing loss, bilateral cataracts and ataxia. While brain MRI was unremarkable in one PCYT2 case with pure HSP, other cases showed progressive, non-extensive T2-weighted white matter hyperintensities with cerebro or cerebellar atrophy.

Case 1 presented with a complex and progressive phenotype partly overlapping with the previously reported PCYT2-related HSP cases including visual impairment, bilateral cataract, sensorineural deafness and seizures (Vaz et al., 2019; Vélez-Santamaría et al., 2020). In this case, we show that confirmed EOSRD could be the early manifestation and a dominant feature of PCYT2-associated disorder. In contrast with the previous PCYT2 reports, where cerebellar ataxia was a part of complex HSP in two affected individuals, Case 1 did not express spastic paraplegia, and severe cerebellar ataxia was the sole locomotor feature. This is not surprising, as various genes involving those involved in phospholipid metabolism have been shown to present with HSP at one end of the disease continuum and ataxia at the other. Because of the overlapping phenotypes and shared disease mechanisms, these genes have been proposed to cause the ataxia-spasticity disease spectrum (ASS) (Synofzik and Schüle, 2017). It should be acknowledged, however, that the suggested association between the compound heterozygous PCYT2 variants in Case 1 and ASS would have been strengthened if we had quantified the wild-type and misspliced mRNA resulting from the splice variant c.1112 + 1G > A or performed lipidomics studies.

SELENOI/EPT1 was first reported in Brain by Ahmed et al. (2017) in four siblings of Omani origin with a core
phenotype similar to that of our case, albeit with a milder course (Table 1). These siblings were verbal and had gradually regressed after delayed achievements of developmental milestones. The spasticity was limited to the lower limbs in all but one sibling. They did not have clinically expressed seizures, but cleft palate, bifid uvula, retinal abnormalities and neurophysiological evidence of a demyelinating peripheral neuropathy were among their distinguishable signs. Ocular examination revealed pigmentary retinopathy and rod-cone dysfunction. The second family with SELENOI/EPT1-linked complex HSP was reported by Horibata et al. (2018) and had a spectrum of main features closer to those seen in our case, although with additional signs such as sensorineural deafness and roving eye movements (Table 1).

Although there were conspicuously overlapping core symptoms between Case 2 and the aforementioned SELENOI/EPT1-linked complex HSP, there were also some notable differences. The first family displayed a more profound developmental delay with delayed achievement of motor milestones, whereas the second family showed a milder course with delayed linguistic milestones.

### Table 1 Summary of genetic findings and clinical features of SELENOI/EPT1 and PCYT2 cases

<table>
<thead>
<tr>
<th>Family</th>
<th>This report</th>
<th>Ahmed et al., 2017</th>
<th>Horibata et al., 2018</th>
<th>This report</th>
<th>Vaz et al., 2019</th>
<th>Vélez-Santamaría et al., 2020</th>
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<tbody>
<tr>
<td><strong>General information</strong></td>
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<tr>
<td>SELENOI/PCYT2 variants</td>
<td>Case 2</td>
<td>4 patients</td>
<td>1 patient</td>
<td>Case 1</td>
<td>5 patients</td>
<td>2 patients</td>
</tr>
<tr>
<td>SELENOI</td>
<td>c.134C&gt;T</td>
<td>(p.Pro45Leu)</td>
<td>SELENOI</td>
<td>c.335G&gt;C</td>
<td>(p.Arg112Pr)</td>
<td>PCYT2</td>
</tr>
<tr>
<td>PCYT2</td>
<td>c.732-2A&gt;G</td>
<td></td>
<td>PCYT2</td>
<td>c.1112+1G&gt;A;</td>
<td>(p.His244Tyr)/</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>c.743T&gt;A;</td>
<td>(p.Val230Glu)</td>
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<td><strong>Development</strong></td>
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<tr>
<td>Age (years)/sex</td>
<td>5/M</td>
<td>1.9–15/3M, 1F</td>
<td>4/M</td>
<td>26/F</td>
<td>2.5–20/4M, 1F</td>
<td>7 and 46/2M</td>
</tr>
<tr>
<td>Consanguinity</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+ (4)</td>
<td></td>
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<tr>
<td><strong>HSP-associated symptoms</strong></td>
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<tr>
<td>Progressive microcephaly</td>
<td>+ (S)</td>
<td>+</td>
<td>+ (S)</td>
<td>–</td>
<td>+ (5), IS</td>
<td>(1)</td>
</tr>
<tr>
<td>Dystrophic features</td>
<td>+ FD (4) CP, HP, BU</td>
<td>HP, BU</td>
<td>–</td>
<td>(S)</td>
<td>+ (5), 2P</td>
<td>(15)</td>
</tr>
<tr>
<td>Seizures</td>
<td>GTCS</td>
<td>–</td>
<td>–</td>
<td>TCS</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Visual impairment</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+ (4)</td>
<td></td>
</tr>
<tr>
<td>Sensorineural deafness</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+ (1)</td>
<td></td>
</tr>
<tr>
<td>Oculomotor abnormalities</td>
<td>+ TU</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+ (15)</td>
<td></td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>NA</td>
<td>+ (1)</td>
<td>NA</td>
<td>–</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Cerebellar ataxia</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+ (1)</td>
<td>(1)</td>
</tr>
<tr>
<td>Bilateral cataract</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+ (1)</td>
<td>(1)</td>
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<tr>
<td><strong>Neurological examination</strong></td>
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<tr>
<td>Nystagmus</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+ (4)</td>
<td>(1)</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
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<tr>
<td>Sparing UL, LL</td>
<td>+ (LL, UL)</td>
<td>+ (3 LL), 1 (UL, LL)</td>
<td>+ (UL, LL)</td>
<td>+ (LL)</td>
<td>+ (1 LL), (1 UL, LL)</td>
<td></td>
</tr>
<tr>
<td>Hyperreflexia</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hypotonia</td>
<td>–</td>
<td>–</td>
<td>Truncal</td>
<td>–</td>
<td>–</td>
<td></td>
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<tr>
<td>Joint contractures</td>
<td>+</td>
<td>NA</td>
<td>+</td>
<td>–</td>
<td>NA</td>
<td></td>
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<tr>
<td><strong>Investigations</strong></td>
<td></td>
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<tr>
<td>Brain MRI</td>
<td>WMH (S), CA, CRA, BSA</td>
<td>WMH</td>
<td>WMH (S), CA, CRA, TCC</td>
<td>CRA</td>
<td>WMH, CA, CRA</td>
<td>WMH (I)</td>
</tr>
<tr>
<td>Ocular examination</td>
<td>NA</td>
<td>RP (2), CRD (1)</td>
<td>Absent VEP; normal ERG</td>
<td>SRD</td>
<td>OA (1)</td>
<td>OA (1)</td>
</tr>
</tbody>
</table>

BS = brainstem atrophy; BU = bifid uvula; CA = cerebral atrophy; CP = cleft palate; CRA = cerebellar atrophy; CRD = cone-rod dysfunction; DD = developmental delay; ER = eye roving; ERG = electroretinography; F = female; FD = facial dysmorphism; FS = focal seizures; GTCS = generalized tonic-clonic seizures; HP = high arched palate; HSP = hereditary spastic paraplegia; ID = intellectual disability; LL = lower limbs; M = male; NA = not available; OA = optic atrophy; P = profound; RP = retinitis pigmentosa; S = severe; SRD = severe retinal dystrophy; TCC = thin corpus callosum; TCS = tonic-clonic seizures; TU = tonic upgaze; UL = upper limbs; VEP = visual evoked potentials; WMH = T2 white matter hyperintensity.

aImpaired word fluidity.
EPT1 reports, the spectrum of the SELENOI/EPT1 phenotype seemed to be more severe in the present case. Features including severe growth failure, unachieved motor milestones and communication skills, severe dysphagia, congenital cataract and facial dysmorphism distinguished our case from the previously published SELENOI/EPT1 families. Cerebellar atrophy, brain stem atrophy and extensive hypomyelination were not observed in the family described by Ahmed et al. (2017), but these features were prominent in Case 2 and the report by Horibata et al. (2018). In addition, tonic upgaze was a peculiar feature in our report that could putatively be ascribed to the observed degree of hypomyelination, pontine/medullary and cerebellar atrophy (Hills et al., 2013; Blumkin et al., 2015).

Of note was the overlapping progressive phenotype between PCYT2 and SELENOI/EPT1. Both expressed a comparable spectrum of associated symptoms and severity, suggesting the importance of the CDP-ethanolamine pathway in the mechanism of complex neurodegenerative disorders. PCYT2 reports have unequivocally demonstrated how ascertaining more cases might expand the disease phenotype towards the milder end of the spectrum to pure HSP, and suggested the possibility of a predominant cerebellar ataxia phenotype with no spastic paraplegia as in Case 1.

Visual impairment was a common feature for almost all affected individuals with biallelic variants in PCYT2 and SELENOI/EPT1. Ocular phenotyping revealed retinal pathology and optic atrophy, suggesting a particular susceptibility of retinal cells to the imbalance in CDP-ethanolamine and related pathways (Rickman et al., 2020).

In summary, we have presented further families with biallelic variants in PCYT2 and SELENOI/EPT1, thereby expanding the clinical spectrum of CDP-ethanolamine-related disorders. Additionally, we have highlighted the remarkable clinical overlap between PCYT2 and SELENOI/EPT1 with retinal involvement being the common sign. Finally, we have drawn attention to the possibility of a broad ASS, which may result from defective PE biosynthesis. Awareness of a possible ASS phenotype and further reports will improve our understanding of the clinical spectrum and disease continuum of emerging CDP-ethanolamine-related disorders.

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

Web resources
The URLs for data presented herein are as follows:
http://www.ensembl.org/index.html
Genome Aggregation Database; http://gnomad.broadinstitute.org/
http://www.iranome.ir/
http://evs.gs.washington.edu/EVS/
https://www.ebi.ac.uk/interpro/protein/UniProt/Q9C0D9/
The 1000 Genomes Browser; http://browser.1000genomes.org/index.html
UCSC Human Genome Database; http://www.genome.ucsc.edu
Combined Annotation Dependent Depletion (CADD); http://cadd.gs.washington.edu/
NeurOmics: http://rd-neuromics.eu
https://www.rcsb.org/

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Competing interests
The authors report no competing interests.

Supplementary material
Supplementary material is available at Brain online.

References


