Acute neurological regression following fever as presenting sign of pontocerebellar hypoplasia type 2D (*SEPSECS* mutation)

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Abstract. Pontocerebellar hypoplasia type 2D (PCH2D) is caused by mutations in the gene encoding O-phosphoseryl-tRNA:selenocysteinyl-tRNA synthase (*SEPSECS*; chromosome 4p15.2). This is a key enzyme in the biosynthesis of selenoproteins, which act in maintaining antioxidant systems. To date, 26 patients with PCH2D have been reported, all with neurological involvement characterized by progressive pontocerebellar and cerebral atrophy. The present study reports on a patient with compound heterozygosity in the *SEPSECS* gene, including a novel missense variant, c.440G>A (p.Ser147Asn). The patient exhibited acute neurological regression following a vaccination-related fever, which is reminiscent of primary mitochondrial disease. In addition, the patient displayed severe spastic tetraparesis, convergent strabismus and postnatal onset of microcephaly, as well as recurrent blood lactate elevation. Brain MRI showed multiple alterations in the peri/supraventricular and subcortical white matter and progressive pontocerebellar and cerebral atrophy. A review of the clinical spectrum associated with *SEPSECS* mutations was conducted and the first report on a patient with *SEPSECS* mutations of acute neurological regression following a catabolic stressor at the onset of PCH2D was provided. This study broadens the genetic background of PCH2D and associated PCH2D phenotype, supporting the causal link between selenoprotein biosynthesis deficiency and mitochondrial disorders.

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Introduction

Pontocerebellar hypoplasia (PCH) refers to a group of genetic, progressive disorders mostly affecting the infratentorial structures with the involvement of the cerebellum, pons and olivary nuclei, associated with atrophic changes of supratentorial structures: The cerebral cortex and basal ganglia. The actual classification is based on the identification of underlying molecular defects leading to the current recognition of 17 PCH types associated with 25 different genes. Based on the underlying molecular pathways, PCH may result from alteration in genes targeting transfer (t)RNA processing (I), other forms of RNA processing (II) or result from mutations in genes not related to RNA processing (1).

PCH2D is caused by mutation of the gene encoding O-phosphoseryl-tRNA selenocysteine (SEC):selenocysteinyl-tRNA synthase [*SEPSECS* (chr.4p15.2)]. This enzyme converts SEC to selenocystenenyl-tRNA in a process relying on the presence of the selenocysteine tRNA (tRNASec) and pyridoxal-5-phosphate. Sec-tRNASec acts as a selenium donor in selenoprotein biosynthesis. Mice with neuronal selenoprotein deficiency show marked cerebellar hypoplasia, associated with Purkinje cell death and decreased granule cell proliferation, indicating that selenoproteins are required for proper cerebellar development (2). The selenoproteins glutathione (GSH) peroxidase (GPXs) and selenoprotein H (SelH) have critical roles in the GSH-dependent and antioxidant systems, providing protection from reactive oxygen species (ROS). SelH increases the expression of the GSH-synthesis enzyme γ-glutamylcysteine synthetase, whereas GPXs catalyse the breakdown of peroxides into water (3).

To date, a total of 26 patients with PCH2D have been reported (1,4-21) with different mutant alleles and variable clinical phenotypes, exhibiting a certain degree of heterogeneity in terms of manifestations and severity. Progressive pontocerebellar and cerebral atrophy, microcephaly and mostly severe developmental disability are prominent neurological features.

The present study reports on a patient harbouring a novel *SEPSECS* mutation and presenting with postnatal microcephaly, progressive motor and intellectual disability, visual

defect and progressive cerebral and pontocerebellar atrophy, as well as recurrent blood lactate elevation. In light of the acute neurological regression following vaccination with fever in the present case, not previously reported in PCH2D, the clinical spectrum associated with *SEPSECS* mutations was reviewed with an emphasis on the causal link between selenoprotein biosynthesis deficiency and mitochondrial disorders.

Case report

Case presentation. The pediatric patient was male and born full-term (40th week of gestation) after an uncomplicated pregnancy, with an uneventful neonatal period. At birth, the body weight was 3.2 Kg (25th pc), and the length was 48 cm (25-50th pc), both in the normal range (25-75th pc) according to the Neonatal Anthropometric Charts for Italy (https://www.siedp.it). Head circumference (HC) was in the low-normal range (33 cm, 10th pc). Early psychomotor development was normal: Head control was gained at 3 months and sitting with support was achieved at 5 months. Following meningococcal vaccination at the age of six months, the patient experienced high fever (39˚C) associated with a seizure episode, followed by the acute onset of convergent strabismus and truncal hypertonia. Neurological examination at the age of 9 months revealed microcephaly (HC, 41 cm; <<3rd pc), internal strabismus, severe developmental delay, poor active movements with limb spasticity, absent handling and inadequate response to environmental stimuli. The patient was unable to keep his head on the trunk, sit, crawl and roll. Electroencephalogram (EEG) showed a prevalence of abnormal background rapid rhythms without epileptic discharges.

Brain MRI, performed at the age of 19 months, showed posterior periventricular white matter signal changes bilaterally and subcortical white matter hyperintensity, particularly in the insular temporal areas. Neuroimaging of the cerebellum and the brainstem were otherwise normal.

At 26 months of age, brain MRI showed slight peritrigonal white matter hyperintensity with a normal corpus callosum and reduction of the cerebral white matter with enlargement of the periencephalic and peritruncal cerebrospinal fluid (CSF) spaces associated with pontobulbar and vermian atrophy (Fig. 1A-C). Between two and five years of age, the patient had recurrent seizures associated with fever. Repeated EEG recording showed hypersynchronous activity in the central posterior regions.

The patient was first evaluated at our department (Child and Adolescent Neurology and Psychiatric Section, University Hospital Policlinico, Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy), at age 6 (November 2021). On physical examination, a sloping forehead, round face, thick nasal alae, low-set ears and thin elongated fingers were noted. Pertinent neurological findings included bilateral convergent strabismus, microcephaly and spastic quadriplegia with fixed fits and absent verbal language. The patient had recurrent episodes with irritability and inconsolable crying when away from home and daytime episodes of perioral tremor. No improvement with gradual titration of baclofen and clonazepam *per os* was observed over time.

Brain MRI showed slight asymmetric enlargement of the lateral ventricles, an enlarged fourth ventricle, multiple areas of altered signal in the peri/supraventricular and subcortical white matter, as well as deepening of the temporo-occipital sulci bilaterally. The white-matter volume was globally reduced with enlargement of subtentorial, pontine and bulbar cisternae, large cisterna magna and a wide pontocerebellar angle. There were overt signs of pontobulbar, cerebellar vermis and hemisphere atrophy with signal alteration in fluid-attenuated inversion recovery sequences (Fig. 1D-F).

Screening methods. Extensive metabolic screening and Array Comparative Genomic Hybridization (aCGH) were performed using the SurePrint G3 Custom CGH Microarray, 4x180K (Agilent Technologies, Inc.) according to the manufacturer's protocol, with appropriate Agilent reference DNAs (Euro male and Euro female). The array data extraction and analysis were performed using CytoGenomics v.5.0.2.5 (Agilent Technologies, Inc.). The extensive metabolic screening included quantitative analysis of urinary organic acids by gas chromatography/mass spectrometry (MS) (22). A blood amino acid (AA) profile was obtained from dried blood spots (DBS) by electrospray ionization-tandem MS (ESI-MS) (25 metabolites, including 14 AAs and 11 AA ratios, were simultaneously measured). The following AAs were determined: Alanine (Ala), arginine (Arg), citrulline (Cit), glutamate (Glu), glutamine (Gln), glycine (Gly), leucine (Leu), methionine (Met), ornithine, phenylalanine (Phe), proline, tyrosine (Tyr) and valine (Val). The following AA ratios were measured: Leu+Val/Phe+Tyr, Cit/Arg, Cit/Phe, Leu/Phe, Met/Leu, Met/Phe, Phe/Tyr, Glu/Gln, Tyr/Leu, Tyr/Met and Val/Phe). The anion gap to check for metabolic acidosis (22) was determined to measure the difference between the primary measured cations (sodium and potassium) and the primary measured anions (chloride and bicarbonate) in serum. Determination of blood acyl-carnitine levels included measures of short-chain, medium-chain and long-chain acyl-carnitines from DBS by using ESI-MS (22). Serum transferrin glycoform analysis was performed by capillary electrophoresis and serum lysosomal enzymes (β-hexosaminidase and β-galactosidase) were measured fluorometrically (23).

Whole blood (3 ml) was collected from the proband and the proband's parents for NGS analysis after informed consent had been provided. NGS analyses were performed by Research & Innovation Genetics Srl (Padua-Italy). DNA library preparation and whole-exome enrichment were performed using the SureSelect All Exon V6 kit (Agilent Technologies, Inc.). The library was sequenced using the NextSeq 2000 Illumina Sequencer (100-bp paired-end reads; Illumina, Inc.) according to the manufacturer's instructions. Bioinformatics analysis included the following: i) NGS reads were aligned to the GRCh37 human reference genome using the Burrows-Wheeler Alignment tool (https://bio-bwa.sourceforge.net/) with the default parameters; ii) PCR duplicate removal using Picard (http://picard.sourceforge.net); iii) identification of single nucleotide polymorphisms and insertions/deletions using the Genome Analysis Toolkit (GATK 4; https://gatk.broadinstitute.org/hc/en-us); iv) variant annotation using snpEff (http://snpeff.sourceforge.net). Whole-exome sequencing (WES) data and read alignment analysis were checked for coverage depth and alignment quality using the Bedtools software package

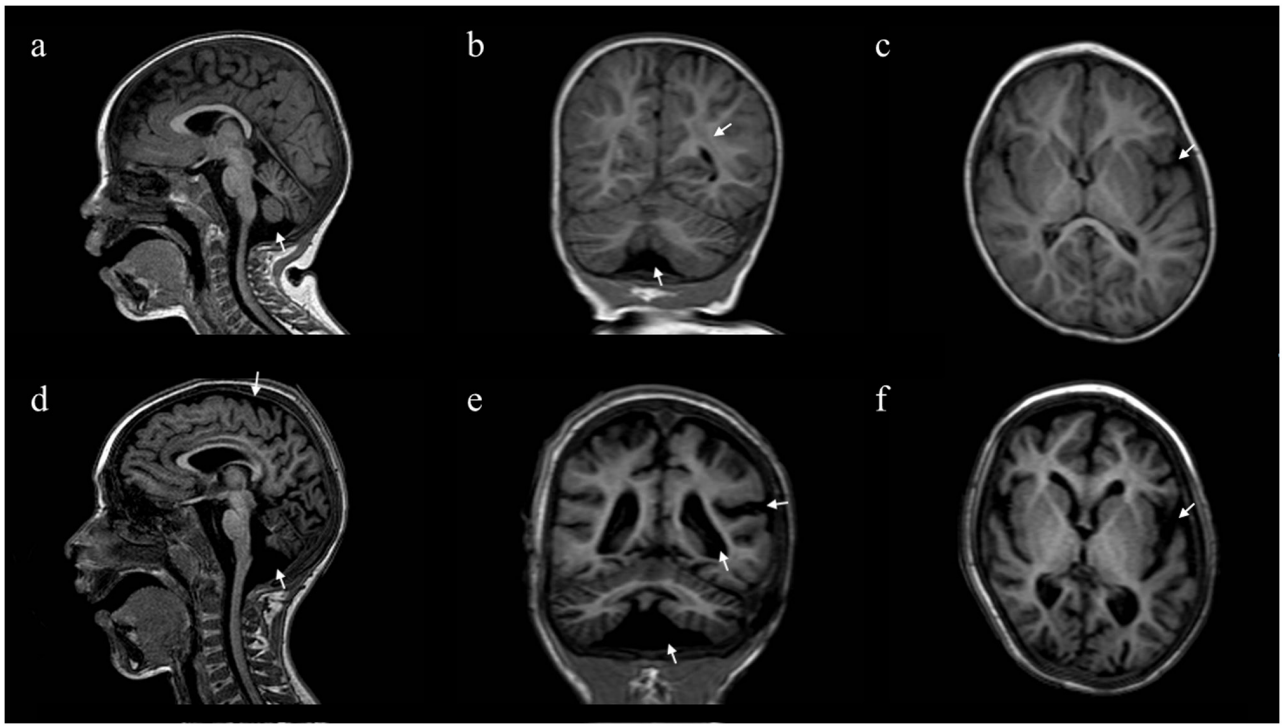

Figure 1. Brain MRI follow-up. Axial, sagittal and coronal T1-weighted sequences of the brain MRI performed at (A-C) 26 months and (D-F) at 6 years of age. Neuroimaging at first evaluation showed (A) ventricular widening with initial signs of cerebellar atrophy, (B) atrophy of the cerebellar vermis relatively sparing cerebellar hemispheres and (C) diffuse cortical atrophy particularly in the temporal lobes bilaterally. Follow-up examination showed (D) enlargement of the cerebrospinal fluid spaces, including sub-tentorial, pontine and bulbar cisternae, (E) global reduction of the bi-hemispheric white matter, enlarged cisterna magna (F) progressive cortical atrophy, particularly in the occipital and posterior temporal lobes bilaterally.

(https://github.com/arq5x/bedtools2). Variant classification was performed in accordance with the guidelines from the American College of Medical Genetics and Genomics (ACMG) (24). Phenotype-driven analysis, coupled with the employment of *in silico* multigene panels specific for microcephaly and neurodevelopmental disorders, was used to filter, select and interpret genetic variants obtained following exome sequencing. The PCR products containing significant variants underwent Sanger sequencing and capillary electrophoresis using an 3100-avant automatic sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions (https://tools.thermofisher.com/content/sfs/manuals/cms_041003.pdf).

Findings of the screening analysis. Extensive metabolic screening, including urinary organic acids and plasma amino acids, anion gap, serum transferrin glycoform analyses and lysosomal enzymes yielded normal results. Isolated, recurrent elevations of lactic acid levels (25 mg/dl at the age of 12 months and 40.2 mg/dl at around the age of 2 years; normal range: 5.7-22.0 mg/dl) were detected (Table I). Additional evidence of mitochondrial dysfunction was found by acyl-carnitine analyses, illustrating a decrease in blood acetyl-carnitine. This is formed by the enzyme carnitine acetyltransferase, which transfers a 2-carbon moiety from acetyl-CoA to L-carnitine. A decrease of acetyl-CoA, the substrate for the synthesis of acetyl-carnitine, is found in the case of increased oxidative stress due to oxidative damage, particularly when several key enzymes of acetyl-CoA and energy metabolism are oxidatively modified (25).

aCGH analysis was normal. Sequencing analysis of the coding exons of the genes included in the genetic panel for the molecular diagnosis of microcephaly and neurodevelopmental disorders showed two variants in *SEPSECS*: A paternal NM_016955.4:c.114+3A>G and maternal NM_016955.4:c.440G>A (p.Ser147Asn) (Fig. 2A).

The variant c.114+3A>G arises in the consensus sequence of the splicing donor site (+3 of exon 1). Functional studies performed on the transcript revealed that it induces exon 1 skipping in the *SEPSECS* gene, including the translation initiation codon ATG, thus resulting in a possible loss of function of the protein (20). The variant is considered pathogenic according to the ACMG criteria (24).

The variant c.440G>A (p.Ser147Asn) has not been reported in the medical literature, to the best of our knowledge. It leads to the substitution of serine at codon 147 by asparagine in a conserved protein position as measured by PhyloP scores (https://ionreporter.thermofisher.com/) which measure evolutionary conservation at individual alignment sites with positive scores indicating sites that are predicted to be conserved (PhyloP-Vertebrate=5.64/6.42; phyloP-Primate=0.56/0.65; PhastCons=1.00/1.00; https://varsome.com/). *In silico* computational analysis indicated the harmful effect probability of p.Ser147Asn amino acid substitution on the structure/activity of the resulting protein [PolyPhen2=0.996/1.00 (PolyPhen-2: Prediction of functional effects of human nsSNPs; http://genetics.bwh.harvard.edu/pph2/); SIFT=0.005/0.00; MutationTaster=1.00/1.00 (https://www.mutationtaster.org/); CADD PHRED=26; Mutation Assessor=2.85/5.00 (http://mutationassessor.org/r3/)].

Table I. Clinical, neuroimaging and genetic data of reported patients with *SEPSECS* mutations.

First author, year	Families	Pts	Sex	Mutation	Effect	Genotype	Brain MRI	Microcephaly	Spasticity	Walking	Epilepsy (seizure type)	Vision	DD/ ID	Language	Other	First signs	Mitochondrial signs	(Refs.)
Ben-Zeev, 2003; Agamy, 2010	2	4	M	c.1001A>G	Missense	Homozygous	Cerebellar/ cerebral atrophy	+ (secondary)	+	ND	+ (generalized, myoclonic)	Nystagmus	+	Non-verbal	-	ND	-	(4,5)
			F	c.1001A>G	Missense	Homozygous	Normal at 5 m; progressive cerebellar/ frontal parasylvian atrophy	+ (secondary)	+	-	-	ND	+	Non-verbal	Chorea	ND	-	
			M	c.1001A>c. 715G>A	Missense missense	Compound heterozygous	Normal at 5 m; progressive cerebellar/ frontal parasylvian atrophy	+ (secondary)	+	-	+ (focal, generalized)	Partial visual pursuit	+	Non-verbal	Chorea	ND	-	
			F	c.1001A>G c.715G>A	Missense missense	Compound heterozygous	w.m..changes thin corpus callosum at 8 m; progressive cerebellar atrophy	+ (secondary)	+	-	-	No visual pursuit	+	Non-verbal	-	ND	-	
Makrythanasis, 2014	1	1	F	c.1466A>7	-	Homozygous	Cerebellar vermis atrophy	+	+	ND	+	ND	ND	ND	-	DD	-	(6)
Alazamy, 2015	1	1	-	c.1027_ 1120del	-	Homozygous	-	ND	+	ND	ND	Nystagmus	ND	Dysarthria	-	ND	-	(7)
Anttonen, 2015	3	3	F	c.974C>G c.1287C>A	Missense nonsense	Compound heterozygous	Early w.m. changes; progressive cerebellar/ cerebral atrophy	ND	+	-	+ (infantile spasms)	Central blindness	+	Delay	High TSH	Opisthotonus (birth)	Elevated blood and CSF lactate	(8)
			F	c.974C>Gc. 1287C>A	Missense nonsense	Compound heterozygous	Early w.m. changes; progressive cerebellar/ cerebral atrophy	ND	+	-	+ (infantile spasms)	Central blindeness	+	Delay	High TSH	Opisthotonus (1 m)	Elevated blood and CSF lactate	

Table I. Continued.

First author, year	Families	Pts.	Sex	Mutation	Effect	Genotype	Brain MRI	Microcephaly	Spasticity	Walking	Epilepsy (seizure type)	Vision	DD/ ID	Language	Other	First signs	Mitochondrial signs	(Refs.)
			M	c.974C>G c.1287C>A	Missense nonsense	Compound heterozygous	Progressive cerebellar and cerebral atrophy	+ (primary)	+	-	+	Central visual defect	+	Delay	-	Opisthotonus (2 m)	Elevated CSF lactate	
Zhu, 2015	1	1	F	c.1A>G c.388+3A>G	Missense splicing	Compound heterozygous	Progressive cerebellar atrophy	+	ND	ND	ND	ND	ND	ND	-	Hypotonia, DD (5 m)	-	(9)
Iwama, 2016	2	2	F	c.356A>G c.77delG	-	Compound heterozygous	Normal at 2 y; progressive cerebellar/ frontoparietal atrophy	+ (secondary)	+	Ataxic gait	-	Nystagmus	+	Slurred speech	-	Downward nystagmus (3 m)	-	(10)
			F	c.356A>G c.467G>A	-	Compound heterozygous	Normal at 5 y; progressive cerebellar/ cerebral atrophy	+ (primary)	+	Ataxic gait	-	ND	ND	Slurred speech	-	DD	-	
Pavlidou, 2016	1	1	M	c.1001T>C	Missense	Homozygosity	Ventricular widening at 18 m; progressive cerebellar/ cerebral atrophy	+ (secondary)	+	Quadriplegia	ND	Optic nerve atrophy	+	Delay	High CK	Hypotonia	Myopathic features; decreased mitochondrial respiratory chain complex IV at muscle biopsy	(11)
Olson, 2017	1	1	M	c.176CC>T	-	Homozygous	w.m changes; cerebral/ ponto-cerebellar atrophy	+	ND	ND	+ (epileptic encephalopathy)	ND	ND	ND	-	Hypotonia DD	-	(12)
Van Dijk, 2018	1	1	F	c.1321G>A	Missense	Homozygous	Progressive cerebellar atrophy	+	-	Broad-based gait	ND	Horizontal nystagmus	+	Able to say few words	-	DD	-	(13)
Hengel, 2020	1	1	-	c.181A>G	-	Homozygous	Ponto-cerebellar hypoplasia	ND	ND	ND	ND	ND	+	ND	-	ND	-	(14)
Arrudi-Moreno 2021,	1	1	F	c.114+3A>G	Splicing disruption	Homozygous	Normal at 14 m; progressive ponto-cerebellar atrophy	+ (secondary)	+	Tetraplegia	-	Convergent strabismus	ND	Able to say few words	-	DD	-	(15)

Table I. Continued.

First author, year	Families	Pts.	Sex	Mutation	Effect	Genotype	Brain MRI	Microcephaly	Spasticity	Walking	Epilepsy (seizure type)	Vision	DD/ID	Language	Other	First signs	Mitochondrial signs	(Refs.)
Nicita, 2022	1	1	M	c.865C>T c.1297T>C	Missense	Compound heterozygous	Cerebral atrophy; thin corpus callosum	+	-	Wide-based gait; bradykinesia	ND	Nystagmus, optic nerve atrophy	ND	Dysarthria	Dystonia, low IgA	Head titubation, ocular nystagmus (13 m)	-	(16)
Martinez-Martin, 2022	1	2	M	c.1321G>A	Missense	Homozygous	Mild atrophy of cerebellar vermis	-	Pyramidal signs	Ataxic gait	-	Convergent strabismus	+	Scanning speech	-	Learning difficulties and motor impairment (7 y)	-	(17)
			M	c.1321G>A	Missense	Homozygous	Normal	-	-	Unable to walk in tandem	-	Nystagmus	+	Scanning speech	-	Motor and language impairment (5 y)	-	
Ramadesikan, 2022	1	2	F	c.1A>T c.846G>A	Loss of function; splicing disruption	Compound heterozygous	w.m. changes at birth; progressive cerebral/ ponto-cerebellar atrophy	+ (secondary)	ND	-	+ (febrile, later focal clusters)	Cortical blindness	+	Non-verbal	-	Abnormal posturing (birth)	Decreased activity of complex I -II at muscle biopsy	(18)
			M	c.1A>T c.846G>A	Loss of function; splicing disruption	Compound heterozygous	Progressive cerebral/ cerebellar atrophy	+ (secondary)	ND	-	+ (febrile, later focal clusters)	Cortical vision imparment	+	Non-verbal	Respiratory failure	Hypotonia	Decreased activity of complex I- II at muscle biopsy	
Rong, 2022	1	1	F	c.701+1G> A c.194A>G	Splicing; missense	Compound heterozygous	Bilateral pallidum signal changes at 15 m; progressive fronto-temporal atrophy	-	ND	ND	- (15 m seizure-like symptoms)	Esotropia	+	Delay	-	Hypotonia	Limbs myogenic changes	(19)
Zhao, 2022	1	1	-	c.628C>T c.770T>C	-	Compound heterozygous	Normal at 28 y; progressive cerebellar atrophy	ND	ND	Ataxic gait	-	ND	+	Slurred speech	Dysph-agia	Bradykinesiat 24 y	-	(21)

Table I. Continued.

First author, year	Families	Pts.	Sex	Mutation	Effect	Genotype	Brain MRI	Microcephaly	Spasticity	Walking	Epilepsy (seizure type)	Vision	DD/ ID	Language	Other	First signs	Mitochondrial signs	(Refs.)
Ghasemi, 2024	2	2	M M	c.208T>C	Missense	Homozygous	ND	ND	ND	Motor delay at 3 y	Febrile seizures	Strabismus	+	Delay	-	ND	-	(1)
				c.1274A>G	Missense	Homozygosity	Cerebellar atrophy at 4 years	ND	+	Ataxia at 4 y	Febrile seizures	Nystagmus	+	Delay	Neuropathy	DD	-	
Present study	1	1	M	c.114+3A> G c.440 G>A	Splicing missense	Compound heterozygous	w.m. changes at 17 m; progressive pontocerebellar and cerebral atrophy	+ (secondary)	+	Tetraplegia	+ (febrile seizures generalized epilepsy)	Convergent strabismus	+	Non-verbal	-	Squint, hypertonia following fever	Elevated blood lactate (25 mg/dl at the age of 12 months and 40.2 mg/dl at around the age of 2 years; normal range: 5.7-22.0 mg/dl)	-

Pts., patients; MRI, magnetic resonance imaging; M, male; F, female; DD, developmental delay; ID, intellectual disability; w.m., white matter; CSF, cerebrospinal fluid; CK, creatine kinase; TSH, thyroid-stimulating hormone; ND, not determined; m, months; y, years; + present; - absent.

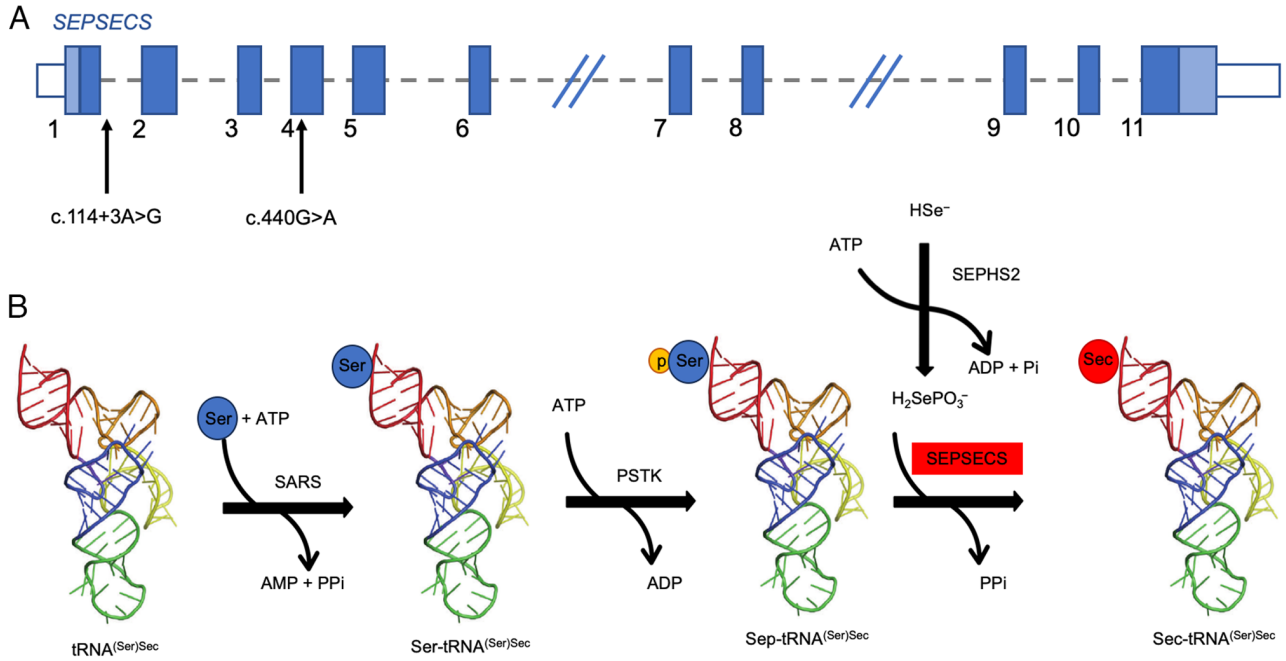

Figure 2. Location of the whole-exome sequencing variants of the patient within the *SEPSECS* gene and role of SepSecS in selenocysteine biosinthesis. (A) The two variants found in the proband are shown in the schematic representation of the *SEPSECS* gene. (B) In humans, Sec-tRNA$^{(Ser)Sec}$ synthesis follows a two-step process via a phosphorylated intermediate: tRNA$^{(Ser)Sec}$ is converted to Ser-tRNA$^{(Ser)Sec}$, which is phosphorylated to Sep-tRNA$^{(Ser)Sec}$. O-phosphoseryl-tRNA:selenocysteinyl-tRNA synthase, encoded by the *SEPSECS* gene, converts Sep-tRNA$^{(Ser)Sec}$ to Sec-tRNA$^{(Ser)Sec}$, which acts as a selenium donor in selenoprotein biosynthesis. SepSeS, O-phosphoseryl-tRNA selenocysteine:selenocysteinyl-tRNA synthase; Sec-tRNA$^{(Ser)Sec}$, selenocysteine tRNA (serine) selenocysteine; Ser-tRNA$^{(Ser)Sec}$, seryl tRNA (serine) selenocysteine; Sep (O-phosphoserine) tRNA, Sec (selenocysteine) tRNA synthase; Sec-tRNA$^{(Ser)Sec}$, selenocysteine-tRNA (serine) selenocysteine.

According to the ACMG criteria PP3 (multiple computational evidence supports a deleterious effect on the gene) and PM3 (absence or extremely low frequency of the variant from controls in the 1000 Genomes Project, Exome Sequencing Project, or other large population datasets), the variant can be classified as likely pathogenic. Both variants were deposited in ClinVar [accession nos. SCV005061756 (https://www.ncbi.nlm.nih.gov/clinvar/variation/984624/?oq=SCV005061756&m=NM_016955.4(SEPSECS):c.114%203A%3EG) and SCV005061752 (https://www.ncbi.nlm.nih.gov/clinvar/variation/3242529/?oq=SCV005061752&m=NM_016955.4(SEPSECS):c.440G%3EA%20(p.Ser147Asn), respectively]. No other significant variants were identified in the whole dataset (dataset available at https://doi.org/10.5281/zenodo.13234362).

Discussion

To date, 27 patients with ascertained *SEPSECS* mutations have been identified, including the present one (Table I). Most patients had an uneventful pre- and perinatal history with the onset of neurological signs in the first 2 years of life. A small minority of patients (10,17,21) had a later onset of neurological symptoms, even in adulthood. In the most severe phenotypes, patients rapidly experienced an obvious worsening of clinical conditions, with a post-natal onset of microcephaly, cerebellar signs and spasticity. Congenital microcephaly was described in 2 subjects (8,10) and a normal cranial circumference throughout the follow-up was reported in 3 patients (17,19). Motor function impairment was variable with most patients experiencing spastic quadriplegia, inability to walk and decreased motor function (12 patients) (4,8,11,15,18) and the current study. Milder cerebellar symptoms were reported in milder affected subjects with later disease onset (4 patients). Choreiform movements (2 patients) (4) and extrapyramidal symptoms resembling dystonia-parkinsonism (1 patient) (16) were infrequent. Intellectual disability was an almost constant feature and verbal communication appeared to be variably affected from the total lack of communicative skills (4,18) to dysarthria and slurred speech (21).

Visual impairment was reported in 9 patients (4,8,11,16,18) with strabismus and nystagmus as common early signs. Epilepsy, reported in 7 patients (4,6,8,12,18), may be either focal or generalized with tonic, myoclonic and tonic-clonic seizures; 2 patients developed febrile convulsions with later onset of non-febrile seizures (18) and an additional patient presented with an early-onset developmental and epileptic encephalopathy with a burst suppression EEG pattern (12).

Brain MRI was normal in the first months of life in certain patients. In milder phenotypes (10,17,21), no alterations were detected even for years, and in one patient, noticeably, neuroimaging was normal until the age of 28 years (21). In the majority of patients, however, white matter changes, particularly in the frontal lobes, were detected early in infancy and preceded the emergence of progressive pontocerebellar atrophy associated with cerebral atrophy. Neuropathological findings included laminar subtotal necrosis of the neocortex, particularly in the parieto-occipital regions, myelin loss and gliosis of white matter, consistent with degeneration secondary to neuronal loss, severe degeneration and atrophy of the brainstem and cerebellar cortex with an olivopontocerebellar atrophy-like

appearance, subtotal striatal degeneration and thalamic atrophy (8).

Pathogenic recessive mutations in the *SEPSECS* gene have been shown to alter conserved residues and to be mostly nonsense, missense or inducing splicing disruption. Among the reported genetic variants, some, like Ala239Thr, Thr325Ser, Tyr334Cys and Tyr429*, are associated with severe early-onset phenotypes, reducing protein stability and increasing misfolding, diminishing the ability of the SepSecS tetramer to bind $tRNA^{Sec}$ and affecting the integrity of the active site (26). Others, such as Gly441Arg, which was found in homozygosity in subjects with milder and late-onset phenotypes, may have a less destructive effect on the catalytic capacity of the enzyme (13).

The current study illustrates a severe early onset PHC2D type caused by compound heterozygous variants c.114+3A>G and c.440G>A (p.Ser147Asn), splicing and missense mutations, respectively. The first variant has been previously described in homozygosity in two unrelated patients with a severe early-onset phenotype and classified as likely pathogenic or pathogenic (15,20). The latter has not been reported in the literature and dedicated databases, to the best of our knowledge; it is rare, arises in a conserved protein position and *in silico* prediction analysis indicated a probably harmful effect.

The trace element Selenium (Se) is a vital micronutrient incorporated into proteins, named selenoproteins, by the amino acid SEC. Serine is added to tRNASec by seryl-tRNA synthetase and then modified to phosphoserine by phosphoseryl-tRNA kinase. Dietary selenium is added to phosphoserine by selenocysteine synthetase to produce SEC (Fig. 2B). Selenoproteins have critical roles in both the GSH-dependent and thioredoxin-dependent antioxidant systems (3).

In the present patient, it appears that acute neurological regression may have been triggered by fever related to vaccination, which is a phenomenon observed in patients with primary mitochondrial diseases (27). Similarities between mitochondrial disorders and selenoprotein biosynthesis defects have been emphasized owing to the consolidated role of selenoproteins in maintaining cellular redox potential and detoxifying H_2O_2 (3). In this regard, recurrent elevation of lactate levels in blood or CSF was observed in 3 out of four studied patients with PCH2D (8) and in the present one. Furthermore, the patient of the present study had a depletion of acetyl-L-carnitine in blood, indicating a deficiency in the regulation of energy metabolism and oxidative stress, since the acetyl moiety is used for producing energy and acts as an antioxidant protecting against oxidative stress (28). Myopathic features and decreased mitochondrial respiratory chain complex I, II and IV were found in three studied patients (11,18). Increased brain protein carbonylation as a sign of oxidative stress was described post-mortem in patients with *SEPSECS* deficiency, as well as moderate liver degeneration resembling mitochondrial encephalopathy (8). In sum, the present study was the first report of acute neurological regression following a catabolic stressor as the presenting sign of PCH2D. In light of the current understanding of the potential relationship between selenoprotein defects and mitochondrial dysfunction (3,8,11,18), it is important to highlight that patients with *SEPSECS* mutations may produce elevated levels of toxic metabolites and ROS during a catabolic state induced by physiological stressors such as fasting, fever, illness, trauma or surgery. Therefore, it may be advisable to consider preventive measures to avoid catabolic stressors in afflicted patients.

Further studies are necessary to characterize the role of *SEPSECS* pathogenic variants in triggering oxidative damage contributing to a severe neurological presentation of PCHD2.

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Availability of data and materials

Original data generated using WES have been deposited in the public database Zenodo (https://doi.org/10.5281/zenodo.13234362, accession no. 10.5281/zenodo.13234362). Detected variants were submitted to ClinVar (https://submit.ncbi.nlm.nih.gov/subs/variation_clinvar; accession nos. SCV005061756 and SCV005061752, respectively). Additional data generated in the present study are included in the figures and tables of this article.

Authors' contributions

RB and FP conceptualized the study. RB, FP, VM, FC and GR performed clinical procedures and acquired data. MF processed the data generated by WES and deposited them in public databases. RB, FP and MF checked and confirmed the authenticity of the raw data. FP and RB were major contributors in writing the manuscript. RR, MDC, MF and RB reviewed and edited the manuscript. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

All procedures performed in this study are part of the routine clinical care of patients with neurodevelopmental disorders, performed in accordance with the ethical standards of the institutional research committee at the University Hospital Policlinico 'G. Rodolico-San Marco' Catania (Catania, Italy) and with the 1964 Helsinki declaration and its later amendments. Written informed consent for all medical procedures was obtained from the patient's parents.

Patient consent for publication

Written consent for the publication of case data, medical images, genetic information and data reported within this article, was obtained from the patient's parents.

Competing interests

The authors declare that they have no competing interests.

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