Selenoprotein biosynthesis defect causes progressive encephalopathy with elevated lactate

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ABSTRACT

Objective: We aimed to decipher the molecular genetic basis of disease in a cohort of children with a uniform clinical presentation of neonatal irritability, spastic or dystonic quadriplegia, virtually absent psychomotor development, axonal neuropathy, and elevated blood/CSF lactate.

Methods: We performed whole-exome sequencing of blood DNA from the index patients. Detected compound heterozygous mutations were confirmed by Sanger sequencing. Structural predictions and a bacterial activity assay were performed to evaluate the functional consequences of the mutations. Mass spectrometry, Western blotting, and protein oxidation detection were used to analyze the effects of selenoprotein deficiency.

Results: Neuropathology indicated laminar necrosis and severe loss of myelin, with neuron loss and astrogliosis. In 3 families, we identified a missense (p.Thr325Ser) and a nonsense (p.Tyr429*) mutation in *SEPSECS*, encoding the O-phosphoseryl-tRNA:selenocysteinyl-tRNA synthase, which was previously associated with progressive cerebellocerebral atrophy. We show that the mutations do not completely abolish the activity of *SEPSECS*, but lead to decreased selenoprotein levels, with demonstrated increase in oxidative protein damage in the patient brain.

Conclusions: These results extend the phenotypes caused by defective selenocysteine biosynthesis, and suggest *SEPSECS* as a candidate gene for progressive encephalopathies with lactate elevation. *Neurology*® 2015;85:306–315

GLOSSARY

PCH2D = pontocerebellar hypoplasia type 2D; **PEHO** = progressive encephalopathy with edema, hypsarrhythmia, and optic atrophy; **RC** = respiratory chain; **SRM-MS** = selected reaction monitoring-mass spectrometry; **T₄** = thyroxine; **tRNA** = transfer RNA; **TSH** = thyroid-stimulating hormone; **T₃** = triiodothyronine.

Mitochondrial dysfunction is a frequent cause of childhood encephalopathy. Besides the typical multisystemic disorders, an increasing number of mitochondrial defects are shown to cause a CNS-specific phenotype.^{1–5} Lactate elevation raises suspicion of mitochondrial involvement and may be observed even in encephalopathies in which muscle biopsies show normal mitochondrial respiratory chain (RC) function.^{1–3,6} Within our cohort of pediatric patients, we identified patients with an undefined cause of cerebellocerebral atrophy, seizures, severe spasticity, and axonal neuropathy with lactate elevation. We report that despite many of the clinical and neuropathologic signs pointing toward mitochondrial impairment, the patients had novel mutations in the *SEPSECS* gene, which functions in cytoplasmic transfer RNA (tRNA)-charging in the selenoprotein biosynthesis pathway. We describe the uniform clinical, neuroradiologic, and neuropathologic features of this entity and a detailed mutation

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Supplemental data
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characterization. Moreover, our results indicate oxidative damage in the brain as part of the pathogenic mechanism resulting from selenoprotein deficiency.

METHODS **Standard protocol approvals, registrations, and patient consents.** All patient and control samples were taken according to the Declaration of Helsinki, with informed consent. The study was approved by the review board of the Helsinki University Central Hospital.

The patients were identified within a cohort of 64 clinically similar patients. One patient (patient 3) was part of the original PEHO (progressive encephalopathy with edema, hypsarrhythmia, and optic atrophy) syndrome patient series⁷ and was included in the neuroradiologic (group A) and ophthalmologic study (patient 11) of that series.

A detailed neuropathologic examination was available for 3 patients (patients 1, 2, and 3), including the spinal cord from patient 1; from patient 4, records pertaining to cerebellum, brainstem, and cerebral hemispheres were available. General autopsy records were available from 3 patients (patients 1, 2, and 4). Fresh-frozen tissue samples of patient 3 were available for the study as well as fibroblasts of patients 1 and 2 and myoblasts of patient 2.

DNA sequencing. For whole-exome sequencing, the exome targets of the patients' DNA were captured with the NimbleGen Sequence Capture 2.1M Human Exome v2.0 array (NimbleGen, Basel, Switzerland) followed by sequencing with the Illumina Genome Analyzer-IIX platform (Illumina, Inc., San Diego, CA) with 2 × 82 base pair paired-end reads. The variant calling pipeline of the Finnish Institute for Molecular Medicine was used for the reference genome alignment and variant calling.⁸ The coding exons of *SEPSECS* were sequenced by Sanger sequencing.

Structural analysis of the mutations. Structural analysis was based on the crystal structure of the human SEPSECS-tRNA^{Sec} binary complex (PDB ID: 3HL2). The SEPSECS mutants p.Thr325Ser and p.Tyr429* were generated in silico and analyzed in Coot.⁹ All figures were produced in PyMOL (The PyMOL Molecular Graphics System, version 1.5.0.4, Schrödinger, LLC).

Oxyblot. The brain protein lysates were extracted using RIPA buffer, and Oxyblot method was performed using an OxyBlot Protein Oxidation Detection Kit (Millipore Corp., Billerica, MA) according to the manufacturer's instructions. The e-Methods on the *Neurology*[®] Web site at Neurology.org include full descriptions of haplotype analysis, in vivo activity assay, and protein analysis methods.

RESULTS **Clinical data.** We investigated 4 children from 3 unrelated Finnish families. Clinical features of the patients are summarized in table 1. These children were born after uncomplicated pregnancies at term to healthy nonconsanguineous parents. Two patients were microcephalic at birth. The children were irritable from birth and presented by the age of 1 to 2 months with opisthotonus posturing, absent head control, tremors, and myoclonic jerks. Severe spastic or dystonic quadriplegia with absent psychomotor development became evident during the first few months. Three patients had epileptic seizures,

including infantile spasms. As a sign of peripheral neuropathy, the deep tendon reflexes attenuated or vanished by the age of 2.5 years. The optic discs were pale but not atrophic. All patients had edema of hands, feet, and face, as well as narrow forehead, tapering fingers, and high palate.

In 2 patients, early EEG studies (younger than 6 months of age) were normal, and later, hypsarrhythmia with infantile spasms was documented. Later EEG recordings showed severe slowing of background activity. Sensory axonal neuropathy was verified by sural nerve biopsy and electroneuromyography (table 1).

Two patients had elevated blood lactate levels and one of them also had elevated lactate in the CSF. Patients 1 and 2 showed mild elevation of thyroid-stimulating hormone (TSH) with normal levels of thyroxine (T₄) (table 1). Triiodothyronine (T₃) levels were not available. Other laboratory evaluations, including liver transaminases, were unremarkable.

Neuroradiology and neuropathology showed neuron and myelin loss. Neuroradiologic examinations showed progressive cerebellar atrophy and less pronounced cerebral atrophy (table e-1). Myelination was delayed in the early MRIs and subsequently arrested. Cerebral white matter showed pronounced and progressive volume loss (figure 1).

Neuropathologic analysis revealed that all 4 patients shared features of progressive neuronal degeneration: laminar subtotal necrosis of the neocortex, which was especially pronounced in the parietooccipital regions (figure 2A), with relative sparing of the hippocampi. The white matter showed myelin loss and pallor with gliosis, consistent with degeneration secondary to the neuronal loss. Patient 1 had subtotal striatal degeneration (figure 2B) and also some thalamic atrophy. All cases shared a quite severe degeneration and atrophy of the brainstem and cerebellar cortex, creating an olivopontocerebellar atrophy–like appearance: basis pontis, inferior olives, and tegmentum of the medulla oblongata were severely atrophic; the cerebellar cortex was severely atrophic as well (figure 2, C and D). Here, the molecular layer was thin, accompanied by a subtotal loss of Purkinje cells and a very thin granule cell layer (figure 2E). The spinal cord, available from one case, showed atrophy and degeneration especially in the posterior columns. Finally, the general autopsy revealed mild to moderate mostly microvacuolar fatty degeneration of the liver parenchyma (figure 2F).

SEPSECS mutations identified as the genetic cause. The genetic cause of the disease was identified by whole-exome sequencing of DNA samples of 2 patients (patients 1 and 2) from 2 unrelated families. The identified variants were first filtered to exclude nongenetic variants and those common in populations.

Table 1 Summary of clinical and laboratory examinations of patients

	Study			
	Patient 1, family 1	Patient 2, family 2	Patient 3, family 3	Patient 4, family 3
Sex	M	F	F	F
Gestational age, wk	41 + 6	39	41	At term
Weight at birth, g	4,000	3,700	3,120	2,820
OFC at birth, cm (SD)	36.5 (+1)	35.5 (+0.5)	31 (−3.3)	32 (−2.3)
Presenting signs (age at onset)	Irritability (neonatal), opisthotonus (2 mo)	Irritability, opisthotonus (neonatal)	Irritability, opisthotonus (1.5 mo)	NA
Tremors/myoclonus	+	+	+	NA
Epileptic seizures (age at onset)	Infantile spasms (12 mo)	Infantile spasms (11 mo)	+	NA
Spastic or dystonic quadriplegia	+	+	+	+
Central visual impairment	+	+	+	+
Optic atrophy	−	−	−	NA
Intellectual disability	Profound	Profound	Profound	Profound
Dysmorphic features				
High arched palate	+	+	+	NA
Tented upper lip	−	+	+	NA
Outward turning ear lobules	+	+	−	NA
Narrow forehead	+	+	+	NA
Edema of hands and feet	+	+	+	NA
Tapered fingers	+	+	+	NA
EEG (age)	N (3 and 5 mo), hypsarrhythmia (12 mo), very slow background (6 y)	N (3 and 4 mo), hypsarrhythmia (13 mo)	Slow background (4 mo), multifocal spikes, very slow background (10 y)	Unspecific slowing (14 mo)
Muscle biopsy	N	Variable fiber size, neurogenic damage	N	NA
Sural nerve biopsy (age at investigation)	Axonal neuropathy (2 y)	Axonal neuropathy (2.3 y)	Axonal neuropathy (10 y)	NA
Neurography	MCV low N, motor ampl N, sensory ampl: radial ^{is} ↓, sural ^{is} —(6 y)	MCV low N, Motor ampl N, Sensory ampl: radial ^{is} ↓, sural ^{is} —(2.3 y)	NA	NA
Blood lactate (ref. 0.7–1.8 mmol/L)	4.9, 0.8, 5.6, 3.1	0.4, 2.8, 5.9, 4.2	N	NA
CSF lactate (ref. 0.6–2.7 mmol/L)	1.5	1.4, 4.2	1.7	NA
Age at death, y	8.5	4.3	15.3	2.7

Abbreviations: ampl = amplitudes; MCV = motor nerve conduction velocity; N = normal; NA = not available; OFC = occipital frontal circumference; ref. = reference.

Symbols: − = absent; + = present; ↓ = decreased.

On the basis that both families were of Finnish ancestry and the clinical manifestation of both patients closely resembled each other, we searched for homozygous or compound heterozygous variants that the patients shared. Two novel heterozygous variants in *SEPSECS* were subsequently identified in both patients, c.974C>G in exon 8 leading to a missense mutation p.Thr325Ser and c.1287C>A in exon 11 leading to a nonsense mutation p.Tyr429* (RefSeq NM_016955.3). The variants were validated by Sanger sequencing (figure 3A). The parents of patient

1 and the mother of patient 2 were heterozygous carriers; the DNA sample of the father of patient 2 was not available. The variants were not present in the 1000 Genomes database (www.1000genomes.org) or in the NHLBI GO ESP Exome Variant Server (evs.gs.washington.edu/EVS/) or in approximately 230 screened Finnish control samples. However, a recent database of 3,323 exome sequences of Finnish individuals provided a heterozygous carrier frequency of 1:277 for the c.1287C>A, p.Tyr429* variant (Sequencing Initiative Suomi, sisu.fimm.fi), suggesting

Figure 1 Brain imaging findings of SEPSECS deficiency

T2 and fluid-attenuated inversion recovery images of patient 1 at age 5 months (A) and 2 years, 10 months (B, C) and patient 2 at age 6 months (D) and 1 year, 8 months (E, F). The early images (A, D) show loss of periventricular white matter volume and almost complete lack of myelin (arrows). Upon disease progression (B, C, E, F), widening of both central and cortical CSF spaces and cerebellar atrophy are present, with no signal for myelin.

enrichment of the nonsense variant in Finland, but without homozygous occurrence.

Next we screened 11 *SEPSECS* exons for mutations in additional patients with mitochondrial encephalopathy and/or other shared features. One patient (patient 3) from an affected sib-pair with similar clinical findings was found to be compound heterozygous for the same *SEPSECS* mutations c.974C>G (p.Thr325Ser) and c.1287C>A (p.Tyr429*). The DNA sample of the affected sib was not available for the study. Investigation of the family histories of the 3 patients with shared *SEPSECS* mutations revealed that they all originated from a restricted area in eastern Finland. Haplotype analysis of the nearby microsatellite markers indicated shared ancestral haplotypes, further supporting the distant common origin of the mutations in our patients (figure 3B).

Predicted effects of the mutations onto the SEPSECS protein structure. *SEPSECS* codes for *O*-phosphoseryl-tRNA:selenocysteinyl-tRNA synthase, the key enzyme in the sole biosynthetic route to selenocysteine (Sec) in eukaryotes and archaea.^{10,11} Residue Thr325, which is affected by a missense mutation in our patients, is a highly conserved amino acid in eukaryotic

SEPSECS (figure 3C). In the *SEPSECS* structure, Thr325 is located in the middle of helix α 12 in the C-terminal domain of the protein (figure 3D). Helix α 12 provides support for the major elements that constitute the active site of *SEPSECS*. Thr325 interacts only with the backbone and side-chain atoms of helix α 12, and its replacement with serine may destabilize the structure of α -helix. This could lead to altered positioning of the cofactor pyridoxal phosphate, and the floor (helix α 10) and the clefts (loop 70 and P-loop) of the active site. These structural rearrangements in the catalytic pocket would ultimately yield an enzyme with reduced catalytic power.

The p.Tyr429* mutant messenger RNA escapes nonsense-mediated decay, and is predicted to lead to truncation of the 73 C-terminal amino acids of *SEPSECS*, the region critical both for tRNA binding and enzyme activity (figure e-1). The full-length *SEPSECS* of approximately 55 kDa was readily detected by Western blotting—even in a higher amount than in the control sample (figure 3E)—in the brain autopsy sample of patient 3, but no evidence of a truncated protein (~47 kDa) was found, indicating that it was either not produced or rapidly degraded.

(A) Parietooccipital cortex displaying edemic transcortical laminar necrosis (ln) with a subtotal neuronal loss (arrows). (B) Putamen (put) is severely neuron-depleted and gliotic (arrows). (C) Pontine basis (pb) is narrow, both neurons and transverse fibers are reduced. (D) Atrophy of the inferior olives (io) and hili with olivocerebellar fibers and narrowed tegmentum seen in low magnification of the medulla oblongata. (E) Atrophic cerebellar cortex, the molecular and granular layers are thin, with practically total loss of Purkinje cells (arrows). (F) Liver parenchyma exhibiting a moderate microvacuolar fatty degeneration (fat visible as white droplets). Paraffin sections, hematoxylin & eosin (A, C, D, F), Luxol fast blue-cresyl violet (B, E); original magnification $\times 40$ (A, E), $\times 100$ (B), $\times 10$ (C, D), $\times 400$ (F).

To functionally confirm the mutation effects, we showed that both mutations severely affected SEPSECS activity in vivo in an anaerobic *Escherichia coli* assay.¹² For the assay, we utilized the $\Delta selA$ strain and inspected the ability of human SEPSECS to restore the benzyl viologen–reducing activity of an *E coli* selenoprotein, the formate dehydrogenase H. As predicted, p.Tyr429* mutant was completely inactive in this assay, while the p.Thr325Ser mutant was active albeit at the decreased level (figure 3F).

Functional consequences of the SEPSECS mutations. Inactivating *SEPSECS* mutations presumably inhibit synthesis of 25 selenoproteins (the human selenoproteome), which participate in diverse biological processes.¹³ We measured selenoprotein levels in the lysate obtained from the autopsy brain material of patient 3 using selected reaction monitoring–mass spectrometry (SRM-MS). Protein levels were normalized against glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase 1. To validate the method, the levels of the glial fibrillary acidic protein,

Figure 3 SEPSECS gene defect with structural and functional consequences of the mutations

(A) SEPSECS mutation sequences. The arrow indicates the mutation site. (B) Haplotypes on the chromosome 4 region containing the SEPSECS gene in 3 patients from 3 unrelated families. Parent samples were used to construct the haplotypes when available. (C) Alignment of the sequence region containing Thr325 (arrow) in eukaryotic SEPSECS proteins. (D) Thr325 is located in the middle of helix $\alpha 12$ in the C-terminal domain of the protein. Helix $\alpha 12$ provides support for the major elements that constitute the active site of SEPSECS by 2 direct interactions: (1) helix $\alpha 12$ (orange) interacts with helix $\alpha 10$ (from monomer 1, in gray); (2) helix $\alpha 10$ carries a catalytic residue Lys284 (orange sticks) to which the cofactor PLP (orange sticks) is covalently attached and forms the floor of the active site. The $\alpha 10$ - $\alpha 12$ interaction places Thr325 (orange sticks) behind the active-site pocket and approximately 15 Å away from PLP (shown with up-and-down arrow). In addition, helix $\alpha 12$ interacts with helix $\alpha 3$ (blue) from the second SEPSECS monomer (monomer 2, in light blue), which is flanked by loop 70 and P-loop (blue) that form the clefts of the active site. The CCA end of tRNA^{Sec} (beige) binds in the proximity of the P-loop, which is also implicated in binding of the phosphoserine group. (E) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis from brain samples; SEPSECS patient (P) and control (C). The full-length SEPSECS of approximately 55 kDa was readily detected, but no signal of the size of the truncated protein (~47 kDa) was present. (F) In vivo dilution series assay of the ability of human SEPSECS variants to restore the benzyl viologen-reducing activity of the selenoprotein formate dehydrogenase H in the *Escherichia coli* Δ selA deletion strain. GAPDH = glyceraldehyde 3-phosphate dehydrogenase; PLP = pyridoxal phosphate; tRNA = transfer RNA.

which resides in astrocytes, were measured and shown to be increased, thus indicating astrogliosis (figure 4, A, D). In contrast, levels of myelin basic protein and neurofilament medium were clearly reduced

(figure 4A), which is consistent with the observed myelin and neuron loss in the patients. Three selenoproteins, thioredoxin reductase TXNRD1 and glutathione peroxidases GPX1 and GPX4, were

abundant enough to be reliably detected. Their levels were decreased by 15% to 40% in the patient brain sample compared with controls (figure 4B). Consistent with a partial defect in selenoprotein production, levels of TXNRD1 and TXNRD2 were decreased in the patient's brain as shown by Western blotting (figure 4D). However, the steady-state level of tRNA^{Sec} was not altered (figure 4F). Of note, the observed defects were tissue-specific, as the patient's fibroblasts,

myoblasts, or differentiated myotubes did not show reduced TXNRD levels (data not shown).

Because of the lactate elevation, we analyzed the amounts of mitochondrial RC complexes I–IV in the autopsy brain samples of patient 3 by blue native electrophoresis, but found them to be similar to controls (figure 4E). In addition, when we quantified the amounts of RC complex subunits by SRM-MS, they were shown to be mostly unaffected (figure 4C).

Figure 4 Selenoprotein and respiratory chain protein amounts in SEPSECS deficiency

(A–C) Selected reaction monitoring–mass spectrometry; autopsy brain sample of patient 3 and 3 controls. The results are shown for (A) glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), and neurofilament medium (NFM), markers of brain cells, for (B) selenoproteins glutathione peroxidases 1 and 4 (GPX1 and GPX4) and thioredoxin reductase 1 (TRXR1) and for (C) mitochondrial respiratory chain complex subunits: NADH dehydrogenase (ubiquinone) Fe-S protein 1 (NDUFS1), NADH dehydrogenase (ubiquinone) Fe-S protein 8 (NDUFS8), ubiquinol-cytochrome c reductase core protein II (QCR2), cytochrome c oxidase subunit Va (COX5A), mitochondrially encoded ATP synthase 6 (ATPA), and citrate synthase (CISY). (D) Western blotting of the brain samples for GFAP and 2 selenoproteins (P, patient 3; C, controls 1–3). (E) Blue native electrophoresis of mitochondrial respiratory chain complexes in the patient (P) and control (C) brain samples. (F) Steady-state levels of tRNA^{Sec} compared with mitochondrial tRNA^{Ala} in the brain sample of patient 3 (P) and controls (C1–C4). (G) Oxyblot shows increased amounts of oxidized proteins in patient brain (P) compared with controls (C1–C3). GAPDH = glyceraldehyde 3-phosphate dehydrogenase; mt-tRNA = mitochondrial transfer RNA; tRNA^{Sec} = selenocysteine-specific transfer RNA.

Because the decreased selenoprotein synthesis affected glutathione peroxidases and thioredoxin reductases—enzymes of antioxidant defense—we analyzed protein carbonylation in the patient's brain, and found protein oxidation to be clearly increased (figure 4G).

DISCUSSION We report here that pathogenic mutations in *SEPSECS* lead to severe cerebellocerebral atrophy by attenuating the synthesis of selenoproteins, which leads to considerable oxidative damage in the patient brains.

SEPSECS mutations were previously described in a single report, underlying progressive cerebellocerebral atrophy with profound mental retardation, progressive microcephaly, severe spasticity, and myoclonic or generalized tonic-clonic seizures, later classified as pontocerebellar hypoplasia type 2D (PCH2D) (MIM 613811).^{12,14} The MRI findings of progressive cerebellar atrophy, followed by cerebral atrophy involving both white and gray matter, mimicked the findings in our patients. In contrast to a normal metabolic profile of patients with PCH2D,¹² our patients had lactacidemia, and they also presented with axonal neuropathy. Similarities between the patients with PCH2D and the patients described here support the disease-causing role of the identified mutations, thus extending the phenotypes caused by defective selenocysteine biosynthesis.

The neuropathologic changes of *SEPSECS* deficiency, such as progressive neuronal degeneration, more pronounced laterally than midline, are reminiscent of Alpers syndrome,¹⁵ which is caused by defects in mitochondrial proteins, most commonly in polymerase gamma, but also in Twinkle helicase and the phenylalanyl-tRNA synthetase.^{4,16,17} The topography of the lesions corresponds to highly energy-dependent regions of the CNS; in case of *SEPSECS* deficiency, this is particularly the parieto-occipital region. The patients with *SEPSECS* mutations described here also showed moderate degeneration of the liver postmortem. These findings, together with elevated lactate, suggest that encephalopathy due to *SEPSECS* deficiency and mitochondrial encephalopathies, such as Alpers syndrome, could share some common pathogenic mechanisms. However, we did not identify significant alterations in the mitochondrial RC complexes in the patient's brain sample that would explain changes typical for RC deficiencies.

Similar manifestations between mitochondrial and selenoprotein disorders could be explained by the role selenoproteins have in maintaining the cellular redox potential and H₂O₂ detoxification.¹³ In other words, selenoproteins are an important part of the antioxidant defense. We showed remarkable increase of

protein carbonylation as a sign of oxidative stress in the brain of patients with *SEPSECS* deficiency. As mitochondria are one of the main sources of cellular reactive oxygen species, *SEPSECS* deficiency could especially damage cells with high mitochondrial activity. Future work on mouse models is needed to clarify this aspect of selenoprotein pathology.

Progressive cerebellar atrophy of our patients together with infantile spasms, dysmorphic features, and edema of hands, feet, and face resembled the findings typically seen in PEHO syndrome.^{7,18} However, unlike patients with PEHO, our patients did not have optic atrophy and were spastic rather than hypotonic. Previously, a connection between pontocerebellar hypoplasias, mitochondrial encephalopathies, and PEHO-like features has been proposed in PCH6 that is caused by *RARS2* mutations.¹⁹ *RARS2* encodes the mitochondrial aminoacyl-tRNA synthetase essential for charging tRNA^{Arg} for protein synthesis of mitochondrial RC complexes.⁶ Of note, *SEPSECS* also affects cellular functions through tRNA, but in a different biological pathway, by catalyzing the conversion of the phosphoseryl-tRNA^{Sec} intermediate into selenocysteinyl-tRNA^{Sec}.^{10,11} Furthermore, PCH2 types A, B, and C are caused by mutations in genes encoding for subunits of the tRNA-splicing endonuclease complex, *TSEN54*, *TSEN2* and *TSEN34*, respectively.²⁰ This complex performs the splicing of intron-containing tRNAs, which comprise approximately 6% of human tRNAs needed for cytoplasmic translation.²¹ Whether a common mechanism exists by which the defects of the different cellular protein synthesis processes lead to similar neurodegenerative phenotypes or whether tRNA involvement in all these entities is purely coincidental remains to be established.

SEPSECS is a tetramer composed of 2 dimers, where each dimer contains 2 active sites formed at the dimer interface.^{10,11} The novel compound heterozygous mutations identified in this study were predicted to have differential effects on the function of the tetrameric *SEPSECS*: p.Thr325Ser introduced Ser in the middle of an α -helix predicting destabilization of the helix,²² thereby modifying the catalytic pocket of the enzyme, whereas p.Tyr429* resulted in a total loss of function. Our dilution series of the bacterial *in vivo* assay verified these predictions. The previously reported *SEPSECS* mutations of patients with PCH2D displayed no detectable activity in an anaerobic *in vivo* assay for *SEPSECS* activity.¹² Thus, the differences in the phenotypes of our patients and those reported previously may be caused by differences in the residual *SEPSECS* activity. The fact that the fibroblasts, myoblasts, and myotubes of our patients did not display decreased selenoprotein levels (not shown) suggests that the residual *SEPSECS*

activity was sufficient to maintain selenoprotein synthesis in these cell types but not in the brain.

The 25 human selenoproteins function in remarkably diverse processes. Besides SEPSECS deficiency, the only known human disease affecting selenoprotein synthesis is caused by mutations in *SECISBP2*, which encodes a protein that recognizes the specific insertion sequence in the selenoprotein messenger RNAs. *SECISBP2* mutations cause either a multisystem disorder²³ or abnormal thyroid hormone metabolism with elevated TSH, T₄, and reverse T₃ and reduced T₃ levels.²⁴ Our patients presented with increased TSH and normal T₄ levels, as measured at ages 2 and 6 years. Thus, although affecting the common metabolic pathway, mutations in *SECISBP2* and *SEPSECS* yield distinct phenotypes.

The selenoproteome is essential for mammals as shown by the early embryonic lethality of the tRNA^{Sec} gene (*Trsp*) knockout mouse.²⁵ The selenoprotein knockout mice have further clarified their importance for brain function.²⁶ The neuron-specific knockout mice of *GPx4*,²⁷ *Txnrd1*,²⁸ and *Trsp*²⁹ are characterized by neurodegeneration, in general, and by cerebellar hypoplasia, in particular.³⁰ In the *Txnrd1* mice, the effect was attributed to decreased proliferation of granule cell precursors within the external granular layer.²⁸ Furthermore, the full knockout of *Selp*,³¹ which encodes a plasma protein SELP that transports selenium from the liver to peripheral tissues, was shown to have a neurologic phenotype with spasticity and seizures.³² It is likely that the lack of several selenoproteins contributes to the neuronal loss in the selenoprotein biosynthesis defects. Our SRM-MS and Western blotting results derived from the analysis of an autopsy brain sample from a patient with *SEPSECS* mutations showed that selenoproteins were present albeit at significantly reduced levels. However, because of the severe neuronal loss and astrogliosis in the patient brain, a comparative analysis has limitations. For instance, neurons may have had more severe reductions of selenoenzyme activities was not feasible with this material. Regardless of these shortcomings, our result of *SEPSECS* mutations causing a selenoprotein deficiency in human brain implicates a specific requirement of selenoproteins in postnatal brain development.

Based on the results of our study, we suggest *SEPSECS* sequencing in progressive early childhood brain atrophies of unknown cause, especially when patients present with sensory axonal neuropathy and elevated lactate.

AUTHOR CONTRIBUTIONS

A.-K.A. collected and analyzed patient data and drafted the manuscript. T.H., A.L., and E.Y. sequenced patients and performed protein and cell culture experiments. T. Linnankivi and P.I. contributed to the clinical analysis. R.L.F. and M. Simonović performed the structural analysis.

Y.L. and D.S. performed the in vivo bacterial assay. D.M.-P. and G.L.C. performed the mass spectrometry analysis. M. Somer, M.L., T. Lönnqvist, and H.P. contributed to the clinical analysis. L.V. performed neuroradiology. A.P. performed neuropathology. H.T. performed the exome sequencing analysis. A.-E.L., A.S., and H.T. designed the study and drafted the manuscript. All authors revised the manuscript.

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DISCLOSURE

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