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ORIGINAL ARTICLE

Milder progressive cerebellar atrophy caused by biallelic *SEPSECS* mutations

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Cerebellar atrophy is recognized in various types of childhood neurological disorders with clinical and genetic heterogeneity. Genetic analyses such as whole exome sequencing are useful for elucidating the genetic basis of these conditions. Pathological recessive mutations in Sep (*O*-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase (*SEPSECS*) have been reported in a total of 11 patients with pontocerebellar hypoplasia type 2, progressive cerebellocerebral atrophy or progressive encephalopathy, yet detailed clinical features are limited to only four patients. We identified two new families with progressive cerebellar atrophy, and by whole exome sequencing detected biallelic *SEPSECS* mutations: c.356A>G (p.Asn119Ser) and c.77delG (p.Arg26Profs*42) in family 1, and c.356A>G (p.Asn119Ser) and c.467G>A (p.Arg156Gln) in family 2. Their development was slightly delayed regardless of normal brain magnetic resonance imaging (MRI) in infancy. The progression of clinical symptoms in these families is evidently slower than in previously reported cases, and the cerebellar atrophy milder by brain MRI, indicating that *SEPSECS* mutations are also involved in milder late-onset cerebellar atrophy.
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INTRODUCTION

Cerebellar atrophy or hypoplasia is recognized in various types of childhood neurological disorders with clinical and genetic heterogeneity. Atrophy and hypoplasia are both strictly characterized: cerebellar atrophy describes a loss of cerebellar tissue with an initially normal structure, whereas cerebellar hypoplasia is defined by a compact cerebellum of reduced volume that does not fill the posterior fossa.[1] However, hypoplasia is often included in atrophy because atrophy is often difficult to be distinguished from hypoplasia.

Cerebellar atrophy is classified into congenital and postnatally acquired cerebellar atrophies. Congenital cerebellar atrophies include several genetic disorders such as pontocerebellar hypoplasia (PCH), spinocerebellar ataxia and inherited metabolic diseases.[1] Pontocerebellar hypoplasia is a clinically and genetically heterogeneous group of inherited neurodevelopmental disorders predominantly characterized by prenatal onset of stunted growth and decay of cerebral structures. The initial classification is based on two subtypes, PCH1 and PCH2, and is determined from neuropathological findings, specifically the presence or absence of anterior horn cell degeneration.[2] Postnatally acquired cerebellar atrophies are caused by cerebral palsy with severe perinatal asphyxia, congenital infection and exposure to teratogens.

Sep (*O*-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase (*SEPSECS*) encodes the SepSecS protein that is important for synthesis of selenocysteine, a selenoprotein component that is essential for mammalian brain development. Pathological recessive *SEPSECS* mutations have been reported in a total of 11 patients with PCH type 2, progressive cerebellocerebral atrophy or progressive encephalopathy, although detailed clinical features are limited to only four patients. Most patients had exhibited a severe neurodevelopment disorder since their infantile periods.[3,4]

In this study, we describe two families with cerebellar atrophy that harbor biallelic *SEPSECS* mutations. Detailed clinical information is described and compared with previously reported cases.

MATERIALS AND METHODS

Patients

A total of 96 families with cerebellar atrophy (including 85 previously described families) were analyzed.[5] Both static and progressive cerebellar atrophy were included. Detailed clinical information was obtained from the clinicians who examined the patients. The institutional review board of Yokohama City University of Medicine approved the experimental protocols. Informed consent was obtained for all patients, in agreement with Japanese regulation requirements.

Whole exome sequencing

Genomic DNA was isolated from peripheral blood leukocytes using QuickGene 610L (Wako, Osaka, Japan), then captured using the SureSelect Human All Exon v4 or v5 Kit (51 Mb; Agilent Technologies, Santa Clara, CA, USA), and sequenced on an Illumina HiSeq2000 or Hiseq2500 (Illumina, San Diego, CA,

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USA) with 101-bp paired-end reads. Exome data processing, variant calling and variant annotation were performed as previously described.[6] Common single-nucleotide polymorphisms (SNPs) with minor allele frequencies ⩾1% in dbSNP 135 and variants observed in >5 of our in-house 575 control exomes database were filtered out. Among the remaining rare variants, we focused on amino acid altering or splicing-affecting variants. Particular attention was given to mutations in previously reported causative genes associated with cerebellar atrophy. *SEPSECS* mutations were validated by Sanger sequencing using genomic DNA from patients and their parents as a template.

RESULTS

Genetic analysis

Using whole exome sequencing, we identified potential biallelic mutations in *SEPSECS* (NM_016955.3) in two individuals from two families: c.356A>G (p.Asn119Ser) and c.77delG (p.Arg26Profs*42) in individual 1, and c.356A>G (p.Asn119Ser) and c.467G>A (p.Arg156Gln) in individual 2 (Figure 1a). These compound heterozygous mutations were verified by Sanger sequencing using patient and parental samples. The amino acids affected by two missense mutations were conserved in eukaryotic SepSecS proteins (Figure 1b) and located on the SepSecS domain (Figure 1c). These mutations were absent in dbSNP 138, our in-house database and the Exome Aggregation Consortium (ExAC) database, and are therefore likely to be extremely rare. Web-prediction tools (specifically, SIFT (http://sift.jcvi.org/), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) and MutationTaster (http://www.mutationtaster.org/)) indicated that both mutations are pathogenic (Supplementary Table 1).

Clinical presentation

Clinical features of individuals with *SEPSECS* mutations are presented in Table 1. Individuals 1 and 2 showed similar clinical courses. During infancy, their development was slightly delayed and initial brain magnetic resonance imaging (MRI) showed normal findings (individual 1 at 6 months of age, and individual 2 at 5 years of age). Ataxia and motor disability slowly progressed. Cerebellar atrophy was first recognized by MRI at 9 years and 18 years of age, respectively.

Individual 1 was a 10-year-old girl, and the first child born to healthy non-consanguineous parents after an uncomplicated 41-week pregnancy. She had no dysmorphic features at birth, and acquired eye pursuits at 2 months of age. She was referred to our hospital at 3 months because of downward nystagmus. Neurological examination revealed that she was floppy but her reflexes were normal. Although her head circumference was within normal range at birth, her head growth was slow. Head circumference at 4 years was 47.0 cm

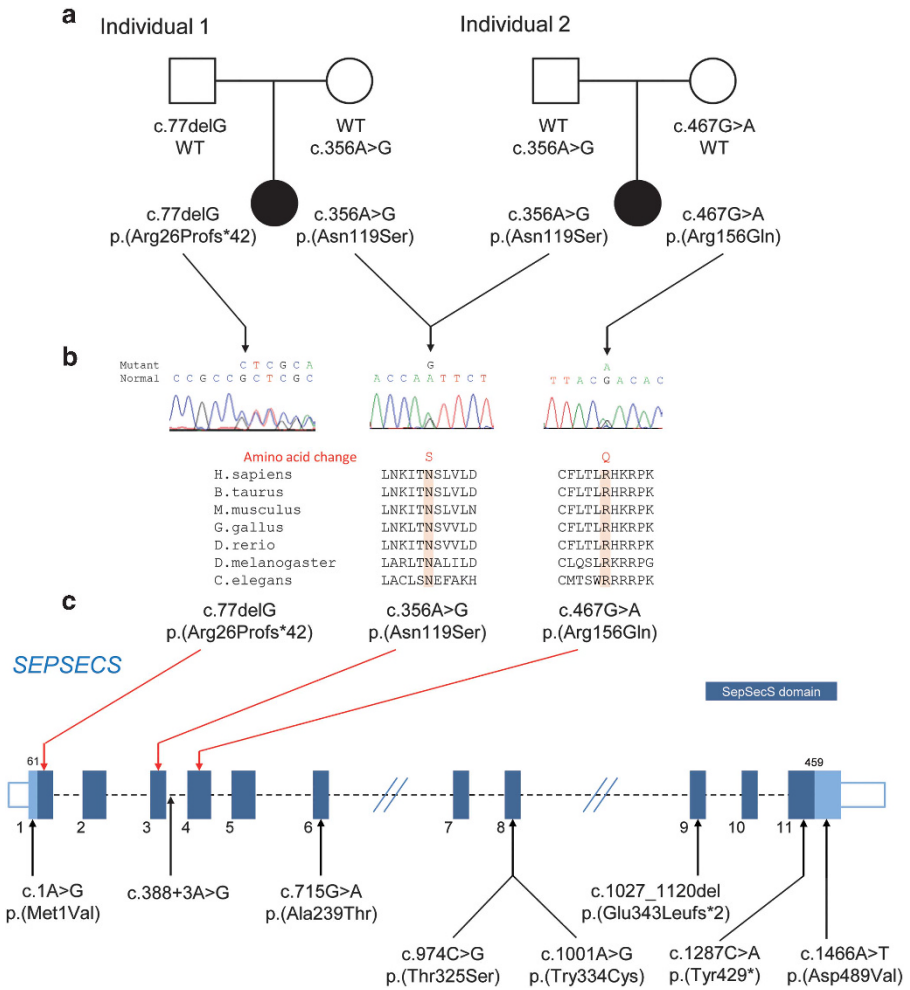

Figure 1 (**a**) Familial pedigrees and *SEPSECS* mutations. (**b**) Electropherograms of mutations and evolutionary conservation of amino acids derived from the missense mutations in *Caenorhabditis elegans* to humans. (**c**) Schematic of the *SEPSECS* gene. Locations of mutations are depicted (upper, novel mutations identified here; lower: previously described mutations). *SEPSECS*, Sep (*O*-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase.

Table 1 Summary of patient clinical examinations

	Individual 1	*Individual 2*
Age, gender	10 Years, female	21 Years, female
Sibling	First child, no sibling	First child, no sibling
SEPSECS mutations	c.77delG (p.Arg26Profs*42) c.356A>G (p.Asn119Ser)	c.356A>G (p.Asn119Ser) c.467G>A (p.Arg156Gln)
Diagnosis	Late-onset progressive cerebellocerebral atrophy	Late-onset progressive cerebellocerebral atrophy
Birth weight	2738 g	2580 g
Head circumference	31.5 cm (−1.7 s.d.)	29.0 cm (−2.9 s.d.)
Initial symptom	Developmental delay	Developmental delay
Seizures	−	−
Ataxic gait	+ (Somehow walking with walker)	+ (18 Years)
Slurred speech	+	+ (18 Years)
Dysmetria	+ (3 Years and 6 months)	+ (18 Years)
Nystagmus	+ (3 Months)	−
Spasticity of limbs	+	+ (18 Years)
Hypotonia	+	+ (3 Months)
Development		
Head control	3 Months	3 Months
Sitting	8 Months	10 Months
Walking	3 Years and 6 months	2 Years
Meaningful words	4 Years	2 Years
Intelligence quotient	38 (3 years and 3 months)	ND
MRI	6 Months: normal; 2 years and 7 months: normal; 9 years: atrophy of cerebellum and frontoparietal lobe of the cerebrum	5 Years: normal; 18 years: cerebellar, vermis and cerebral atrophy
Blood lactate	16.9 mg dl^{-1} (range 4.0–16.0)	12.8 mg dl^{-1} (range 3–17)
CSF lactate	11.8 mg dl^{-1} (range 4.0–16.0)	ND

Abbreviations: CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; ND, no data; *SEPSECS*, Sep (*O*-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase.

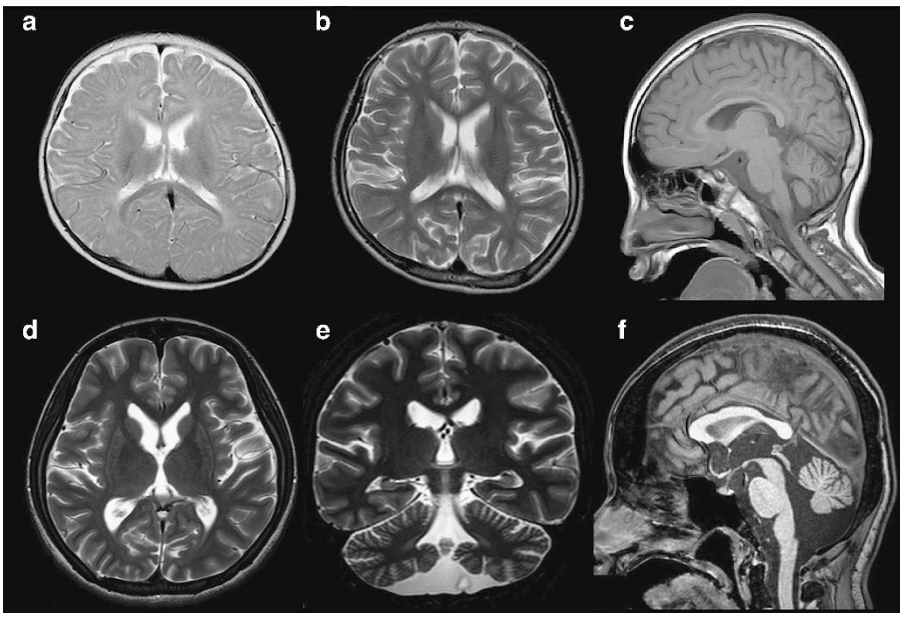

Figure 2 Brain magnetic resonance imaging (MRI) of individual 1 at 6 months (**a**) and 9 years (**b**–**c**) of age, and individual 2 at 18 years (**d**–**f**) of age. Axial (**a**, **b**) and sagittal (**c**) T2-weighted images show mild atrophy of the frontoparietal lobes and mild cerebellar atrophy at 9 years (**b**) of age, but not at 6 months (**a**) of age. Axial (**d**) and coronal (**e**) T2-weighted images show mild cerebral atrophy and cerebellar lobe atrophy, respectively. White matter around posterior horn of lateral ventricle showed slightly hyperintensity. Sagittal T1-weighted image (**f**) shows cerebellocerebral and vermis atrophy.

(−2.1 s.d.). Brain MRI at 6 months of age was normal (Figure 2a). She was able to sit alone at 8 months, and her nystagmus disappeared by 9 months. She moved by bottom shuffling before walking. She walked with support and alone at 1 year and 3 months, and 3 years and 6 months, respectively. She was able to speak several meaningful words, but could not speak in sentences at 3 years and 6 months. Around the same time, rotating and horizontal nystagmus appeared and she had intention tremors and dysmetria.

At 9 years of age, she was admitted to hospital because of respiratory infection. Brain MRI revealed progressive atrophy of the cerebellum

Table 2 Summary of *SEPSECS* mutations

Patient	*Ethnic group*	*Zygosity*	*Mutation*	*Amino acid change*	*Phenotype*	*Reference*
1	Japanese	Compound heterozygosity	c.77delG	p.Arg26Profs*42	Late-onset PCCA	This report (individual 1)
			c.356A>G	p.Asn119Ser		
2	Japanese	Compound heterozygosity	c.356A>G	p.Asn119Ser	Late-onset PCCA	This report (individual 2)
			c.467G>A	p.Arg156Gln		
3, 4	Mixed Iraqi-Moroccan	Compound heterozygosity	c.1001A>G	p.Tyr334Cys	PCCA	Agamy *et al.*[3]
			c.715G>A	p.Ala239Thr		
5, 6	Jewish Iraqi	Homozygosity	c.1001A>G	p.Tyr334Cys	PCCA	Agamy *et al.*[3]
7–10	Finnish	Compound heterozygosity	c.974C>G	p.Thr325Ser	Progressive encephalopathy	Anttonen *et al.*[4]
			c.1287C>A	p.Tyr429*		
11	ND	Compound heterozygosity	c.1A>G	p.Met1Val	PCH type 2D	Zhu *et al.*[11]
			c.388+3A>G			
12	Jordan (Consanguineous family)	Homozygosity	c.1466A>T	p.Asp489Val	Syndromic ID/DD	Makrythanasis *et al.*[12]
13	ND	ND	c.1027_1120del	p.Glu343Leufs*2	Neurodegenerative disease	Alazami *et al.*[13]

Abbreviations: DD, developmental delay; ID, intellectual disorder; ND, no data; PCCA, progressive cerebellocerebral atrophy; PCH, pontocerebellar hypoplasia; *SEPSECS*, Sep (*O*-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase.
SEPSECS variants were annotated based on the transcript, NM_016955.3.

and frontoparietal cerebral lobe (compared with 6 months) (Figures 2b and c). Electroencephalography, peripheral conduction study, cerebrospinal fluid examination and somatosensory evoked potentials were normal. Neurological examination revealed progressive spasticity and ataxia with an unstable walk, even using a walking aid.

Individual 2, a 21-year-old girl, was the first child born to healthy non-consanguineous parents. There was no familial history. Although she showed congenital microcephaly at birth, her initial development was normal: she obtained head control at 3 months of age. Thereafter, her motor development was slightly delayed but she could sit and pull to stand at 10 months, and 1 year and 6 months, respectively, but could not walk until 2 years.

She was able to speak meaningful words and sentences at 2 and 5 years of age, respectively. After she graduated from special support school (instead of regular senior high school), she attended our medical institution because she had difficulty in walking without support. Neurological examination revealed that she had ataxia, coordination disturbance, dysmetria, hypotonia and deep tendon hyperreflexia. Brain MRI revealed cerebellar and vermis atrophy and slight cerebral atrophy (Figures 2d–f).

DISCUSSION

Previous studies have reported clinical features of patients with *SEPSECS* defects. Common symptoms among most patients, including the two described here, include developmental delay, hypotonia and cerebellar ataxia. Brain MRI frequently shows atrophy of the cerebellum, vermis and cerebrum. Nystagmus, microcephaly, seizure, spasticity and encephalopathy are also observed in some patients. Brain atrophy is recognized at various developmental stages, but mostly at birth to childhood. In our patients, cerebellar atrophy in individuals 1 and 2 was recognized at the age of 9 and 18 years, respectively. This is later than in previous cases, and the degree of atrophy is also milder. This indicates that the phenotype of *SEPSECS* defects includes milder cases than previously reported. In addition, the fact that our milder cases presented with microcephaly and developmental delay implied that *SEPSECS* plays important roles in brain development.

SEPSECS consists of 12 exons and encodes the SepSecS protein of 501 amino acids that catalyzes the last step in the conversion of Sep-tRNA to Sec-tRNA.[7] This reaction is the sole route to selenocysteine biosynthesis in humans.[8] Selenocysteine is a component of selenoproteins, and human selenoproteins include 25 members with biological functions implicated in diverse human diseases ranging from cardiovascular to immunoreactive disorders.[9] Conditional *Trsp* knockout mice, in which neuronal selenoproteins are deficient, show cerebellar hypoplasia and Purkinje cell loss,[10] suggesting that selenoproteins are essential for mammalian brain development. In addition, selenoproteins are suggested to play an important role in antioxidant defense.[9] Decreasing selenoprotein synthesis may damage organs with high mitochondrial activity because mitochondria are one of the main sources of cellular reactive oxygen species.[4] In a previous study, *SEPSECS* mutations caused clinical features similar to those of mitochondrial disease such as lactacidemia.[4] Although blood and/or cerebrospinal fluid lactate levels were almost within the normal range in our two patients, possibly consistent with the milder phenotype, elevated lactates might be a key clinical feature in patients with *SEPSECS* mutations.

To date, five missense, one nonsense, one splice site change and a gross deletion have been reported in *SEPSECS* (Figure 1c and Table 2). Four patients (in three families) with compound heterozygous mutations (c.974C>G (p.Thr325Ser) and c.1287C>A (p.Tyr429*)) showed progressive encephalopathy with microcephaly and infantile epileptic seizures.[4] Four patients with biallelic missense mutations (c.1001A>G (p.Tyr334Cys) and c.715G>A (p.Ala239Thr)) were diagnosed with progressive cerebellocerebral atrophy. In one of them, cerebral atrophy was recognized at the age of 18 months.[3] A patient with a missense mutation (first methionine) and a splice site change (c.1A>G and c388+3A>G) showed PCH type 2D.[11] In this study, individual 1 with missense and frameshift mutations showed late-onset progressive cerebellocerebral atrophy, whereas individual 2 had biallelic missense mutations and showed markedly late-onset progressive cerebellocerebral atrophy. This suggests that the genotypes do not clearly indicate phenotypic difference and severity. Considering the common mutation (c.356A>G (p.Asn119Ser)) in our two individuals, it is possible that c.77delG (p.Arg26Profs*42) (individual 1) has a more deleterious effect on *SEPSECS* function compared with c.467G>A (p.Arg156Gln) (individual 2), supporting the fact that individual 2 shows a much milder phenotype than individual 1.

In conclusion, we have identified two families with cerebellar atrophy arising from biallelic novel *SEPSECS* mutations. Late-onset and milder phenotypes were recognized, indicating that *SEPSECS* mutant phenotypes show a wide range of clinical phenotypes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)