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BRIEF COMMUNICATION

A novel missense *SNAP25b* mutation in two affected siblings from an Israeli family showing seizures and cerebellar ataxia

Hiroyuki Fukuda[1] · Eri Imagawa[1] · Kohei Hamanaka[1] · Atsushi Fujita[1] · Satomi Mitsuhashi[1] · Satoko Miyatake[1] · Takeshi Mizuguchi[1] · Atsushi Takata[1] · Noriko Miyake[1] · Uri Kramer[2] · Naomichi Matsumoto[1] · Aviva Fattal-Valevski[2]

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Abstract

SNAP25 is a core component of the soluble N-ethylmaleimide-sensitive factor attachment receptor complex, which plays a critical role in synaptic vesicle exocytosis. To date, six de novo *SNAP25* mutations have been reported in patients with neurological features including seizures, intellectual disability, severe speech delay, and cerebellar ataxia. Here, we analyzed an Israeli family with two affected siblings showing seizures and cerebellar dysfunction by whole-exome sequencing, and identified a novel missense *SNAP25* mutation (c.176G > C, p.Arg59Pro) inherited from their unaffected father. Two *SNAP25* isoforms are known, *SNAP25a* and *SNAP25b*, which each contain a different exon 5. The c.176G > C mutation found in this study was specific to *SNAP25b*, while five previously reported mutations were identified in exons common to both isoforms. Another was previously reported to be specific to *SNAP25b*. Comparing clinical features of reported patients with *SNAP25* mutations, the current patients demonstrated apparently milder clinical features with normal intelligence, and no magnetic resonance imaging abnormality or facial dysmorphism. Our results expand the clinical spectrum of *SNAP25* mutations.

Introduction

SNAP25 on chromosome 20p12.2 encodes a synaptosomal-associated protein of 25 kDa (SNAP25), which is mainly expressed in neurons and neuroendocrine cells [1, 2]. SNAP25 is a plasma membrane soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE) protein that forms a specific SNARE complex together with syntaxin and synaptobrevin proteins [3]. In synaptic vesicle exocytosis, the SNARE complex enables neuronal vesicles to release their neurotransmitters by mediating calcium-triggered vesicle fusion with the plasma membrane [4, 5].

SNAP25 transcripts exist as two isoforms: *SNAP25a* and *SNAP25b*. *Snap25a* and *Snap25b* were differentially expressed during mouse development, and were predominantly localized in the embryonic and adult brains of mice, respectively [6]. *Snap25b*-deficient mice (with protected *Snap25a* expression) demonstrated neurological hyperactivity, anxiety, learning deficits, and spontaneous seizures [7]. Therefore, *Snap25b* might be required for synaptic maturation and neuronal function, though the functional difference between the two isoforms remains elusive [8]. To date, six de novo mutations in *SNAP25* have been reported in individuals with different types of seizures [9–12]. Here, we report on two affected siblings with a novel *SNAP25b*-specific mutation detected by whole-exome sequencing (WES), and discuss its clinical phenotype together with those of previously reported mutations.

Subjects and genetic analysis

An Israeli family with two affected siblings showing seizures and cerebellar dysfunction was recruited in this study

These authors contributed equally: Hiroyuki Fukuda, Eri Imagawa.
These authors contributed equally: Naomichi Matsumoto, Aviva Fattal-Valevski.

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✉ Naomichi Matsumoto
naomat@yokohama-cu.ac.jp

[1] Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan

[2] Pediatric Neurology Unit, Dana-Dwek Children's Hospital, Tel Aviv Medical Center & Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

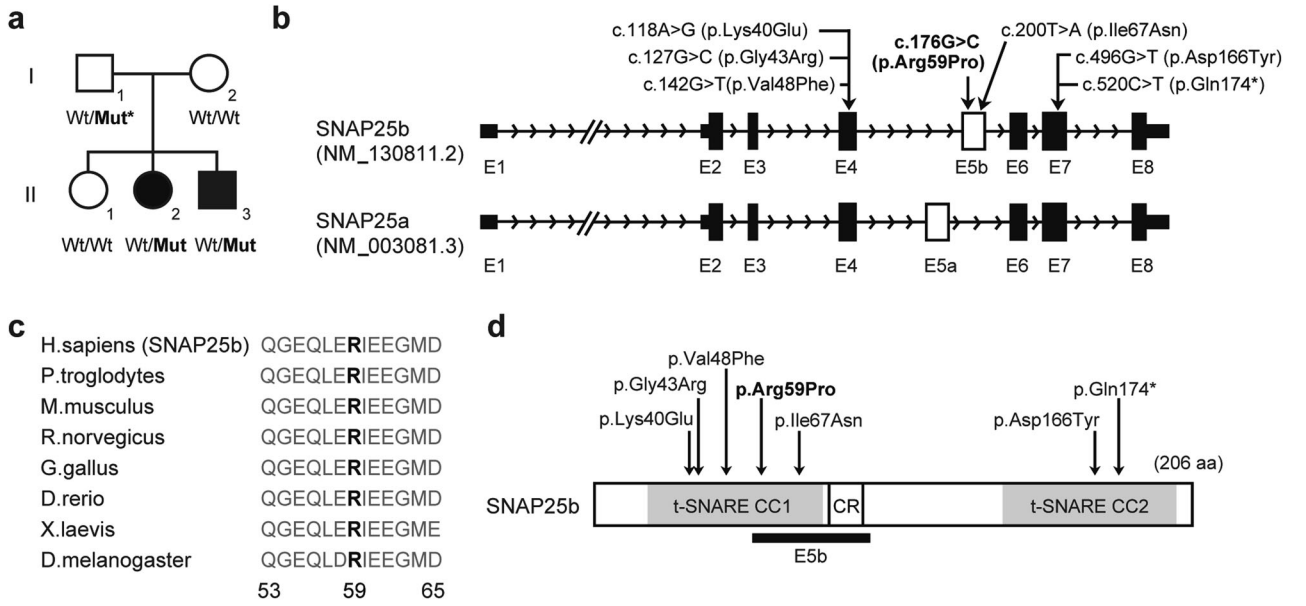

Fig. 1 Familial pedigree and *SNAP25* mutation. **a** Familial pedigree. The bold asterisk shows the somatic mosaic c.176G > C mutation in I-1. **b** The two *SNAP25* isoforms are shown with previously reported mutations listed above. The novel p.Arg59Pro mutation is shown in bold. E1–E4 exon 1–exon 4, E5a or E5b Exon 5 of *SNAP25a* or *SNAP25b,* E6–E8 exon 6–exon 8. **c** Evolutionary conservation of p.Arg59 in *SNAP25*. **d** Human SNAP25b (NP_570824.1) protein structure. t-SNARE CC1/2 t-SNARE coiled-coil homology 1/2, CR cysteine-rich domain

(II-2 and II-3; Fig. 1a). The study protocol was approved by Institutional Review Boards of Yokohama City University School of Medicine. Clinical features are summarized in Table 1 and Supplementary information. Samples were collected from familial members after parental consent was given. WES was performed using DNA extracted from peripheral blood leukocytes from one of the affected individuals (II-3) as described in the Supplementary information. We focused on variants under the autosomal dominant or autosomal recessive inheritance model. Stepwise variant selection is described in Supplementary Table S1. Candidate variants were sequenced in all family members (I-1, I-2, II-1, II-2, and II-3) by Sanger sequencing. As for the mosaic c.176G > C mutation in *SNAP25* found in the father (I-1), deep sequencing was performed using Miseq on PCR products amplified from DNA of blood leukocytes, saliva, hair roots, and nails (Supplemental information).

Mutation detection

In WES of individual II-3, 94.7% of RefSeq coding DNA sequence (CDS) was covered by 20 reads or more, and the average read depth for the CDS was 75.1×. After variant selection, only the missense *SNAP25* variant c.176G > C (p.Arg59Pro; NM_130811.2) remained (Supplementary Table S1 and Fig. 1b). This variant was likely to be pathogenic because it was predicted to be deleterious by three prediction tools (SIFT = 0.001, Polyphen2 = 0.998, and MutationTaster = disease causing). The variant was absent in our in-house Japanese exome data ($n = 575$), dbSNP build 138, the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/), the Exome Aggregation Consortium (http://exac.broadinstitute.org/), the Human Genetic Variation Database (http://www.hgvd.genome.med.kyoto-u.ac.jp/), and Tohoku Medical Megabank Organization database (https://ijgvd.megabank.tohoku.ac.jp/). The p.Arg59 residue is highly evolutionarily conserved from flies to humans in the t-SNARE coiled-coil homology 1 domain (Fig. 1c, d). Sanger sequencing revealed heterozygous changes in the affected individuals and the absence of this mutation in their unaffected mother and elder sister (Supplementary Figure S1a). The father's electropherogram showed a lower peak for the mutant allele (Supplementary Figure S1a and S1b). Targeted deep sequencing produced read fractions containing the mutant allele from the father's DNA extracted from peripheral blood leukocytes, saliva, hair roots and nails of 24.9, 30.8, 52.4, and 26.9%, respectively.

Discussion

The p.Arg59Pro mutation within exon 5 is specific to *SNAP25b* (Fig. 1b). Two splicing variants of *SNAP25* contain alternative exon 5s (each 118 bp in size). SNAP25a is highly homologous to SNAP25b, though nine amino acid residues corresponding to different exon 5 are different

Table 1 Summary of clinical features in individuals with *SNAP25* mutations

References	This study		Rohena et al. (2013)	Shen et al. (2014)	Handam et al. (2017)	Heyne et al. (2017)		
Subject	II-2	II-3	Proband	Proband	HSJ0745	DDD1	DDD2	DDD3
Gender	Female	Male	Female	Female	Male	Male	Female	Female
Age at last examination	11y 2m	2y 5m	15y	11y	23y	7y	2y 5m	11m
Mutation	c.176G > C, p.Arg59Pro	c.176G > C, p.Arg59Pro	c.142G > T, p.Val48Phe	c.200T > A, p.Ile67Asn	c.496G > T, p.Asp166Tyr	c.118A > G, p.Lys40Glu	c.127G > C, p.Gly43Arg	c.520C > T, p.Gln174*
Inheritance	Inherited from mosaic father	Inherited from mosaic father	De novo	De novo	De novo	De novo	De novo	De novo
Age at onset of seizures	1y 5m	1y 1m	5m	Early childhood	1y 6m	ND	ND	Infant
Seizure type at onset	GTCS, FS	GTCS, FS	Repetitive blinking, upward eye deviation, IA	Intermittent eyelid ptosis, blank stare, unresponsiveness	GTCS, focal seizure with IA	NS	NS	NS
Other seizure types	MC, drop attacks	CPS	GTCS, FCS	ND	ND	AS	GTCS	GTCS
EEG	Normal	Frontocentral discharges with focal spike wave	Generalized slowing (mild), generalized spike-and-slow wave complex	Generalized atypical polyspike and wave discharges, background diffuse slowing	Generalized spike-wave discharges during sleep, intermittent generalized discharges during drowsiness	ND	ND	ND
Response to therapy	Responsive (clonazepam)	Responsive (levetiracetam)	Intractable	Intractable	Responsive (clobazam, valproic acid)	ND	ND	ND
ID	−	−	+ (Severe)	+	+ (Moderate)	ND	ND	ND
Verbal ability	Delayed speech	Normal	No words	Mostly single words (at 11y)	Single words (at 2y), full sentences (at current)	ND	ND	ND
Neurological features	Cerebellar ataxia (mild), intention tremor, motor clumsiness, hypotonia, learning disabilities, attention deficit disorder	Motor clumsiness	Diminished oralmotor tone, spastic quadriparesis	Fatigable weakness, ataxic dysarthria, paretic and ataxic gaits, areflexia, multiple joint contracture	Speech dyspraxia	ND	Cerebellar ataxia, hand flapping, resting and intention tremor	ND
Brain MRI	Normal	Normal	Delayed myelination	Normal	Mild diffuse cortical atrophy	ND	ND	ND
Craniofacial dysmorphism	Normal	Normal	Microcephaly	ND	Upslanted short palpebral fissures	Epicanthus, coarse hair with upsweep	Deep-set eyes	ND
Other features	ND	ND	Severe scoliosis	ND	Apneas, bradycardia, severe constipation	Abnormal dentition, bilateral talipes equinovarus	ND	ND

AS absence seizures, *CPS* complex partial seizures, *EEG* electroencephalograms, *FCS* focal clonic seizures, *FS* febrile seizures, *GTCS* generalized tonic–clonic seizures, *IA* impaired awareness, *ID* intellectual disability, *MC* myoclonic seizures, *MRI* magnetic resonance imaging, *ND* not described, *NS* not specified, *m* month(s), *y* year(s), + present, − not present

(Supplementary Figure S2). Of the six previously reported mutations in *SNAP25*, four missense mutations (p.Lys40-Glu, p.Gly43Arg, p.Val48Phe, and p.Asp166Tyr), and one nonsense mutation (p.Gln174*) were found in exons common to the two isoforms, whereas one missense mutation (p.Ile67Asn) was seen in exon 5 of *SNAP25b* (Fig. 1b) [9–12].

Most patients reported to carry *SNAP25* mutations presented with various types and severities of seizures, intellectual disability, severe speech delay, neurological features including ataxia and muscle weakness, and abnormal brain electroencephalograms (EEGs) or abnormal magnetic resonance imaging (MRI) (Table 1). Although the current affected siblings had generalized tonic–clonic seizures and cerebellar dysfunction with muscle clumsiness, their intelligence were normal with no facial dysmorphism, and no abnormal MRI findings. Furthermore, one affected individual (II-2) had no abnormality in their interictal EEG. Their symptoms were therefore much milder than those of previously reported patients. It attracts our attention that a patient with the p.Ile67Asn mutation showed intellectual disability, severe speech delay and intractable seizures [10]. Thus the exon 5b specific mutations (p.Arg59Pro and p.Ile67Asn) are not necessarily associated with mild clinical phenotypes. *SNAP25* with a high pLI score of 0.96 indicates that haploinsufficiency is the most likely mechanism of pathogenicity. Indeed, *Snap25*$^{+/-}$ mice (with both isoforms affected) showed cognitive and learning deficits, were susceptible to seizures, and showed an abnormal EEG pattern, which was similar to clinical findings in patients with *SNAP25* mutations (Table 1) [13, 14]. Interestingly, p.Ile67Asn was reported to act in a dominant-negative manner [10], possibly explaining the difference of clinical features between p.Arg59Pro and p.Ile67Asn.

In conclusion, we report a novel missense *SNAP25* mutation in an Israeli family with affected siblings with seizures. This mutation is the second *SNAP25b*-specific mutation. Further studies are needed to investigate genotype–phenotype correlations in *SNAP25* mutations as well as their pathomechanisms.

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Compilance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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