Mutant SNAP25B causes myasthenia, cortical hyperexcitability, ataxia, and intellectual disability

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ABSTRACT

Objective: To identify and characterize the molecular basis of a syndrome associated with myasthenia, cortical hyperexcitability, cerebellar ataxia, and intellectual disability.

Methods: We performed in vitro microelectrode studies of neuromuscular transmission, performed exome and Sanger sequencing, and analyzed functional consequences of the identified mutation in expression studies.

Results: Neuromuscular transmission at patient endplates was compromised by reduced evoked quantal release. Exome sequencing identified a dominant de novo variant, p.Ile67Asn, in SNAP25B, a SNARE protein essential for exocytosis of synaptic vesicles from nerve terminals and of dense-core vesicles from endocrine cells. Ca^{2+} -triggered exocytosis is initiated when synaptobrevin attached to synaptic vesicles (v-SNARE) assembles with SNAP25B and syntaxin anchored in the presynaptic membrane (t-SNAREs) into an α -helical coiled-coil held together by hydrophobic interactions. Pathogenicity of the Ile67Asn mutation was confirmed by 2 measures. First, the Ca^{2+} triggered fusion of liposomes incorporating v-SNARE with liposomes containing t-SNAREs was hindered when t-SNAREs harbored the mutant SNAP25B moiety. Second, depolarization of bovine chromaffin cells transfected with mutant SNAP25B or with mutant plus wild-type SNAP25B markedly reduced depolarization-evoked exocytosis compared with wild-type transfected cells.

Conclusion: Ile67Asn variant in SNAP25B is pathogenic because it inhibits synaptic vesicle exocytosis. We attribute the deleterious effects of the mutation to disruption of the hydrophobic α -helical coiled-coil structure of the SNARE complex by replacement of a highly hydrophobic isoleucine by a strongly hydrophilic asparagine. *Neurology*® 2014;83:2247-2255

GLOSSARY

AChR = acetylcholine receptor; **cDNA** = complementary DNA; **CMS** = congenital myasthenic syndrome; **EP** = endplate; **MEPP** = miniature endplate potential; **SNAP25B** = synaptosomal-associated protein, 25B; **SNARE** = soluble N-ethylmaleimide-sensitive factor attachment protein receptor; **t-SNAREs** = target membrane-attached SNAP25B and syntaxin; **v-SNARE** = synaptic vesicle-attached synaptobrevin.

The SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) proteins are core components of the synaptic vesicle fusion machinery. Synaptobrevin attached to the synaptic vesicles is referred to as a v-SNARE, and SNAP25 (synaptosomal-associated protein of 25 kD) and syntaxin attached to the target plasma membrane are designated as t-SNAREs.¹ The assembled complex is a coiled-coil in which α -helices are held together by strong hydrophobic interactions. The complex is stabilized by complexin, a small soluble neuronal protein.² In the resting state, Munc18 binds to a closed form of syntaxin and blocks formation of the SNARE complex but assists the SNARE complex to effect exocytosis in the active state.³

SNAP25 also participates in endocytosis at hippocampal synapses⁴ and negatively modulates the neuronal voltage-gated calcium channel during intense activity.⁵ Also, by calcium-dependent interaction with synaptotagmin, SNAP25 has a role in vesicle docking and priming as well as in triggering fast exocytosis.⁶ In the absence of SNAP25, vesicle docking at the presynaptic active zones persists, but the pool of vesicles primed for release is empty, and fast calcium-triggered exocytosis is abolished.⁷

Supplemental data
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SNAP25 has 2 splice variants, SNAP25A and SNAP25B, generated by obligate alternative splicing of 2 similar exon 5 sequences and a ubiquitously expressed homolog, SNAP23.⁸ Except for 9 residues in exon 5, the 2 SNAP25 isoforms are identical and their expression is developmentally regulated. In the mouse brain, Snap25a is expressed before Snap25b, but by the second postnatal week, Snap25b becomes the major splice variant and Snap25a is weakly expressed.^{8,9} Snap25b accumulates in the plasma membrane of nerve terminals at motor endplates (EPs) and central synapses consistent with its being an essential SNARE protein for regulated exocytosis, whereas Snap25a is diffusely distributed in the neuronal cytoplasm and axoplasm.^{9,10}

Herein, we report identifying by exome sequencing a de novo dominant mutation of a conserved residue in exon 5 of SNAP25B. The mutation causes a congenital myasthenia, cortical hyperexcitability, cerebellar ataxia, and intellectual disability. The mutant SNAP25B compromises quantal release at patient EPs, hinders fusion of liposomes containing t-SNAREs and v-SNAREs, and markedly inhibits catecholamine release from transfected chromaffin cells.

METHODS **Standard protocol approvals, registrations, and patient consents.** All human studies were approved by the institutional review board of the Mayo Clinic, and the parents gave informed consent to participation in the study.

In vitro electrophysiology studies. Intercostal muscle specimens were obtained from origin to insertion from the patient, and from patients without muscle disease undergoing thoracic surgery serving as controls. The amplitude of the miniature EP potential (MEPP), the quantal content of the EP potential (m), estimates of the probability of quantal release (p), the number of readily releasable quanta (n), and the EP acetylcholine receptor (AChR) content were determined as previously described.^{11–14}

Genetic analysis. Exome sequencing of genomic DNA isolated from blood of the patient and her parents was performed at the Mayo Clinic. We used Ingenuity software to filter variants at intergenic and intronic sites. Because there was no family history of similarly affected relatives, we searched for variants compatible with recessive as well as de novo variants and searched for variants in genes that affect quantal release from motor nerve terminals. The identified mutation was confirmed by Sanger sequencing of genomic DNA isolated from blood and muscle. Nucleotides of isoform B of *SNAP25* complementary DNA (cDNA) were according to GeneBank accession number NM_130811.2.

Expression of wild-type and mutant SNAP25B: Construction of vectors expressed in mammalian cells. We isolated cDNA from spinal cord anterior gray column of an

accident victim, amplified the entire coding region of wild-type SNAP25B, and cloned it into pmCherry-N1 vector as well as into pZsGreen-N1 vector (Clontech, Mountain View, CA) with the tags at the C-terminal position. The mutation was verified by Sanger sequencing of the plasmid. The mutation was engineered into wild-type SNAP25B-pmCherry-N1 plasmid using the QuikChange II XL Site-Directed Mutagenesis kit (Agilent Technologies, Santa Clara, CA). COS-7 cells were transfected with mCherry-tagged wild-type or with ZsGreen-tagged wild-type plus SNAP25B mutant plasmids using TransIT-LT1 transfection reagent (Mirus Bio LLC, Madison, WI). Cells were harvested after 24 hours of transfection. Extracts of transfected cells were immunoblotted with SNAP-25 antibody (rabbit immunoglobulin G fraction; Sigma-Aldrich, St. Louis, MO) followed by alkaline phosphatase-labeled donkey anti-rabbit immunoglobulin G antibodies (Jackson ImmunoResearch, West Grove, PA) and with mouse monoclonal GAPDH antibody (EMD Millipore, Billerica, MA) to control for loading. The blots were developed by the alkaline phosphatase method and quantitated using NIH Image 1.63.

Liposome fusion assays. Construction of bacterial expression vectors. Coding regions of wild-type and mutant SNAP25 and the entire coding region of human synaptobrevin 2 (GenBank accession no. NM_014232.2) were cloned into the bacterial-expression vector pET100/D-TOPO (Life Technologies, Grand Island, NY). Isoform 1 of human syntaxin 1A (GenBank accession no. NM_004603.3) was cloned into pET200/D-TOPO (Life Technologies). Both vectors include 6 N-terminal histidine residues. Primers are available on request.

Expression and purification of bacterially expressed SNARE proteins. Protein expression and purification procedures were based on modification of previously reported methods.^{15–17} Details of the procedure are described in appendix e-1 on the *Neurology*® Web site at Neurology.org.

Liposome fusion studies. Protein reconstitution into liposomes was done by minor modifications of a previously reported method.¹⁸ Details of the assay are described in appendix e-2.

Amperometric assay of exocytosis from chromaffin cells: Primary culture and transfection of bovine chromaffin cells. Adult bovine adrenal glands were obtained from a local slaughter house. Chromaffin cells were prepared by collagenase digestion of medullary tissue followed by further separation from debris and erythrocytes by centrifugation on a Percoll gradient (GE-Amersham Biosciences, Uppsala, Sweden) as described.¹⁹ Details of transfection, preparation of carbon fiber electrodes, and amperometric recordings are described in appendix e-3.

RESULTS **Clinical data.** The patient, currently 11 years of age, was hypomotile in utero during the third trimester and born by induced labor after 42 weeks of gestation. She was stiff and cyanotic at birth, required oxygen therapy, and had multiple joint contractures. She achieved full head control at age 2 years. Although she walked a few steps independently at age 7, subsequently she could only walk with a walker. Since early childhood, she had intermittent eyelid ptosis and episodes of blank stare and unresponsiveness. The EEG showed generalized atypical polyspike and wave discharges and diffuse slowing of the background rhythm, which did not change

during the staring episodes. Treatment with valproic acid between 5 and 8 years was ineffective. MRI of the brain was normal. Multiple orthopedic procedures corrected some joint deformities. At age 11 she speaks mostly single words, she is echolalic, and her development is at the level of a 3- to 4-year-old child. She has fatigable weakness, fluctuating eyelid ptosis, ataxic dysarthria, a paretic and ataxic gait worsened by flexion contractures at the knees, and she is areflexic (figure 1A). The EMG revealed an 18% to 50% decrement of the fourth compared with the first evoked compound muscle action potential in different muscles. Injection of 1 mg of neostigmine reduced the decrement to less than half in the trapezius muscle, but pyridostigmine therapy was ineffective. Therapy with 3,4-diaminopyridine, along with levetiracetam to prevent seizures, improved the patient's strength but not her ataxia. There was no history of similarly affected relatives.

Structural studies. Conventional histochemical studies of intercostal and serratus anterior muscles were

normal. The cholinesterase-reactive synaptic contacts on intercostal muscle fibers were pretzel-shaped or occasionally ovoid. Paired fluorescence localization of the AChR and AChE (acetylcholinesterase) showed normal expression of both proteins at patient EPs. Qualitative inspection and morphometric analysis of electron micrographs of 22 EPs revealed no abnormality (see table e-1). The density and distribution of AChR on the junctional folds were normal. The nerve terminals harbored abundant synaptic vesicles, and many were docked at active zones (figure 1B).

In vitro analysis of neuromuscular transmission. The frequency of the spontaneous MEPPs was reduced to 31% of normal (table 1). The synaptic response to acetylcholine, reflected by the MEPP amplitude, was slightly reduced, but the number of AChRs per EP fell in the normal range. The observed values for the acetylcholine quanta released by nerve impulse (m) (figure 1C) and for the readily releasable quanta (n) (figure 1D) were not normally distributed, with some

Figure 1 Patient at age 10 years and EP studies

(A) The patient cannot rise from the floor or walk without assistance. (B) Electron micrograph of patient EP shows the nerve terminal harbors abundant synaptic vesicles. Asterisks indicate vesicles focused on the active zones. The postsynaptic region is well developed. Bar = 0.5 μm. (C) Histograms of the quantal content of the EPP (m). The patient values are not normally distributed, with some values much lower and some normal or high normal. (D) Vertical scatterplot of readily releasable quanta (n) at patient and control EPs. Some values at patient EPs are much lower and some as high or higher than at control EPs. EP = endplate; EPP = EP potential.

Table 1 Microelectrode studies of neuromuscular transmission and α -bungarotoxin binding sites per EP

	Controls	Patient (no. of EPs)	p
MEPP amplitude, mV ^a	1.00 ± 0.03 (165)	0.78 ± 0.10 (14)	<0.02 ^b
MEPP frequency, min ⁻¹	10.7 ± 0.6 (11)	3.3 ± 0.73 (11)	<0.001 ^b
Quantal content of EPP at 1 Hz (m) ^c	26.9 ± 1.0 (91)	Low values: 6.2 ± 3.1 (12)	<0.001 ^d
		High values: 25.5 ± 4.9 (10)	NS ^d
No. of readily releasable quanta (n)	268 ± 20 (25)	Low values: 32.5 ± 22.5 (2)	<0.025 ^d
		High values: 323 ± 135 (3)	NS ^d
Probability of quantal release (p)	0.14 ± 0.04 (25)	0.09 ± 0.01 (5)	<0.01 ^b
[¹²⁵ I]α-Bungarotoxin binding sites per EP ^e	12.8 ± 0.8 E6 (13); normal range 9.1–18.7	9.92 E6	

Abbreviations: EP = endplate; EPP = EP potential; MEPP = miniature EP potential; NS = not significant.

Values indicate mean $\pm$ SE. Numbers in parentheses indicate number of EPs except for control [125 I] α -bungarotoxin binding sites where they indicate number of patients.

^aCorrected for membrane potential of -80 mV and fiber diameter of $50\text{ }\mu\text{m}$; 30°C .

^b Determined by 2-tailed t test.

^c Corrected for membrane potential of -80 mV, nonlinear summation, and non-Poisson release.

^d Determined by Mann-Whitney rank sum test.

^eEach acetylcholine receptor binds 2 molecules of α -bungarotoxin.

values significantly lower and some normal or higher than normal suggesting somatic mosaicism as can be observed with mutations arising in a postzygotic cell.²⁰ The probability of quantal release (p) was reduced to 63% of normal.

Mutation analysis. To identify the molecular basis of the defect in the exocytotic machinery, we performed whole-exome sequencing using DNA isolated from the patient and her parents. We scrutinized the identified putative variants with Ingenuity Variant Analysis software (Qiagen, Redwood City, CA) and focused on mutations affecting the exocytotic machinery. This revealed a de novo variant, p.Ile67Asn (c.200T>A), in the highly conserved exon 5 of SNAP25B (figure 2A). Sanger sequencing confirmed the mutation in the patient and its absence in her parents (figure 2C). The observed variant is not present in NHLBI GO Exome Variant Server (NHLBI GO Exome Sequencing Project, Seattle, WA, 10/2013; URL: <http://evs.gs.washington.edu/EVS>), 1000 Genomes project (<http://browser.1000genomes.org>), and dbSNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP/>).

Ile167 is conserved among all vertebrate species as well as in *Drosophila melanogaster* (figure 2B). SIFT, PolyPhen-2, and MutationTaster predict the variant to be damaging or disease-causing. The mutated isoleucine points toward the center of the coiled-coil structure of the assembled SNARE complex²¹ (figure 2D). We found no mutations in any heretofore identified congenital myasthenic syndrome (CMS) genes, namely, *CHRNA1*, *CHRB1*, *CHRND*, *CHRNE*, *RAPSN*, *CHAT*, *COLQ*, *DOK7*, *MUSK*, *AGRN*, *LRP4*, *SCN4A*, *DPAGT1*, *GFPT1*, *ALG2*, *ALG14*, *LAMB2*, and *PLEC*.

Expression of wild-type and mutant SNAP25B in COS-7 cells. Immunoblots of extracts of COS-7 cells transfected with mCherry-tagged mutant and wild-type SNAP25B probed with anti-SNAP 25B antibodies showed no difference in expression of wild-type and mutant SNAP25B. ZsGreen-tagged and mCherry-tagged wild-type SNAP25B plasmids were also similarly expressed.

Mutant SNAP25B hinders the fusion of liposomes containing t-SNAREs and v-SNAREs. Fusion of the synaptic vesicles with the presynaptic membrane requires interaction of the synaptic vesicle-associated synaptobrevin (v-SNARE), with the presynaptic membrane-associated syntaxin and SNAP25B (t-SNAREs). When v- and t-SNAREs are reconstituted into separate liposomal vesicles, they assemble to form SNARE-pins that link adjacent vesicles.¹⁵ This process is inefficient but is accelerated when a stabilized syntaxin/SNAP-25 acceptor complex is used.²² In this system, Ca^{2+} drives the fusion of v-SNARE and t-SNAREs in opposing bilayers in a circular array to form a conducting pore in vitro.²³ As a first assessment of the pathogenic effects of the Ile67Asn substitution in SNAP25B, we performed 20-minute liposome fusion experiments in triplicate in the presence and absence of 10 mM Ca^{2+} , mixing liposomes incorporating synaptobrevin either with liposomes containing wild-type or mutant SNAP25B plus syntaxin. The resulting changes in particle size distribution were evaluated using the Kolmogorov–Smirnov test.²⁴

In the absence of Ca^{2+} , mixing synaptobrevin liposomes with liposomes incorporating wild-type or mutant SNAP25B plus syntaxin caused no change

Figure 2 Analysis of the identified mutation

(A) Scheme of SNAP25B complementary DNA indicating protein domains and position of the mutation. (B) The mutated residue is conserved across vertebrates and *Drosophila*. (C) Sanger chromatograms of patient and parental genomic DNA shows T>A change in patient but not in parents. (D) Stereo view of the crystal structure of the rat SNARE complex normal to its long axis at the level of the mutated Ile67 based on Protein Data Bank 1SFC. The mutated isoleucine points to center of the complex. Its replacement by a hydrophilic asparagine predicts disruption of hydrophobic interactions between α -helices. F = father; M = mother; Pt = patient; Sb = synaptobrevin; SNAP25B = synaptosomal-associated protein, 25B; SNARE = soluble N-ethylmaleimide-sensitive factor attachment protein receptor; Sn1 = α -helix near N-terminal end of SNAP25B; Sn2 = α -helix near C-terminal end of SNAP25B; Sx = syntaxin.

in particle size distribution. In the presence of 10 mM Ca^{2+} , particle size distribution shifted to the right on mixing synaptobrevin liposomes with wild-type SNAP25B-syntaxin liposomes ($p < 0.05$) (figure 3A), but mixing with mutant SNAP25B-syntaxin liposomes caused no significant change (figure 3B).

Transfection of chromaffin cells with mutant or with mutant plus wild-type SNAP25B inhibits exocytosis of catecholamine-containing vesicles. Because SNAP25B is highly expressed in both chromaffin cells and neurons,²⁵ we measured depolarization-triggered exocytosis of

dense-core vesicles from wild-type, mutant, and mutant plus wild-type SNAP25B-transfected chromaffin cells by amperometry.²⁶ In this system, exocytosis of each vesicle is signaled by a single temporally resolved spike.^{27–29} The area of each spike is a measure of the charge generated by oxidation of the released catecholamine and the number of released molecules is obtained by dividing the generated charge by Faraday's constant.

During the first minute after depolarization, non-transfected and wild-type SNAP25B-transfected chromaffin cells exocytose vesicles at similar rates (figure 3D, open and solid circles). In contrast, mutant SNAP25B-transfected cells exocytose vesicles at a much slower rate and generate lower-amplitude spikes (figure 3C, middle panel and figure 3D, lower curve, solid triangles) reducing the released catecholamine molecules to only 11% of that released by wild-type transfected cells (table 2). Cells cotransfected with both wild-type and mutant SNAP25B identified by dual green and red fluorescence exocytose vesicles at essentially the same rate as cells transfected with only mutant SNAP25B (figure 3C, lower tracing, and figure 3D, lower curve, open triangles) and reduce the number of catecholamine molecules to essentially the same extent as cells transfected with only mutant SNAP25B (table 2).

DISCUSSION CMS are heterogeneous disorders in which the safety margin of neuromuscular transmission is compromised by one or more mechanisms. The disease proteins reside in the presynaptic region, the synaptic basal lamina, or the postsynaptic region. Among more than 300 patients with CMS with identified mutations investigated at the Mayo Clinic, only 5% had an exclusive presynaptic defect that was caused by mutations in choline acetyltransferase.³⁰ Thus, the Ile67Asn mutation in *SNAP25B* causes a novel presynaptic CMS associated with cortical hyperexcitability, cerebellar ataxia, and intellectual disability. The identified Ile67Asn mutation is pathogenic by a dominant negative effect rather than by haplotype insufficiency because cotransfection of chromaffin cells with mutant cDNA or with wild-type plus mutant cDNA suppresses depolarization-evoked exocytosis to the same extent.

A heterozygous Val48Phe mutation in exon 4 of *SNAP25B* was recently reported in a 15-year-old girl with intractable epilepsy and severe encephalopathy but no neuromuscular symptoms. Expression studies to scrutinize the effects of the mutation were not performed.³¹

Mutations in *Snap25b* had also been detected in animal models. Of note, a heterozygous Ile67Thr mutation in *Snap25b* had been observed in the blind-drunk (+/Bdr) mouse that showed a mild

(A) Mixing liposomes incorporating wild-type v- and t-SNAREs causes significant right shift in particle size distribution. (B) Mixing liposomes incorporating wild-type v-SNARE with t-SNARE liposomes harboring mutant Snap25B causes no significant shift in particle size distribution. (C) Representative amperometric traces from depolarized chromaffin cells. Each spike represents a single exocytotic event. Mutant SNAP25B and mutant SNAP25B plus wild-type transfected cells generate fewer and lower-amplitude spikes than wild-type SNAP25B-transfected cells. (D) Cumulative exocytotic events during the first minute after depolarization from 15 nontransfected, 11 wild-type SNAP25B-transfected, 14 mutant SNAP25B-transfected, and 17 mutant SNAP25B plus wild-type transfected cells. For each group of cells, each point indicates the mean number of spikes over 5 seconds. Vertical lines indicate 1 SE. Compared with nontransfected or wild-type transfected cells, mutant transfected, or mutant plus wild-type transfected, cells exocytose vesicles at similar markedly reduced rates. SNAP25B = synaptosomal-associated protein, 25B; t-SNAREs = target membrane-attached SNAP25B and syntaxin; v-SNARE = synaptic vesicle-attached synaptobrevin.

ataxic gait around age 4 weeks and impaired sensorimotor gating.³² In silico modeling and melting point studies indicated the mutation increased affinity for syntaxin that would hinder rapid disassembly of the SNARE complexes after exocytosis. The mutation also decreased the frequency of the miniature excitatory

postsynaptic current in cortical brain slices to approximately 30% of wild-type, caused marked short-term depression of the excitatory postsynaptic current after high-frequency stimulation, and reduced the release of secretory vesicles from depolarized pancreatic island β cells. Whereas Snap25^{-/-} mice die at birth of

Table 2 Amperometry studies of bovine chromaffin cells transfected with wild-type, mutant, and mutant plus wild-type SNAP25B

	Wild-type	Mutant; p vs wild-type	Mutant + wild-type; p vs wild-type
Fusion events, min ⁻¹	78.3 ± 11.1 (11)	23.8 ± 4.6 (14); p < 0.001 ^a	27.2 ± 3.7 (17); p < 0.001 ^a
Spike amplitude, pA	49.6 ± 3.7 (369)	20.9 ± 3.0 (107); p < 0.001 ^a	20.2 ± 2.2 (206); p < 0.001 ^a
Total charges detected, pC min ⁻¹	59.9 ± 17.5 (7)	6.4 ± 1.7 (8); p < 0.001 ^b	6.1 ± 1.6 (8); p < 0.001 ^b
Total catecholamine molecules released, ^c min ⁻¹	187 ± 54.6 E6 (7)	19.8 ± 5.1 E6 (8); p < 0.001 ^b	17.8 ± 4.6 E6 (8); p < 0.001 ^b

Abbreviation: SNAP25B = synaptosomal-associated protein, 25B.

Values indicate mean ± SE. Numbers in parentheses indicate number of cells except for spike amplitude where they indicate number of spikes.

^aDetermined by 2-tailed t test.

^bDetermined by Mann-Whitney rank sum test.

^cDerived by Faraday law: $Q = zFM/N_A$, where Q is charge in redox transfer; z, electrons transferred per molecule (2 for catecholamine); F, faraday constant; M, number of reactant molecules; and N_A , Avogadro number.

respiratory failure, heterozygous (Snap25^{+/-}) mice develop cognitive deficits and cortical hyperexcitability.³³ Still other studies indicate that prenatal replacement of the mature Snap25b isoform with Snap25a isoform results in developmental defects, seizures, and impaired short-term plasticity, while the introduction of the isoform change in adult mice results in severe learning impairment, morphologic changes in hippocampus, and altered neuropeptide expression.³⁴ Finally, in *Drosophila melanogaster* with a wild-type background, a heterozygous Arg206Ala (198 in vertebrates) mutation in Snap25b at a botulinum toxin A cleavage site curtails the MEPP frequency at the neuromuscular junction and is predicted to hinder contacts within rosettes of SNARE complexes that assemble to effect fusion of the synaptic vesicle with the presynaptic membrane.³⁵

SNAP25B has also been implicated in the pathogenesis of schizophrenia by studies showing altered transcript or protein levels in brain.^{36,37} Moreover, genetic variations at *SNAP25* may be associated with schizophrenia or attention deficit hyperactivity disorders.³⁷

In the Ile67Asn mutation in our patient, a highly hydrophilic asparagine (hydropathy index, -3.5) replaces a highly hydrophobic isoleucine (hydropathy index, +4.5).³⁸ This likely disrupts or disfigures the coiled-coil configuration of the SNARE complex wherein α -helices are held together by strong hydrophobic interactions²¹ (figure 2D). Consistent with this assumption, the liposome fusion assay, which mimics the partially zippered trans-SNARE complex, shows that the Ile67Asn mutation hinders liposome fusion (figure 3, A and B). Its effects thus differ from that of Ile67Thr in the blind-drunk mouse where the newly introduced threonine is hydrophobic, which enhances the stability of the complex hindering its disassembly after exocytosis.

Recordings from the patient's EPs showed a 30% reduction of the MEPP frequency compared with controls, which is similar to the decrease of miniature excitatory postsynaptic current frequency in cortical recordings of the blind-drunk mouse. Thus, the spontaneous exocytosis of single vesicles as well as the depolarization-evoked fast-synchronous release of many vesicles are governed by the SNARE complex. It is of interest that recent studies indicate that whereas Ca²⁺ binding to synaptotagmin triggers fast-synchronous exocytosis, another Ca²⁺ sensor, Doc2, operating on a different time scale is tuned to regulate spontaneous exocytosis.³⁹ The probability of quantal release at patient EPs was reduced to 67% of normal. A likely explanation for this is that mutant SNAP25B reduces the store of readily releasable vesicles.⁴⁰

AUTHOR CONTRIBUTIONS

Dr. Shen contributed to study concept and design, data acquisition, interpretation and analysis, and manuscript preparation. Dr. Selcen contributed patient evaluation, study concept and design, data acquisition, interpretation and analysis, and manuscript preparation. Joan Brengman contributed to data acquisition. Dr. Engel contributed patient evaluation, study concept and design, data acquisition, interpretation and analysis, and manuscript preparation.

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DISCLOSURE

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