

Synaptotagmin 2 Mutations Cause an Autosomal-Dominant Form of Lambert-Eaton Myasthenic Syndrome and Nonprogressive Motor Neuropathy

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Synaptotagmin 2 is a synaptic vesicle protein that functions as a calcium sensor for neurotransmission but has not been previously associated with human disease. Via whole-exome sequencing, we identified heterozygous missense mutations in the C2B calcium-binding domain of the gene encoding Synaptotagmin 2 in two multigenerational families presenting with peripheral motor neuron syndromes. An essential calcium-binding aspartate residue, Asp307Ala, was disrupted by a c.920A>C change in one family that presented with an autosomal-dominant presynaptic neuromuscular junction disorder resembling Lambert-Eaton myasthenic syndrome. A c.923C>T variant affecting an adjacent residue (p.Pro308Leu) produced a presynaptic neuromuscular junction defect and a dominant hereditary motor neuropathy in a second family. Characterization of the mutation homologous to the human c.920A>C variant in *Drosophila* Synaptotagmin revealed a dominant disruption of synaptic vesicle exocytosis using this transgenic model. These findings indicate that Synaptotagmin 2 regulates neurotransmitter release at human peripheral motor nerve terminals. In addition, mutations in the Synaptotagmin 2 C2B domain represent an important cause of presynaptic congenital myasthenic syndromes and link them with hereditary motor axonopathies.

Synaptotagmin 2 (SYT2), a synaptic vesicle protein, is the major isoform of the synaptotagmin family at mammalian neuromuscular junctions (NMJs) and functions as a calcium sensor for neurotransmission.^{1,2} The C2B domain of SYT2 is essential for neurotransmitter release at the NMJ in several animal models, although mutations in SYT2 (MIM 600104) have not been previously linked to human disease.^{1,3} Here we describe two multigenerational families with dominant mutations of adjacent residues in the SYT2 C2B domain that cause a disorder with features including presynaptic NMJ dysfunction resembling Lambert-Eaton myasthenic syndrome (LEMS) and motor neuropathy. We model and validate the identified dominant defects of the protein in a *Drosophila* model that recapitulates key synaptic dysfunctions found in affected individuals.

In the three-generational index family USA1, affected family members developed foot deformities in childhood including pes cavus and hammer toes (Figure 1). These individuals had a variable degree of proximal and distal limb weakness, muscle fatigue that improved with rest, mild gait difficulties, and reduced or absent deep tendon reflexes that could be elicited after brief exercise. Acetylcholine receptor binding, blocking and modulating antibodies, and voltage-gated calcium channel antibodies

were negative in the proband in USA1. In a second family (UK1), affected individuals (Figure 1) also presented with childhood-onset foot deformities, but had congenital hip dislocation (in affected females), distal, often asymmetric lower extremity muscle atrophy, lower limb predominant weakness, and absent deep tendon reflexes. A summary of the clinical features of each person in both families is included in Table 1. Electrophysiological studies in USA1 (Figure 2, Table S1 available online) showed presynaptic NMJ dysfunction with low-amplitude compound muscle potentials (CMAPs) and marked CMAP amplitude increments after brief exercise. Concentric needle electromyography was normal in weak muscles in affected USA1 family members. In UK1, electrophysiologic abnormalities included low-amplitude lower-limb CMAPs with a variable degree of postexercise amplitude facilitation and slight re-innervation on needle electromyography of distal muscles, consistent with motor neuropathy (Figure 2, Table S2). Sensory nerve conduction studies were normal in all individuals in each family (Tables S1 and S2).

A whole-exome sequencing study was undertaken in each family, under Institutional Review Board approval, and after written informed consent. Whole-exome sequencing in USA1 family members II.2, III.2, and II.1 (unaffected) was performed with the 50Mbases Agilent

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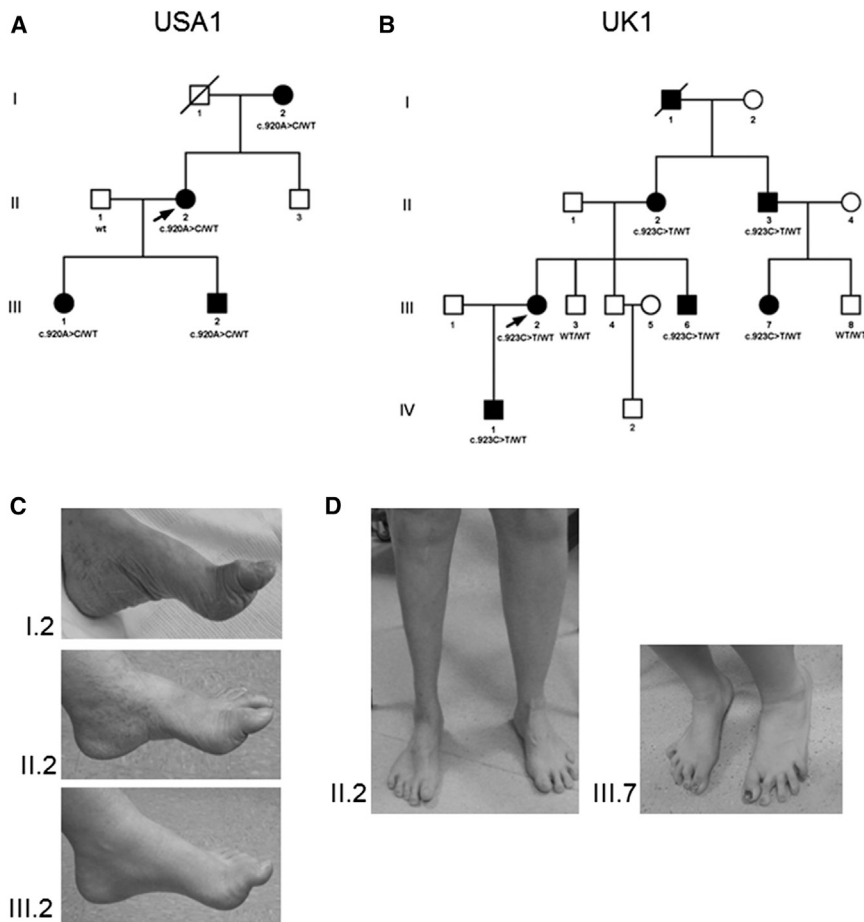


Figure 1. Pedigrees of Affected Families and Lower Limb Deformities

(A and B) Family pedigrees for USA1 (A) and UK1 (B). Filled symbols are clinically affected individuals, arrows denote the probands.

(C) Foot and toe deformities in three generations in the USA1 family.

(D) Asymmetric distal lower limb muscle atrophy (individual II.2 shown here) was noted in affected UK1 females. Splayed toes (family member III.7) were notable in the UK1 kindred.

SureSelect v.4 capture kit combined with an Illumina HiSeq2000 sequencing instrument. Raw data were aligned with the BWA software and variants were called using GATK. All resulting variants and also genotype calls at non-variant positions were annotated, and segregation patterns were calculated with the GEDI protocol.⁴ After import into the GEM.app software, we filtered for dominantly segregating variants and excluded variants that were present in the NHLBI EVS data set of 6,500 individuals, the local GEM.app data set of 3,000 individuals, variants with GERP conservation scores of less than 3, and genes with a residual variation intolerance score of >95 percentile.⁵ We considered only nonsynonymous variants, splice site changes, and indels. Using five different protein prediction algorithms (Polyphen, MutationTaster, MutationAssessor, SIFT, LRT), we created a composite score where each “damaging” assessment receives one point. Of the six remaining genes from this analysis, only one gene, *SYT2*, had a full score of 5 (Table S3). Full Sanger-based segregation analysis of all six genes in the USA1 family reduced the gene list to only two candidates, including a heterozygous c.920A>C (p.Asp307Ala) missense mutation (RefSeq accession number NM_177402.4) in the gene encoding *SYT2* (Figure 2, Table S3). *SYT2* represents an enticing candidate given its well-established role in calcium activation of neurotransmitter release at NMJs.^{1–3} Further,

Asp307, an aspartate residue, is the second of five residues that coordinate calcium binding to the C2B domain (Figure 2).⁶ Disruption of these residues leads to a loss of calcium binding and dominant-negative disruption of exocytosis in *Drosophila*.^{7–10}

Whole-exome sequencing in family UK1 (individuals III:2 and IV:1) was performed with the Illumina TruSeq 62 Mb exome capture and sequenced on an Illumina HiSeq 2000 instrument. The in-house bioinformatics pipeline at Newcastle University included alignment to the human reference genome (UCSC hg19), reformatting, and variant detection (Varscan v.2.2, Dindel v.1.01), as described previously.¹¹ On-target variant filtering excluded those with minor allele frequency greater than 0.01 in several databases. Rare homozygous and compound heterozygous variants were defined, and protein altering and/or putative “disease-causing” mutations, along with their functional annotation, were identified using ANNOVAR. Lists of on-target variants were filtered against data from NHLBI-6500-ESP, the 1000 Genomes project, and the exome sequences of 334 unrelated in-house control exomes to identify rare homozygous variants with a MAF < 0.01. Putative pathogenic variants were confirmed by Sanger sequencing and segregation analyses was performed. This sequencing revealed a heterozygous segregating missense mutation affecting the amino acid residue adjacent to USA1 (c.923C>T [p.Pro308Leu]) (Figure 2). This change was also predicted to be deleterious and not present in controls. No other segregating variants were identified in this family (Table S3).

To determine how these *SYT2* mutations might alter synaptic function, we focused on the c.920A>C (p.Asp307Ala) variant given its established role in calcium binding to the C2B domain. We generated transgenic *Drosophila* containing wild-type (WT) and mutant Synaptotagmin genes using the GAL4-UAS expression system. *Drosophila* contains a single isoform (DSYT1) of the

Table 1. Clinical Features in Family Members in USA1 and UK1 Affected by SYT2 Mutations

	USA1 (c.920A>C [p.Asp307Ala])				UK1 (c.923C>T [p.Pro308Leu])					
	I.2	II.2 ^a	III.1	III.2	II.2	II.3	III.2 ^a	III.6	III.7	IV.1
Age (years)	70	49	23	15	44	42	27	16	12	7
Gender	F	F	F	M	F	M	F	M	F	M
Foot or Toe Deformity, Joint Abnormalities^b										
Feet/Toes	C,H	C,H,S	C,H	C,H	C,S	H	HA,H	C,H	C,H,S	P
Hip dysplasia	–	–	–	–	+	–	+	–	+	–
Lower Limb Features^c										
Weakness	P = D	P = D,F	–	–	R > L; R: D,P; L: D	D	D	–	R > L,D	–
Wasting	–	–	–	–	R: D,P; L: D	D	D	–	–	–
Tendon reflexes	A	A,F	A, Red	A,F	A	A,F	A	A	A	A
Upper Limb Features^c										
Weakness	D	p = D,F	–	–	D	–	D	–	–	–
Tendon reflexes	A	A,F	A,F	A,F	A	A	A	A	A	A
Motor Development and Gait^d										
Delayed milestones	–	–	–	–	–	–	–	–	–	+
Rapid fatigue with activity	–	++	–	+	–	–	–	–	–	–
Gait abnormalities	W	TW, HW	–	+	W, HW, TW	HW, TW	W, HW, TW	–	HW	W, HW
Orthotics	–	–	–	–	KO	–	AFO	–	–	FO
Orthopedic surgeries	–	T	–	–	K,F	A,T	F	–	–	–
Difficulty with sports	–	+	–	+	+	–	+	+	–	+
Other Clinical Findings										
Hearing loss	+	+	–	–	–	–	–	–	–	–
Benign positional vertigo	–	+	–	–	–	–	–	–	–	–
Sensory loss (hands or feet)	–	–	–	–	+	–	+	–	–	+
Electrophysiological Findings^e										
CMAP amplitudes	Red	Red	Red	Red	Red	Red	Red	N	Red	ND
CMAP facilitation	–	+++	+++	+++	–	+	+++	ND	++	ND
SNAP amplitudes	N	N	N	N	N	N	N	N	N	N
EMG	N	N	ND	ND	R	R	R	R	R	ND

^aProband^bAbbreviations are as follows: C, pes cavus; P, pes planus; HA, high arch; H, hammer toes; S, splayed toes.^cAbbreviations are as follows: P, proximal; D, distal; R, right; L, left; A, absent; N, normal; ND, not done; F, facilitation with exercise; Red, reduced. Minus sign (–) indicates not present; plus sign (+) indicates present.^dAbbreviations are as follows: AFO, ankle foot orthotic; FO, foot orthotics; KO, knee orthotic; K, knee surgery; A, ankle surgery; F, foot surgery; T, toe surgery; W, waddling gait; HW, unable/impaired heel walk; TW, impaired toe walk. Minus sign (–) indicates not present; plus sign (+) indicates present.^eSee Tables S2 and S3 for detailed electrophysiologic findings. Facilitation denotes an increase in tendon reflexes, strength, or CMAP amplitude after 10 s of maximal voluntary contraction. USA II.1, a 49-year-old male and the husband of the proband, UK III.3 (a 24-year-old male and brother of the proband), and UK III.8 (a 9-year-old female cousin of the proband) are unaffected with no neurologic symptoms or signs and no foot deformities. Abbreviations are as follows: CMAP, compound muscle action potential; Red, reduced; SNAP, sensory nerve action potential; EMG, needle electromyography; R, reinnervation; N, normal; ND, not done. Single plus sign (+) indicates 30%–49% CMAP amplitude increase after 10 s of exercise; double plus sign (++) indicates 50%–99% CMAP amplitude increase; triple plus sign (+++) indicates >100% CMAP amplitude increase.

mammalian synaptic vesicle SYT subfamily (SYT1, SYT2, SYT9). DSYT1 is highly homologous to human SYT2, with perfect conservation of the five C2B calcium binding residues, including Asp307 (corresponding to Asp362 in *Drosophila*) (Figure 3A, Figure S1). We generated transgenic WT and c.1527A>C (p.Asp362Ala) *UAS-DSyt1* lines and

induced these genes using the pan neuronal GAL4 driver *elav^{C155}* (Figures 3B and 3C). The p.Asp362Ala protein localized normally to synapses in both the *sytl^{-/-}* null and wild-type backgrounds (Figure 3C). Neurotransmitter release was analyzed at third instar *Drosophila* NMJs using current recordings of postsynaptic responses in voltage

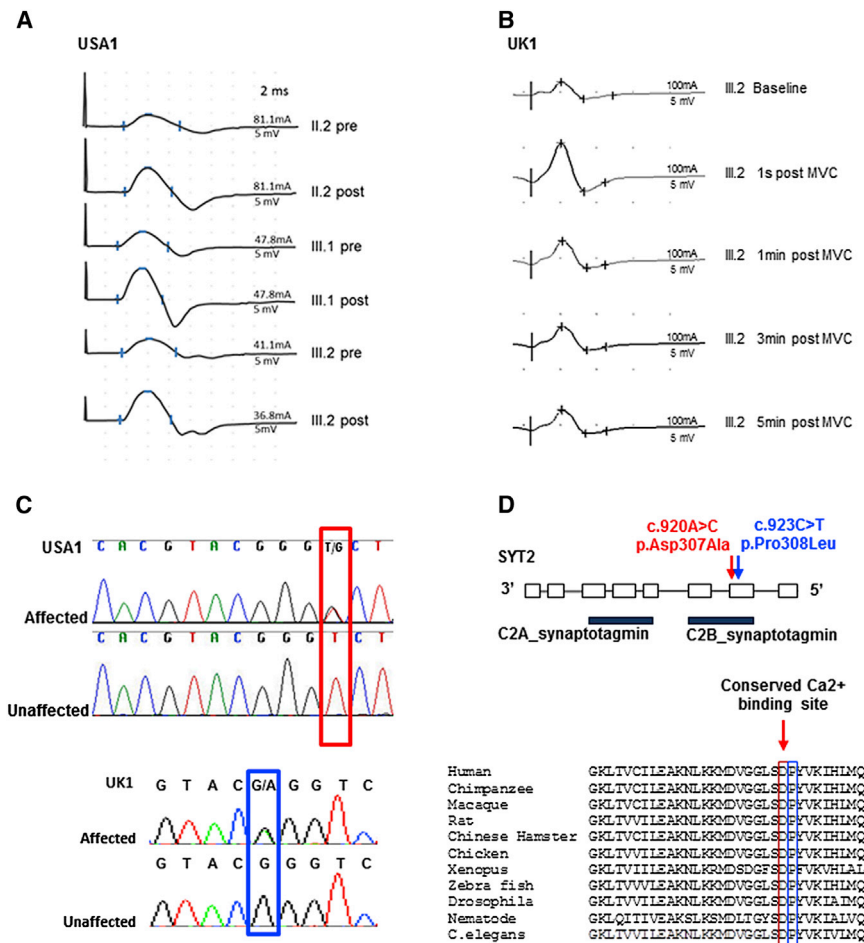


Figure 2. Postexercise Compound Muscle Action Potential Amplitude Facilitation in USA1 and UK1 and Family Genetic Studies

(A) Median nerve distal CMAP before and immediately after 10 s of maximal voluntary contraction for USA1 proband II.2 and her affected offspring III.1 and III.2. Each of these individuals demonstrated a greater than 100% increment in median nerve distal compound muscle action potential amplitude following brief exercise. Individual II.2 also demonstrated a 139% increment in the peroneal motor distal compound muscle action potential amplitude recording from the tibialis anterior muscle. Individuals III.1 and III.2 additionally demonstrated 110% and 200% distal ulnar compound muscle action potential amplitude increments, respectively, after 10 s of exercise. See Table S1 for full electrophysiological data for USA1.

(B) Amplitude and area increment of the peroneal CMAP (recording from tibialis anterior) in the UK1 proband (III.2) after 10 s of maximal voluntary contraction (MVC), which persisted beyond 5 min. See Table S2 for electrophysiologic data for UK1.

(C) Sanger traces demonstrating the c.920A>C missense mutation in SYT2 in USA1. This produces an amino acid change from aspartate to alanine at the second aspartate residue (p.Asp307Ala) in the cytoplasmic C2B domain of SYT2. A c.923C>T missense mutation in SYT2 in affected UK1 individuals results in a

line-to-leucine amino acid change at the adjacent residue (p.Pro308Leu). Note that the SYT2 gene is encoded at the opposite strand. (D) Position of the identified mutations in each family on the gene structure of SYT2 and the conservation of the affected residues across species.

clamp. p.Asp362Ala failed to rescue any of the defects observed in the *DSyt1*-null background (Figures 3D–3H). Rescued animals with p.Asp362Ala DSYT1 lacked synchronous neurotransmitter release and displayed enhanced asynchronous release and elevated spontaneous fusion rates as previously described in *DSyt1*-null mutants.^{8,10} These results indicate that the p.Asp362Ala mutation completely abolishes the ability of the protein to support calcium-triggered neurotransmitter release.

We next assayed the effects of the p.Asp362Ala protein on synaptic transmission in the presence of endogenous DSYT1, mimicking the dominant human condition. Four independent transgenic p.Asp362Ala lines were lethal when driven with the pan neuronal GAL4 driver *elav*^{C155} in *Drosophila* neurons. Two transgenic lines, C2B#1 (with induced levels of p.Asp362Ala protein similar to WT DSYT1) (Figure 3B) and C2B#2 (induced at a slightly higher level), were further characterized. Induction of p.Asp362Ala DSYT1 in the presence of endogenous DSYT1 caused a striking dominant-negative effect on neuromuscular transmission, while WT DSYT1 had no effect (Figures 4, S2, and S3). p.Asp362Ala induction reduced evoked release (Figures 4A and 4B) and increased the frequency

of spontaneous fusion events (Figures 4C and 4D). The p.Asp362Ala C2B#2 transgenic line produced a more severe phenotype, indicating dosage-dependent disruptions in synaptic transmission. High-frequency stimulation revealed increased facilitation of evoked release in the presence of p.Asp362Ala (Figures 4E and 4F), consistent with a reduction in initial release probability caused by the mutant protein and similar to the observation of enhanced CMAP amplitude after exercise in the USA1 family. These findings indicate that the SYT mutant exerts a strong dominant-negative effect on synaptic transmission that mimics many aspects of the defects observed at the NMJs of affected USA1 family members.

How does the SYT2 mutant protein dominantly interfere with neurotransmitter release? We previously demonstrated that SYT functions as a multimer in vivo in *Drosophila*, consistent with structural studies of the protein.^{9,12,13} The dominant effect observed in the c.920A>C family and the phenotypes of p.Asp362Ala in *Drosophila* suggest that mutant SYT2 is likely to multimerize with endogenous SYT2 and disrupt its normal properties, resulting in the defects in synaptic transmission we observe in both systems. Synaptotagmins have been

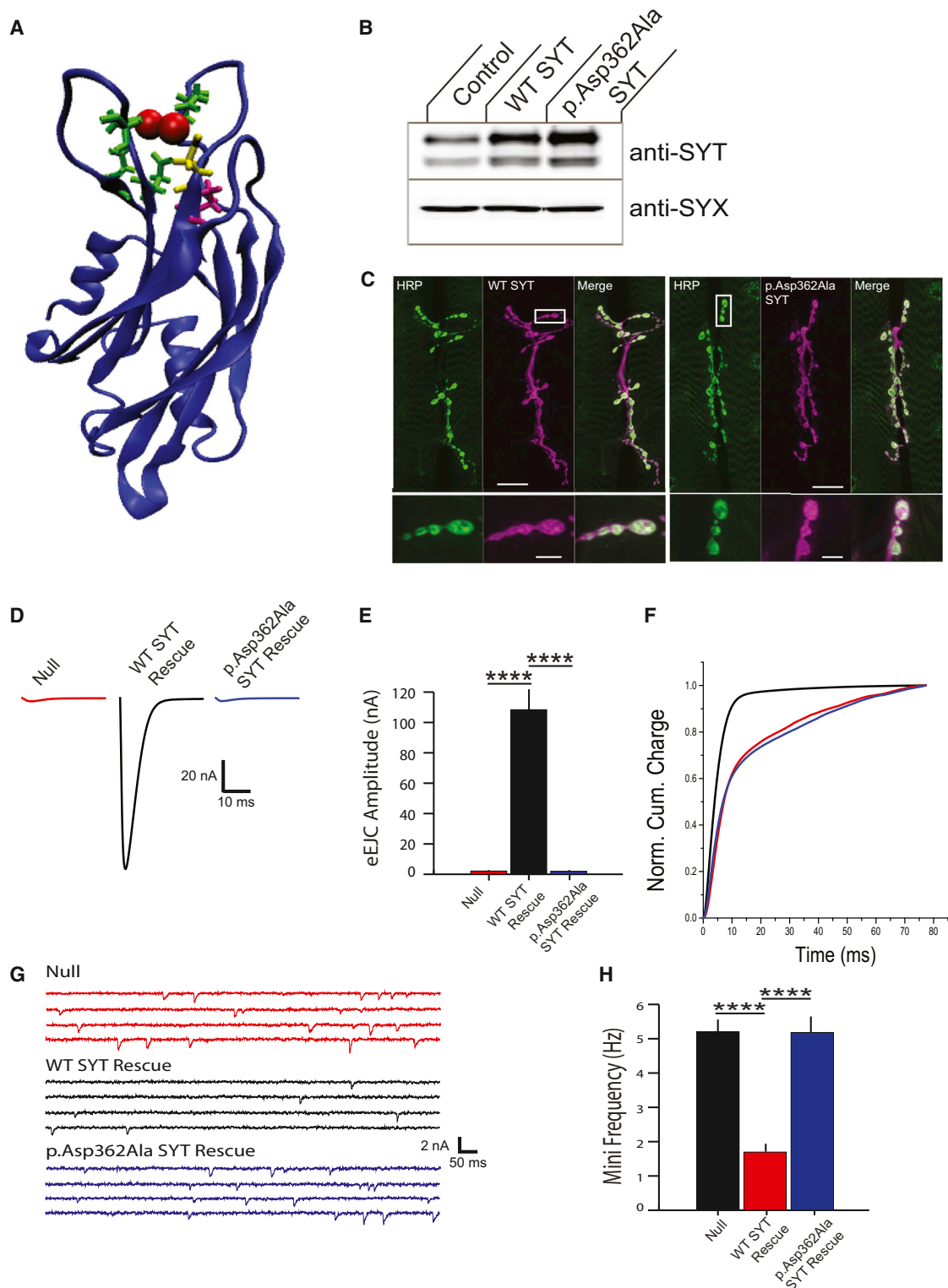


Figure 3. p.Asp362Ala DSYT1 Fails to Support Neurotransmitter Release in a *synaptotagmin*-Null Mutant

(A) Stereoview of the Asp307 (yellow) and Pro382 (magenta) residues modeled on the rat SYT1 C2B crystal structure. The five essential Ca^{2+} -binding residues are highlighted in green and Ca^{2+} ions in red.

(B) Immunoblot with anti-SYT (top) or anti-Syntaxin (SYX; control) antisera from head extracts of control or transgenic *Drosophila* following wild-type (WT) or p.Asp362Ala DSYT induction by *elav^{C155}*-GAL4.

(C) Representative larval NMJs stained with anti-Myc antisera (green) for animals with Myc-tagged WT or p.Asp362Ala SYT1. The axon is stained with the neuronal marker anti-HRP (magenta). The boxed area is magnified in the bottom panel. Mutant SYT1 targets normally to presynaptic terminals. Scale bars represent 20 μ m (top) or 5 μ m (bottom).

(D) Representative EPSCs recorded in 2 mM external Ca^{2+} in *Dsyt1*^{-/-} null larvae (red) and null mutants rescued with WT DSYT1 (black) or p.Asp362Ala DSYT1 (blue).

(legend continued on next page)

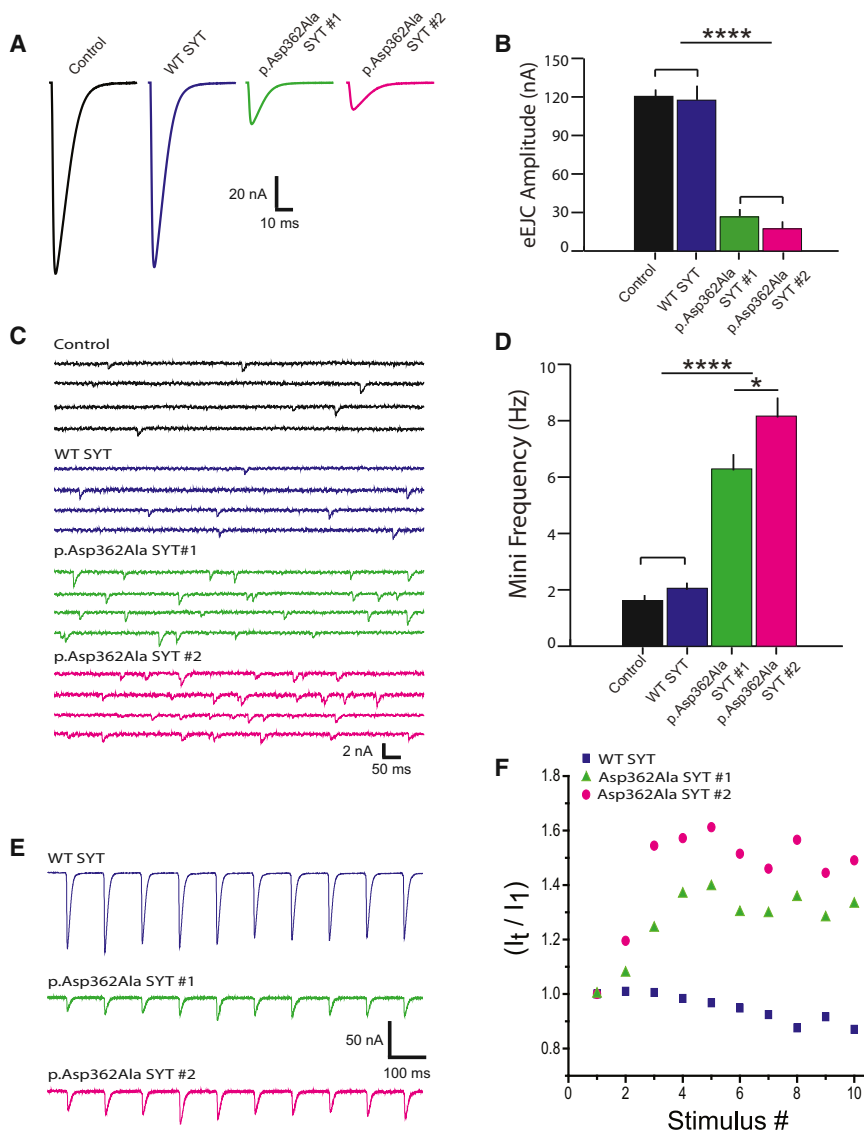


Figure 4. Mutant Synaptotagmin Disrupts Neurotransmitter Release in the Presence of Endogenous Synaptotagmin

(A) Representative EPSCs recorded in 0.2 mM extracellular Ca^{2+} at third instar larval muscle 6 synapses for the indicated genotypes (control, induction of WT or p.Asp362Ala DSYT1 by *elav^{C155}-GAL4*).

(B) Quantification of mean eEJC amplitudes in the indicated genotypes (*w^{-/-}*, 120.5 ± 5.3 nA, n = 16; *elav^{C155}-GAL4*; UAS-SYT1, 117.5 ± 11 nA, n = 15; *elav^{C155}-GAL4*; UAS-SYT1 p.Asp362Ala#1, 26.6 ± 4.7 nA, n = 16; *elav^{C155}-GAL4*; UAS-SYT1 p.Asp362Ala#2, 17.3 ± 3.2 nA, n = 16).

(C) Postsynaptic current recordings of spontaneous release for the indicated genotypes.

(D) Quantification of average mini frequency for the indicated genotypes (*w^{-/-}*, 1.6 ± 0.1 Hz, n = 10; *elav^{C155}-GAL4*; UAS-SYT1, 2.1 ± 0.2 Hz, n = 10; *elav^{C155}-GAL4*; UAS-SYT1 p.Asp362Ala#1, 6.3 ± 0.5 Hz, n = 16; *elav^{C155}-GAL4*; UAS-SYT1 p.Asp362Ala#2, 8.2 ± 0.7 Hz, n = 13).

Student's t test: *p < 0.05; ****p < 0.0001. Error bars represent SEM.

(E) Representative EPSCs during a short 10 Hz tetanic stimulation of the nerve in 0.2 mM external Ca^{2+} for the indicated genotypes.

(F) The average for the first ten responses normalized to the first response during the tetanus is shown following induction of WT DSYT 1 (blue), DSYT1 p.Asp362Ala #1 (green), and DSYT1 p.Asp362Ala #2 (red). The average fast tetanic facilitation after ten responses for each genotype is: WT DSYT1 = 0.94 ± 0.02; DSYT1 p.Asp362Ala#1 = 1.29 ± 0.03; DSYT1 p.Asp362Ala#2 = 1.49 ± 0.04. Student's t test revealed significant differences between WT and p.Asp362Ala (p < 0.0001).

suggested to function as fusion clamps to prevent spontaneous exocytosis, in addition to their role as calcium sensors for evoked release, which likely explains why spontaneous fusion is increased in the *Drosophila* model.^{1,2} We postulate that the SYT2 p.Pro308Leu residue will alter calcium binding to the C2B domain as well.

While the clinical presentation in the two families shows similarities, including foot deformity, areflexia, and limb weakness, there are also features with variable

degrees of penetrance. In family USA1, harboring the c.920A>C (p.Asp307Ala) variant, three of four individuals (II.2, III.1, and III.2) showed a pure presynaptic NMJ disorder with low-amplitude CMAs and a marked postexercise increment of CMAP amplitude. Intriguingly, this resembles findings in an autoimmune-induced clinical form of presynaptic dysfunction called Lambert-Eaton myasthenic syndrome.¹⁴ In this rare condition, antibodies target presynaptic voltage-gated calcium channels, but

(E) Quantification of mean excitatory junctional current (eEJC) amplitudes in the indicated genotypes (*Dsyt1^{-/-}* null, 2.1 ± 0.3 nA, n = 10; *elav^{C155}-GAL4*; *Dsyt1^{-/-}*; UAS-SYT1, 108.4 ± 13.9 nA, n = 18; *elav^{C155}-GAL4*; *Dsyt1^{-/-}*; UAS-SYT1 p.Asp362Ala, 2.0 ± 0.5 nA, n = 13).

(F) Cumulative vesicle release defined by charge transfer normalized for the maximum in 2.0 mM Ca^{2+} for each genotype (same color code as in D). Each trace was adjusted to a double exponential fit. Both the null and p.Asp362Ala rescued animals display a prominent increase in the slow asynchronous phase of release.

(G) Postsynaptic current recordings of spontaneous release at muscle 6 synapses in *Dsyt1^{-/-}* null larvae (red) and null mutants rescued with WT DSYT1 (black) or p.Asp362Ala DSYT1 (blue).

(H) Quantification of mini frequency in the indicated genotypes (*Dsyt1^{-/-}* null, 5.2 ± 0.6 Hz, n = 8; *elav^{C155}-GAL4*; *Dsyt1^{-/-}*; UAS-SYT1, 1.7 ± 0.2 Hz, n = 16; *elav^{C155}-GAL4*; *Dsyt1^{-/-}*; UAS-SYT1 p.Asp362Ala, 5.2 ± 0.4 Hz, n = 12).

Student's t test: ****p < 0.0001. Error bars represent SEM.

antibodies against synaptotagmins have also been identified in some individuals.¹⁵ Family members from UK1, harboring the c.923C>T (p.Pro308Leu) variant, also had low-amplitude CMAPs, but only one person (III.2) exhibited marked, postexercise CMAP amplitude increments that were longer lasting than in individuals from USA1. Some UK1 individuals also had muscle wasting, and needle electromyography disclosed mild chronic distal muscle reinnervation. Mutation in the presynaptic choline transporter has also been shown to cause a lower motor neuron phenotype, suggesting that presynaptic dysfunction may be a more general mechanism for peripheral axonopathies.¹⁶ *SYT2* is also expressed in other tissues outside of the NMJ, including the cerebellum and cochlea. Analysis of additional families will be necessary to define the spectrum of human phenotypes associated with *SYT2* mutations.

In summary, mutations in the *SYT2* C2B domain represent an important cause of disorders of the human peripheral motor nerve terminal; foot deformities are a hallmark, with phenotypes ranging from a dominant NMJ syndrome resembling Lambert-Eaton myasthenic syndrome to mixed manifestations of distal hereditary motor neuropathy and presynaptic NMJ dysfunction.

Supplemental Data

Supplemental Data include three figures and three tables and can be found with this article online at <http://dx.doi.org/10.1016/j.ajhg.2014.08.007>.

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Web Resources

The URLs for data presented herein are as follows:

1000 Genomes, <http://browser.1000genomes.org>

ANNOVAR, <http://www.openbioinformatics.org/annovar/>

GEM.app, <https://genomics.med.miami.edu>

Mutation Assessor, <http://mutationassessor.org/>

MutationTaster, <http://www.mutationtaster.org/>

NHLBI Exome Sequencing Project (ESP) Exome Variant Server, <http://evs.gs.washington.edu/EVS/>

Online Mendelian Inheritance in Man (OMIM), <http://www.omim.org/>

PolyPhen-2, <http://www.genetics.bwh.harvard.edu/pph2/>

SIFT, <http://sift.bii.a-star.edu.sg/>

UCSC Genome Browser, <http://genome.ucsc.edu>

References

1. Pang, Z.P., Melicoff, E., Padgett, D., Liu, Y., Teich, A.F., Dickey, B.F., Lin, W., Adachi, R., and Südhof, T.C. (2006). Synaptotagmin-2 is essential for survival and contributes to Ca²⁺ triggering of neurotransmitter release in central and neuromuscular synapses. *J. Neurosci.* 26, 13493–13504.
2. Littleton, J.T., Stern, M., Schulze, K., Perin, M., and Bellen, H.J. (1993). Mutational analysis of *Drosophila* synaptotagmin demonstrates its essential role in Ca²⁺-activated neurotransmitter release. *Cell* 74, 1125–1134.
3. Wen, H., Linhoff, M.W., McGinley, M.J., Li, G.L., Corson, G.M., Mandel, G., and Brehm, P. (2010). Distinct roles for two synaptotagmin isoforms in synchronous and asynchronous transmitter release at zebrafish neuromuscular junction. *Proc. Natl. Acad. Sci. USA* 107, 13906–13911.
4. Gonzalez, M.A., Lebrigo, R.F., Van Booven, D., Ulloa, R.H., Powell, E., Speziani, F., Tekin, M., Schüle, R., and Züchner, S. (2013). GENomes Management Application (GEM.app): a new software tool for large-scale collaborative genome analysis. *Hum. Mutat.* 34, 842–846.
5. Petrovski, S., Wang, Q., Heinzen, E.L., Allen, A.S., and Goldstein, D.B. (2013). Genic intolerance to functional variation and the interpretation of personal genomes. *PLoS Genet.* 9, e1003709.
6. Sutton, R.B., Davletov, B.A., Berghuis, A.M., Südhof, T.C., and Sprang, S.R. (1995). Structure of the first C2 domain of synaptotagmin I: a novel Ca²⁺/phospholipid-binding fold. *Cell* 80, 929–938.
7. Mackler, J.M., Drummond, J.A., Loewen, C.A., Robinson, I.M., and Reist, N.E. (2002). The C(2)B Ca²⁺-binding motif of synaptotagmin is required for synaptic transmission in vivo. *Nature* 418, 340–344.
8. Yoshihara, M., and Littleton, J.T. (2002). Synaptotagmin I functions as a calcium sensor to synchronize neurotransmitter release. *Neuron* 36, 897–908.
9. Lee, J., Guan, Z., Akbergenova, Y., and Littleton, J.T. (2013). Genetic analysis of synaptotagmin C2 domain specificity in

- regulating spontaneous and evoked neurotransmitter release. *J. Neurosci.* 33, 187–200.
10. Jorquera, R.A., Huntwork-Rodriguez, S., Akbergenova, Y., Cho, R.W., and Littleton, J.T. (2012). Complexin controls spontaneous and evoked neurotransmitter release by regulating the timing and properties of synaptotagmin activity. *J. Neurosci.* 32, 18234–18245.
 11. Pfeffer, G., Elliott, H.R., Griffin, H., Barresi, R., Miller, J., Marsh, J., Evilä, A., Vihola, A., Hackman, P., Straub, V., et al. (2012). Titin mutation segregates with hereditary myopathy with early respiratory failure. *Brain* 135, 1695–1713.
 12. Littleton, J.T., Stern, M., Perin, M., and Bellen, H.J. (1994). Calcium dependence of neurotransmitter release and rate of spontaneous vesicle fusions are altered in *Drosophila* synaptotagmin mutants. *Proc. Natl. Acad. Sci. USA* 91, 10888–10892.
 13. Wu, Y., He, Y., Bai, J., Ji, S.R., Tucker, W.C., Chapman, E.R., and Sui, S.F. (2003). Visualization of synaptotagmin I oligomers assembled onto lipid monolayers. *Proc. Natl. Acad. Sci. USA* 100, 2082–2087.
 14. Titulaer, M.J., Lang, B., and Verschuuren, J.J. (2011). Lambert-Eaton myasthenic syndrome: from clinical characteristics to therapeutic strategies. *Lancet Neurol.* 10, 1098–1107.
 15. Takamori, M., Maruta, T., and Komai, K. (2000). Lambert-Eaton myasthenic syndrome as an autoimmune calcium-channelopathy. *Neurosci. Res.* 36, 183–191.
 16. Barwick, K.E., Wright, J., Al-Turki, S., McEntagart, M.M., Nair, A., Chioza, B., Al-Memar, A., Modarres, H., Reilly, M.M., Dick, K.J., et al. (2012). Defective presynaptic choline transport underlies hereditary motor neuropathy. *Am. J. Hum. Genet.* 91, 1103–1107.