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A Family with Spinocerebellar Ataxia Type 5 Found to Have a Novel Missense Mutation Within a *SPTBN2* Spectrin Repeat

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Abstract

OBJECTIVE—Identification of a novel missense mutation in the *SPTBN2* gene of a family with a clinical diagnosis of spinocerebellar ataxia type 5 (SCA5).

METHODS—A family with late-onset autosomal dominant pure cerebellar ataxia, consistent with SCA5 but lacking previously reported *SPTBN2* mutations, was identified. DNA was collected from seven individuals across two generations and the *SPTBN2* gene on chromosome 11 was sequenced.

RESULTS—A nonsynonymous heterozygous substitution in exon 12 was detected in individuals diagnosed with SCA5 while unaffected family members did not possess this variant. The identified c.1415C>T variant results in a p.T472M substitution in the second SPEC domain of the beta-III spectrin protein. The threonine at position 472 is not in close proximity to the characteristic residues that define the SPEC domain and is variable across diverse SPEC domains, yet is highly conserved in *SPTBN2*. Consistent with these observations, bioinformatic analysis of the p.T472M variant suggests it to be pathological.

CONCLUSION—Two deletions within the *SPTBN2* SPEC domains (E532_M544del and L629_R634delinsW) have been previously reported to cause SCA5, but this is the first missense mutation in this region of the protein shown to likely be pathogenic.

Keywords

ATAXIA; SCA5; SPTBN2

INTRODUCTION

Spinocerebellar ataxia 5 (SCA5) is an autosomal dominant neurodegenerative disorder that targets the cerebellum¹. SCA5 has a worldwide prevalence of <1% and is rare among dominant ataxias^{2,3}. SCA5 patients primarily exhibit limb and gait ataxia (>90%), but truncal ataxia, sensory deficits, abnormal eye movements, dysarthria, and hyperactive deep tendon reflexes are also prevalent (25-90%)¹. Individuals generally struggle with fine motor coordination but rarely require a wheelchair until late in the disease course¹. Onset typically occurs in the early 30s and is progressive throughout life. It is known that mutations in the *SPTBN2* gene on chromosome 11 cause SCA5^{1,4}. *SPTBN2*, primarily expressed in the brain, encodes the beta-III spectrin protein consisting of 2390 amino acids comprising 2 calponin homology domains, 10 SPEC domains (consisting of 17 triple helical spectrin

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repeats), and a pleckstrin homology domain^{1, 4}. Three mutations known to cause SCA5 have previously been reported, consisting of one missense mutation interfering with the actin-binding site in the second calponin homology domain (p.L253P) and two deletions, p.E532_M544del and p.L629_R634delinsW, within the third SPEC domain of the protein that are believed to disrupt the triple alpha-helical structure of the repeats^{1, 4}. In this study we report the identification of a novel missense mutation within the second SPEC domain of the *SPTBN2* gene present in a family with clinical SCA5.

PATIENTS

The proband is a 67-year-old woman who developed progressive gait ataxia in her early 50s with frequent falls. MRI of the brain showed cerebellar atrophy, most prominent in the midline vermis. Laboratory testing was negative for a complete workup of acquired causes of ataxia⁵. Genetic testing showed no repeat expansions in the genes for Huntington disease, dentatorubral-pallidoluysian atrophy (DRPLA), and spinocerebellar ataxia (SCA) types 1, 2, 3, 6, 7, 8, 10, and 17. Gene sequencing was normal for the SCA14 gene. Sequencing of the *SPTBN2* gene showed a heterozygous C>T transition at nucleotide position 1415 (c.1415C>T) altering codon 472 and changing a threonine to methionine (p.T472M) (see Figure).

The family was of Norwegian descent. Medical history was notable for late-onset (age > 40 years) pure cerebellar ataxia in her mother and 4 of her mother's 13 siblings (see Figure). Her father exhibited no balance symptoms. The patient herself has 5 siblings, one of whom has balance problems.

METHODS

Blood samples were obtained from seven members of this family across two generations. DNA was extracted and amplified using published exon-spanning intronic primers⁴. Sequencing was performed using a 3730 DNA Analyzer (Applied Biosystems; Foster City, USA) and compared to the *SPTBN2* reference sequence and its translation (NM_006946.2 and NP_008877.1). Bioinformatic analysis was performed using publically available resources SIFT (<http://sift.jcvi.org/>)⁶, PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>)⁷, and PMut (<http://mmb.pcb.ub.es/PMut/>)⁸. Additional databases utilized included the Single Nucleotide Polymorphism Database (dbSNP) (<http://www.ncbi.nlm.nih.gov/projects/SNP/>)⁹, the 1000 Genomes Project (<http://browser.1000genomes.org/index.html>)¹⁰, and the National Heart Lung and Blood Institute (NHLBI) Exome Variant Server (<http://evs.gs.washington.edu/EVS/>)¹¹.

RESULTS

All individuals diagnosed with cerebellar ataxia tested were found to possess the heterozygous c.1415C>T substitution in the *SPTBN2* gene. We performed bioinformatic analyses using three separate classifiers (PMut, PolyPhen-2, and SIFT; see Methods) which all predicted the resulting p.T472M variant to be deleterious. We further examined the equivalent position across SPEC domains from diverse proteins and found it to be unrestricted (consensus residue is alanine), whereas sequence alignment of *SPTBN2* across multiple species (including the Sumatran orangutan, giant panda, dog, rabbit, horse, mouse, chicken, zebra finch, western clawed frog, Japanese puffer fish, Florida lancelet, and acorn worm) showed the threonine residue at position 472 to be highly conserved (data not shown). The p.T472M variant was not observed in any databases of normal sequence variation (dbSNP, 1000 Genomes Project, or the NHLBI Exome Variant Server; see Methods).

DISCUSSION

The p.T472M variant identified in this family is the first missense mutation in a *SPTBN2* spectrin repeat likely associated with SCA5. While the two previously reported pathogenic deletions, p.L629_R634delinsW and p.E532_M544del, likely disrupt protein structure involving the characteristic leucine and tryptophan residues of the SPEC domain, p.T472M is distinct and located in an adjacent helix¹². Bioinformatic analysis using multiple classifiers suggests this to be a detrimental substitution. Consistent with this, missense variants in other proteins with similar domains have been reported to cause neurodegenerative disease - for example, point mutations in spectrin-like repeats of the protein strumpellin are associated with spastic paraplegia¹³. The observation of a nonsynonymous polymorphism in *SPTBN2* within the same codon (rs145249947, c.1416G>A, p.T472T) suggests pathogenicity is unlikely to be at the nucleotide level. In contrast, conservation of threonine at position 472 in beta-III spectrin across multiple evolutionarily diverse species supports a critical role for this residue in protein function and in the absence of functional data provides the closest inference¹⁴. The nature of this mutation may potentially explain the milder late-onset phenotype observed in our patients. Although, without functional studies, we cannot confirm that this variant is truly pathogenic and not a rare or private polymorphism, it does appear to co-segregate with disease in this family. In a previous screen of 310 patients of German descent, Zühlke and colleagues identified three novel missense variants in *SPTBN2* as potentially pathogenic, but determined them unlikely to be causal due to either lack of disease segregation, co-association with a known pathogenic mutation in a separate gene, or presence in normal controls³. We tested these three variants using the same bioinformatic analysis as described above and found the overall consensus to be benign for all (data not shown). In contrast, the c.1415C>T variant reported here is deemed pathogenic by multiple bioinformatic analyses, segregates with disease in this family, and is not found in databases of normal variation representing over 6,000 individuals, suggesting a high probability of pathogenicity. This observation indicates that missense mutations in the *SPTBN2* SPEC domains should be considered in clinically appropriate families lacking deletions or previously reported mutations in other regions of the protein.

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FIGURE.

A) Genomic organization of the *SPTBN2* gene. Depicted are the calponin homology domains (CH), the SPEC domains (1-10), and the pleckstrin homology domain (PH). The N-terminal region is magnified to illustrate the location of published mutations causing spinocerebellar ataxia type 5 (SCA5) and the variant identified in this study.

B) Pedigree of the family described in this report. Two generations of the family are depicted. Affected individuals are shaded in gray. Seven patients (I-2, I-3, I-4, II-3, II-4, II-5, II-6) were sequenced for the *SPTBN2* variant in this study. The proband is indicated by an arrow. Participants with the T472M missense mutation are indicated. WT = wild type sequence.