RE-ANALYSIS OF AN ORIGINAL CMTX3 FAMILY USING EXOME SEQUENCING IDENTIFIES A KNOWN BSCL2 MUTATION

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ABSTRACT: CHARCOT–MARIE–TOOTH (CMT) disease is a group of peripheral neuropathies affecting both motor and sensory nerves. CMTX3 is an X-linked CMT locus, which maps to chromosome Xq26.3–q27.3. Initially, CMTX3 was mapped to a 31.2-Mb region in 2 American families. We have reexamined 1 of the original families (US-PED2) by next generation sequencing. METHODS: Three members of the family underwent exome sequencing. Candidate variants were validated by PCR and Sanger sequencing analysis. CONCLUSION: No pathogenic coding variants localizing to the CMTX3 region were identified. However, exome sequencing identified a known BSCL2 mutation (N88S). This study demonstrates the power of exome sequencing as a tool to identify gene mutations for a small family in the absence of statistically significant linkage data.

METHODS

Exome sequencing and variant calling was performed as a tool to identify gene mutations for a small family in the absence of statistically significant linkage data.

RESULTS

In this study we performed exome sequencing analysis on family members from US-PED2. Several candidate variants were identified; however, a previously reported autosomal mutation for distal hereditary motor neuropathy (dHMN) was discovered in this family.

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Abbreviations: AFO, ankle–foot orthotics; BSCL2, Berardinelli–Seip congenital lipodystrophy 2; CMT, Charcot–Marie–Tooth; dHMN, distal hereditary motor neuropathy; DTR, deep tendon reflexes; ER, endoplasmic reticulum

Key words: BSCL2; CMTX3; exome sequencing; peripheral neuropathy; X-linked Charcot–Marie–Tooth

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There was a stocking-glove sensory loss to touch and proprioception. Deep tendon reflexes (DTRs) were 2 in arms and 3 in legs with upgoing toes. There was a mild postural tremor. Nerve conduction velocities were normal in sensory nerves but slowed in median and fibular motor nerves (12 and 31 m/s). His course is one of slowly progressive distal weakness and sensory loss. At 36 years of age, he remains ambulatory with the assistance of ankle–foot orthotics (AFO). Osteoarthritic pain in hips and knees limits her activity.

Exome sequencing identified a total of 78,536 variants in the affected man. Initially, the CMTX3 region (Xq26.3–27.1) was analyzed for the presence of candidate variants, but none were identified. Having eliminated the CMTX3 locus, a total of 393 novel variants still required validation. To further filter the variants, we analyzed the patient’s exome data for variants in known peripheral neuropathy genes. This filtering strategy identified 1 reported variant on chromosome 11 in the gene Berardinelli–Seip congenital lipodystrophy 2 (BSCL2/seipin). The base change corresponded to a previously identified disease-associated missense mutation, c.263A>G (NM_032667). Coverage analysis of this variant in the BSCL2 gene showed a total of 78 reads, with 48 reads, being the alternate base. Sanger sequencing confirmed the base change, and the variant segregated with the affected man/carrier woman and was absent in the unaffected sibling (Fig. 1b and c).

**DISCUSSION**

In this study we performed exome analysis on 1 of the original American families (US-PED2) that contributed to establishing linkage to the CMTX3 locus. In the

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**FIGURE 1.** Identification and validation of a known BSCL2 gene mutation in US-PED2. (a) Filtering strategy to identify shared variants in the affected man and obligate carrier woman. This method enabled the identification of potential pathogenic mutations in the CMTX3 region as well as in known peripheral neuropathy genes. (b) Mapped exome sequence reads in the Integrative Genomics Viewer (IGV) for the affected man from US-PED2. The gray bar represents the reference allele (T), and the colored base indicates the alternative allele (C). (c) Sequence traces showing the validation of the change identified by Sanger sequencing. Both the affected and obligate carrier individuals were heterozygous for the change c.263A>G (NM_032667). Other regions represent the X chromosome, apart from the CMTX3 region, and the autosomes. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
original study this family was not large enough to demonstrate significant linkage independently.6 We therefore undertook exome analysis for US-PED2 to screen for variants in genes located on both the autosomes and sex chromosomes.

We obtained DNA samples from 3 of the 12 family members of the original pedigree, and confirmed that coding variants were not present in the CMTX3 region. The family was subsequently assessed for variants in known peripheral neuropathy genes located on the autosomes, and 1 known pathogenic mutation, N88S, in the BSCL2 gene, was identified. This mutation has been reported previously for phenotypes associated with Silver syndrome, dHMN type 5, CMT type 2, and hereditary spastic paraplegia.15 Studies of the N88S mutation suggest a toxic gain of function that causes improper folding of the BSCL2 protein. The incorrectly folded protein accumulates in the endoplasmic reticulum (ER), which leads to ER stress and eventually cellular dysfunction.16–19

Mutations in BSCL2 show a wide spectrum of clinical features, which can affect both motor and sensory neurons.15 One of the more unique clinical symptoms reported in US-PED2 was spastic paraplegia.6 This clinical feature has been reported in families with BSCL2 mutations.15 Interestingly, recent examination of US-PED2 (K.M.) revealed that the carrier woman with the BSCL2 mutation was clinically normal, thus reflecting the phenotype for the obligate carriers of an X-linked CMT disease.20 However, variability in penetrance of the BSCL2 phenotype has been reported previously in families with BSCL2 mutations in which individuals carrying the disease mutation appear to be clinically normal.15,21,22

Incomplete penetrance of the N88S mutation is clearly demonstrated in US-PED2. For over a decade, US-PED2 had been classified as X-linked CMT with evidence indicating linkage to the CMTX3 locus.6 We therefore assumed a novel gene in the CMTX3 locus may lead to the phenotype observed in this family. The reported X-linked inheritance for this family did not make screening genes associated with spasticity a priority. Given the outcome of this study, however, families with signs of spasticity, but that are too small to show significant linkage, may benefit from this approach. In this study, exome sequencing proved to be a fast and cost-efficient method, as it gave us the opportunity to examine both the region of interest and the entire coding exome.

In conclusion, we have identified a known BSCL2 mutation in an original CMTX3 family (US-PED2) with the aid of exome sequencing. Despite not finding a pathogenic variant in the CMTX3 region, this family can be revisited once the gene mutation is identified in larger families showing significant linkage to the CMTX3 locus.12,13 This study demonstrates the power of exome analysis to identify gene mutations in the absence of statistically significant linkage in small nuclear families.

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