**Participants**

This study includes three patients from two families. Index cases were referred to our center with initial diagnoses of ataxia and / or HSP by expert clinicians. All patients tested negative for the trinucleotide repeat expansions for FA, SCA1, 2, 3, 6, and 17 genes (*FXN*, *ATXN1, ATXN2*, *ATXN3*, *CACNA1A*, and *TBP*, respectively). The protocol was approved by the Ethics Committee on Research with Human Participants (INAREK) at Bogazici University, Istanbul, Turkey. Genetic counseling was offered and informed consent was obtained from all individuals who participated in the study.

The patients had an age at onset of 15, 21 (index cases) and 32. Both families were from the central Anatolia region of Turkey and close consanguinity was present. All patients presented with lower limb spasticity, ataxic gait, and cerebellar dysarthria. Detailed clinical features are listed in Table 1.

Peripheral blood was collected from all participants and stored in EDTA tubes. Genomic DNA was isolated from blood using MagNA Pure Compact Instrument for genetic analysis. After elimination of the repeat expansions, the samples were prepared for whole exome sequencing. Only index case was subjected to WES from Family 1, whereas two affected sisters and the parents were selected for WES in Family 2.

**Sequencing and data analysis**

Paired-end sequencing was performed on Illumina Hiseq2000 and 2500 DNA sequencing platforms (Roche SeqCap EZ Whole Exome Kit v2 and v3). Raw reads were mapped to GRCh37 plus the decoy using Burrows-Wheeler Aligner and PCR duplicates were marked by Picard tools. Single nucleotide variant and small indel calling was performed by Genome Analysis Toolkit (GATK) and variants were annotated by ANNOVAR package based on RefSeq genes and dbSNPv1381,2. Fully processed and annotated vcf files were visualized by Varsifter software (version 1.7)3. Due to consanguinity in the parents and lack of known disease history in the upper generations, homozygous variants were selected. This included homozygous variants for Patient 1. For Patients 2 and 3, shared homozygous variants in two sisters, where their parents were heterozygous, were selected. Initially, only variants resulting in a truncated protein, or an altered protein sequence and disrupting splice site were considered. Variations with minor allele frequencies higher than 1% in 1000 Genomes (April 2012 release), the National Lung and Blood Institute (NIHLBI) Exome Sequencing Project (ESP), the Exome Aggregation and Consortium (ExAC) Browser and the Genome Aggregation Database (gnomAD) were excluded from further analysis. Online prediction and conservation tools PolyPhen-2, MutationTaster, CADD and GERP++ were used to estimate the pathogenicity of the variants while their scores were not used to filter out any variations. The candidate list was screened for presence of variants in known hereditary spastic paraplegia and ataxia genes pointing to homozygous mutations in *CAPN1*. The presence of the variants and segregation in the families were confirmed by Sanger sequencing (primers available upon request).

**References**

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