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BRIEF COMMUNICATION

A novel homozygous *FBXO38* variant causes an early-onset distal hereditary motor neuronopathy type IID

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Abstract

Distal hereditary motor neuronopathies (dHMN) are a genetically heterogeneous group of neuromuscular disorders caused by anterior horn cell degeneration and progressive distal muscle weakness. A heterozygous missense variant in *FBXO38* has been previously described in two families affected by autosomal-dominant dHMN. In this paper, we describe a homozygous missense variant in *FBXO38* (c.1577G>A; p.(Arg526Gln)) in a young Turkish female, offspring of consanguineous parents, with a congenital mild neuronopathy with idiopathic toe walking, normal sensory examination, and hearing loss. This work is the first to describe a novel homozygous variant and a suggested loss of function mechanism in *FBXO38*, expanding the dHMN type IID phenotype.

Introduction

Distal hereditary motor neuronopathy (dHMN), also known as distal spinal muscular atrophy (dSMA), is characterized by the degeneration of alpha motor neurons leading to progressive muscle wasting and weakness [1]. To date, several genes have been associated with the autosomal-dominant (*HSPB1, HSPB8, GARS, DYNC1H1, BSCL2, HSPB3, DCTN1, TRPV4, SETX, BICD2, FBXO38*), autosomal-recessive (*IGHMBP2*), and X-linked (*ATP7A*) forms of dHMN [2]. However, despite the advances in next generation sequencing techniques, a significant percentage of cases are still genetically unexplained.

A heterozygous variant in the F-box protein 38 *(FBXO38)* gene has been described as a cause of autosomal-dominant dHMN type II phenotype in two families [1]. Here, we report a patient with a homozygous *FBXO38* variant that is characterized by slowly progressive distal weakness, hearing loss, and multiple organ anomalies (duplex collective system, arcuate uterus, and choanal atresia).

Case presentation and methods

A 20-year-old Turkish female was referred to our laboratory with childhood-onset slowly progressive motor neuropathy (Fig. 1). She initially presented to neurology at the age of 15 years with walking difficulties for three years. Her parents noted that she had a tendency to walk on tip-toes. Her past medical history was significant with sensorineural hearing loss, duplex collective system, arcuate uterus, and a surgical correction of choanal atresia. Her initial neurological examination revealed bilateral mild weakness in foot dorsiflexors and toe extensors (Medical Research Council [MRC)] grade: 4/5). She was unable to walk on heels. She had a bilateral pes cavus deformity. Cranial magnetic resonance imaging and deep tendon reflexes were normal. There were no sensorial abnormalities and the rest of the neurological examination was unremarkable. Nerve conduction studies revealed reduced

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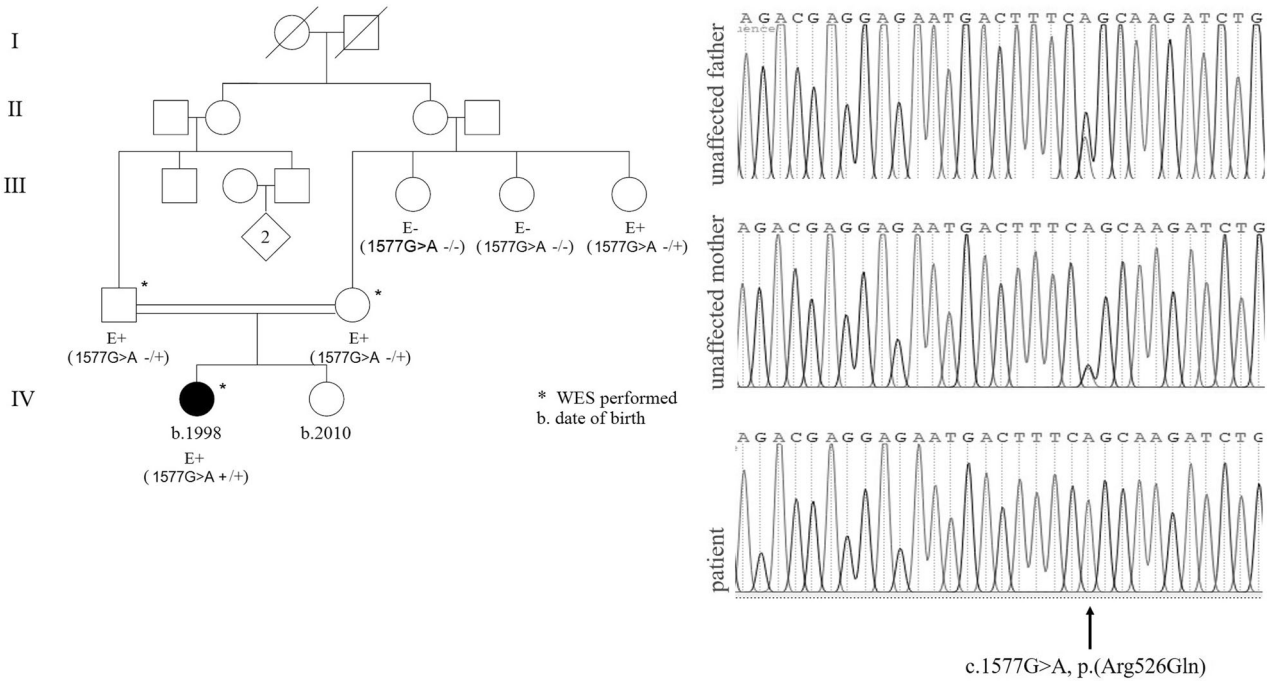

Fig. 1 Pedigree of the family with *FBXO38* variant. Segregation of the variant E+(*FBXO38* c.1577G>A; p.(Arg526Gln)) is confirmed by Sanger sequencing

compound muscle action potential (CMAP) amplitudes in the lower extremities with the normal sensory conduction findings. Electromyography (EMG) showed a chronic neurogenic change and active reinnervation and denervation in the L3-S1 innervated muscles. During the follow-up of five years, her distal lower limb weakness progressed (MRC grade 3/5) and extended to distal muscles of upper extremities (MRC grade: 4/5).

Whole-exome sequencing (WES) was performed for the index case, her unaffected mother and father. Alignment of paired-end sequencing reads to the human reference genome GRCh37 was carried out by the Burrows-Wheeler Aligner (BWA-MEM) algorithm [3]. Quality control assessment and variant calling were performed using the HaplotypeCaller and Variant Quality Score Recalibration tools of the Genome Analysis Toolkit v.3.5 (GATK) [4, 5]. Variants were annotated for predicted protein alterations and population frequencies using ANNOVAR software [6]. For potentially pathogenic variants, the evolutionary conservation rate was estimated using GERP++ [7] and the deleteriousness of the protein alteration was predicted using SIFT (http://sift.bii.a-star.edu.sg) and PolyPhen2 (http://genetics.bwh.harvard.edu/pph2). Due to the consanguinity of the parents and the lack of known disease history in the past generations, homozygous variants were prioritized. The presence of the variant and its segregation across the pedigree were confirmed by Sanger sequencing (primer sequences available upon request). Homozygosity mapping from the WES data was performed via PLINK v1.9.0 [8] (Table 1S).

Results

WES analysis identified 1286 variants compatible with autosomal recessive inheritance. Of these, 21 were protein-altering and had a frequency lower than 0.01 in ExAC and gnomAD (Table 1). Runs of homozygosity revealed homozygous regions harboring 10 of the prioritized variants (Table 1S, Fig. 1S). Among these, a novel homozygous missense variant (chr5: 147796726, G>A; p.(Arg526Gln)) in the *FBXO38* gene (NM_001271723), previously only associated with autosomal dominant dHMN (MIM #608533), was identified. The variant (rs376255193) was not reported in the homozygous state in either ExAC nor in gnomAD. Sanger sequencing confirmed the segregation of the variant in homozygous state in the index case and in heterozygous form in the unaffected parents (Fig. 1). The amino acid position 526 of *FBXO38* was estimated to be evolutionarily highly conserved by GERP++ with a score of 5.47. The arginine to glutamine change at this position was predicted to be deleterious and possibly damaging by SIFT and PolyPhen2 (Table 1).

Discussion

The *FBXO38* gene encodes for a member of the F-box family of proteins and is known as a coactivator of the Kruppel-like factor 7 (KLF7), which is implicated in axonal outgrowth and regeneration, cytoskeletal dynamics, synaptic vesicle

Table 1 List of rare, protein-altering variants homozygous in the index case and heterozygous in the unaffected parents

Chr:Position (hg19)	Gene	ExAC MAF	gnomAD MAF	gnomAD Hoz	nt change	aa change	dbSNP ID	SIFT	PolyPhen2	GERP++
1:152275876	*FLG*	2.09E−03	2.06E−03	4	c.11486G>A	p.(Arg3829His)	rs145079750	D	P	−2.3
1:152275883	*FLG*	9.97E−04	1.10E−03	0	c.11479G>T	p.(Gly3827Trp)	rs140464988	D	B	−0.756
5:7789874	*ADCY2*	2.54E−04	2.92E−04	1	c.2589C>G	p.(His863Gln)	rs199773760	D	D	0.966
5:10236714	*FAM173B*	1.38E−03	1.35E−03	1	c.320C>T	p.(Ala107Val)	rs114646426	NA	D	3.87
5:147796726	***FBXO38***	**7.49E−05**	**1.02E−04**	**0**	**c.1577G>A**	**p.(Arg526Gln)**	**rs376255193**	**D**	**P**	**5.47**
5:148596547	*ABLIM3*	2.85E−03	2.51E−03	2	c.695C>T	p.(Thr232Ile)	rs116226381	D	B	1.06
5:149511562	*PDGFRB*	5.56E−04	5.93E−04	1	c.1223C>G	p.(Ser408Cys)	rs200203294	D	P	5.17
9:86571126	*C9orf64*	1.76E−03	1.83E−03	1	c.290G>A	p.(Ser97Asn)	rs183493508	T	P	2.51
9:90501554	*SPATA31E1*	8.29E−06	7.22E−06	0	c.2152C>T	p.(Arg718Trp)	rs567122633	D	D	4.64
9:95228676	*ASPN*	6.78E−04	6.54E−04	0	c.565G>C	p.(Asp189His)	rs146775001	T	P	4.57
9:110084385	*RAD23B*	NA	NA	NA	c.740C>T	p.(Thr247Ile)	NA	T	P	5.23
11:119168156	*CBL*	5.11E−04	4.01E−04	1	c.C2216T	p.(Ser739Phe)	rs2227986	D	P	5.27
11:119183244	*MCAM*	2.18E−03	1.72E−03	4	c.854G>A	p.(Ser285Asn)	rs138873873	T	B	0.657
12:11244438	*TAS2R43*	4.63E−03	5.57E−03	132	c.391G>A	p.(Val131Met)	rs186718859	T	B	NA
12:20790041	*PDE3A*	NA	NA	NA	c.1043C>A	p.(Pro348Gln)	NA	D	D	3.45
17:73921443	*FBF1*	1.00E−03	1.12E−03	0	c.911G>A	p.(Arg304His)	rs190439091	NA	NA	NA
17:76888360	*CEP295NL*	8.36E−03	9.57E−03	12	c.226T>C	p.Trp76Arg	rs145329239	D	B	NA
17:79660609	*HGS*	9.04E−04	9.96E−04	0	c.739C>G	p.Gln247Glu	rs145607073	D	D	4.34
18:7231881	*LRRC30*	4.92E−03	4.67E−03	10	c.745A>G	p.Ser249Gly	rs144753731	T	B	3.22
18:22040837	*HRH4*	2.47E−05	4.09E−06	0	c.145C>T	p.Arg49Ter	rs765269581	NA	NA	4.66
18:22806981	*ZNF521*	1.95E−03	2.02E−03	1	c.241G>A	p.Glu81Lys	rs114155230	T	B	5.55

Chr chromosome, *MAF* minor allele frequency, *Hoz* number of homozygotes, *nt* nucleotide, *aa* aminoacid, *SIFT* (*D* deleterious, *T* tolerated), *PolyPhen2* (*P* possibly damaging, *D* probably damaging, *B* benign), *NA* not available

The *FBXO38* variant is shown in bold

trafficking, and neurotransmission [9, 10]. Previous studies have shown that an alteration of FBXO38 protein sequence causes a disruption in the activation of KLF7, which in turn leads to an impaired axonal development and repair [1]. Due to its effect on synaptogenesis and neurotransmission that are a part of the auditory system [11], disruption of KLF7 function may play a role in excitatory and inhibitory neurotransmission at synapses; therefore, can result in hearing impairment and dysfunction. Further functional studies are warranted to investigate the potential causality of the FBXO38 and KLF7 for the described clinical outcome.

Here, we report a homozygous *FBXO38* p.(Arg526Gln) variant as a cause of dHMN type IID in a female with a very early-onset slowly progressive distal motor neuronopathy accompanied by hearing loss and multiple organ anomalies. To date, only one heterozygous missense variant (p.(Cys206Arg)) in the *FBXO38* gene has been shown to cause dHMN with calf-predominant weakness in two families. A loss of function of altered FBXO38 (haploinsufficiency), as well as a dominant-negative effect have been suggested as possible mechanisms [1]. Our study validates that variations in the *FBXO38* gene cause a dHMN type II phenotype, but still leaves the pathogenic mechanism open. The mild phenotype in the homozygous girl reported here, similar to the heterozygous pathogenic variants could suggest dominant negative effects in both or the slightly younger and broader phenotype may suggest loss of function in the homozygous patient.

Although prediction tools suggested that the variant has a deleterious effect, we acknowledge as a limitation of the current study that there is not enough functional evidence to explain the clinical findings complete satisfaction. Another limitation of the study is that WES cannot detect structural variations and the non protein-coding regions of the genome that may also lead to abnormal phenotypes. Future genetic and functional analyses in this and possibly other families will hopefully reveal the effect of the *FBXO38* variant on the phenotype of our patient, but they will also evaluate the impact of the additional 21 variants homozygously present in her.

In the previous report, the average age at onset is 27.7, ranging from 13 to 48 and in contrast, in our case, the onset is at early childhood. In addition, her parents who are heterozygous for the variant are asymptomatic. An investigation of this finding, e.g., a further search into the modifier genes would be the focus of a future study. To the best of our knowledge, this is the first report of a homozygous *FBXO38* variant that causes a very early-onset and mild form of distal HMN type IID. Therefore, we suggest, clinicians to consider an analysis for *FBXO38* variant in an early-onset distal motor neuronopathy. Future studies will establish the prevalence of autosomal recessive form of distal HMN type IID and whether other homozygous *FBXO38* variants can cause a similar phenotype.

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Compliance with ethical standards

Ethical approval This work was approved by The Ethics Committee of Boğaziçi University, where the study was started.

Conflict of interest The authors declare that they have no conflict of interest.

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