Title: 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 from a consanguineous family, causing 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. **Keywords:** distal hereditary motor neuronopathy, *FBXO38* gene variant, whole-exome sequencing, homozygosity mapping

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 (Figure 1). She initially presented to neurology at the age of 15-years-old 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 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 5 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 1,286 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, Figure1S). 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 (Figure 1). The amino acid position 526 of *FBXO38* was estimated to be evolutionarily highly conserved by GERP++ with a score of 5.47 out of 6, and the arginine to glutamine change at this position was predicted to be deleterious and possibly damaging by SIFT and PolyPhen2 (Table 1).

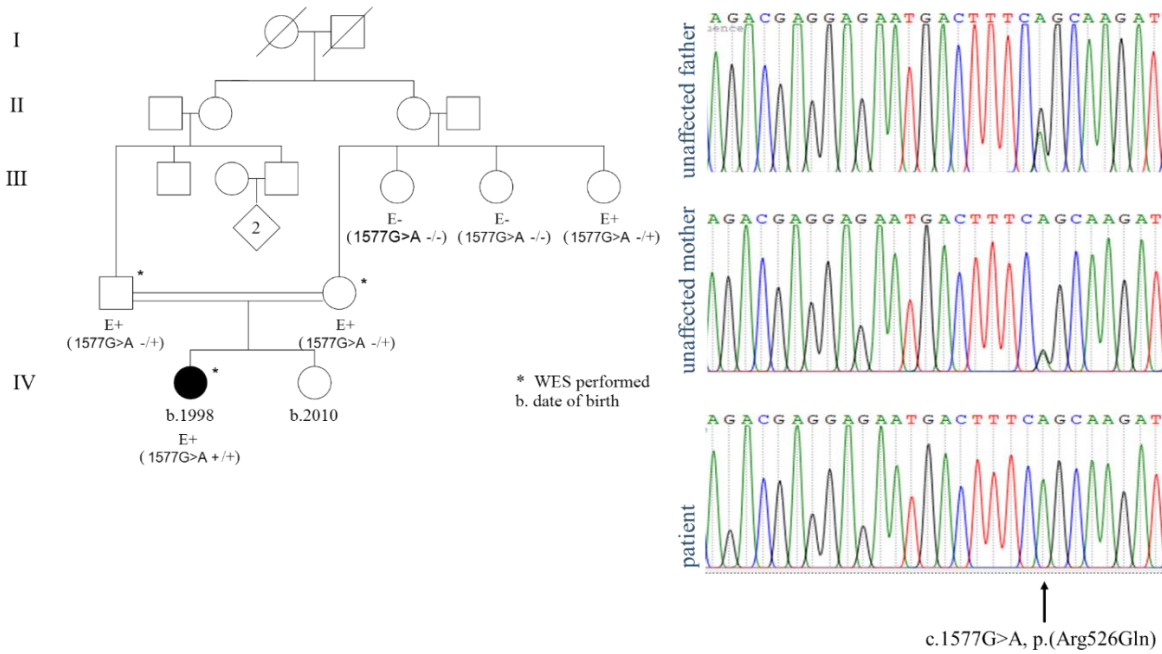

Figure 1. Pedigree of the family with *FBXO38* variant. Segregation of the variant E+ (*FBXO38* c.1577G>A; p.(Arg526Gln)) is confirmed by Sanger sequencing.

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

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 [9]. 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].

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 with 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 not only validates that variationss 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. Future functional analysis will not only reveal the effect of the *FBXO38* variant on the phenotype of the patient, but it will also evaluate the impact of the additional 21 variants homozygously present in the index case.

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 from early childhood and the symptoms. 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

This work was approved by The Ethics Committee of Boğaziçi University in which the study was started.

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Supplementary Information

Table 1S. List of homozygous stretches in the index case using PLINK v1.9 with the following parameters: --minimum GQ:20, --homozygous kb:500, --homozygous SNP:10, --homozygous window SNP:20, --homozygous window SNP missing:10, --homozygous window threshold:0.05, --homozygous density:500, --homozygous gap:2000, --homozygous window het:1.

Chromosome	Start position	End position	Length	PHOM	PHET	Prioritized variants
1	93353719	94443811	1090.093	0.429	0.048	
2	214015179	216264197	2249.019	0.5	0	
2	233198373	234293244	1094.872	0.467	0.033	
5	**147585805**	**149312215**	**1726.411**	**0.448**	**0**	**FBXO38:p.(Arg526Gln),** ABLIM3:p.(Thr232Ile)
5	150005108	152419405	2414.298	0.4	0.04	
5	154778196	157215027	2436.832	0.45	0.05	
5	167626968	170149573	2522.606	0.474	0	
7	18913857	22430437	3516.581	0.417	0.083	
9	94885073	96356817	1471.745	0.45	0.05	ASPN:p.(Asp189His)
9	100594227	103576043	2981.817	0.438	0.031	
9	106856775	110084385	3227.611	0.45	0.05	RAD23B:p.(Thr247Ile)
11	117265512	120742059	3476.548	0.508	0.048	CBL:p.(Ser79Phe), MCAM:p.(Ser285Asn)
11	123396844	125495521	2098.678	0.432	0.054	
11	127562818	130536523	2973.706	0.417	0.028	
12	8075942	9578922	1502.981	0.481	0.037	
12	10131939	15089665	4957.727	0.491	0.057	TAS2R43:p.(Val131Met)
12	16089898	21198794	5108.897	0.367	0.033	PDE3A:p.(Pro348Gln)

12	21325814	24737061	3411.248	0.478	0	
17	32614846	34398495	1783.65	0.379	0.034	
17	38520054	39637244	1117.191	0.48	0.04	
17	40314213	41886190	1571.978	0.545	0.045	
17	50235187	55065530	4830.344	0.45	0.05	
17	72294857	74779365	2484.509	0.466	0.034	FBF1:p.(Arg304His)
17	75730446	76486579	756.134	0.476	0.048	
17	77215049	78797066	1582.018	0.484	0.065	
17	79617830	81006286	1388.457	0.5	0.02	HGS:p.(Gln247Glu)
18	13460037	15322073	1862.037	0.462	0.038	
18	19069636	20716014	1646.379	0.467	0.067	

*PHOM: Proportion of homozygous sites, PHET: Proportion of heterozygous sites.

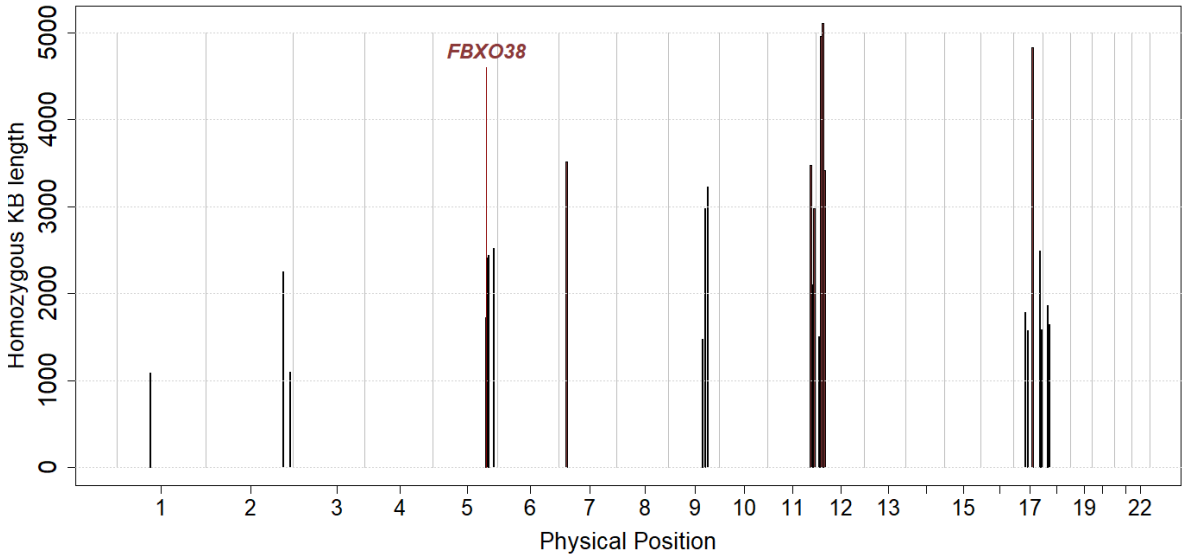

Figure 1S. The graphical representation of homozygous stretches detected by PLINK 1.9.