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ORIGINAL ARTICLE

MYO1H is a novel candidate gene for autosomal dominant pure hereditary spastic paraplegia

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Abstract

In this study, we aimed to determine the genetic basis of a Turkish family related to hereditary spastic paraplegia (HSP) by exome sequencing. HSP is a progressive neurodegenerative disorder and displays genetic and clinical heterogeneity. The major symptoms are muscle weakness and spasticity, especially in the lower extremities. We studied seven affected and seven unaffected family members, as well as a clinically undetermined member, to identify the disease-causing gene. Exome sequencing was performed for four affected and two unaffected individuals. The variants were firstly filtered for HSP-associated genes, and we found a common variant in the *ZFYVE27* gene, which has been previously implied for association with HSP. Due to the incompletely penetrant segregation pattern of the *ZFYVE27* variant, revealed by Sanger sequencing, with the disease in this family, filtering was re-performed according to the mode of inheritance and allelic frequencies. The resulting 14 rare variants were further evaluated in terms of their cellular functions, and three candidate variants in *ATAD3C*, *VPS16*, and *MYO1H* genes were selected as possible causative variants, which were analyzed for their familial segregation. *ATAD3C* and *VPS16* variants were eliminated due to incomplete penetrance. Eventually, the *MYO1H* variant NM_001101421.3:c.2972_2974del (p.Glu992del, rs372231088) was found as the possible disease-causing deletion for HSP in this family. This is the first study reporting the possible role of a *MYO1H* variant in HSP pathogenesis. Further studies on the cellular roles of Myo1h protein are needed to validate the causality of *MYO1H* gene at the onset of HSP.

Keywords Hereditary spastic paraplegia · *MYO1H* · WES · Candidate gene · Novel gene

Introduction

The aim of this study was to identify the disease-causing variant/gene by exome sequencing in a Turkish family presenting symptoms compatible with pure hereditary spastic paraplegia (HSP), predominantly in male cases.

HSP is a group of clinically and genetically heterogeneous progressive neurological disorders characterized by muscle weakness and spasticity of lower limbs due to degeneration of motor neurons in the corticospinal tract (Harding 1981; Murala et al. 2021). To this date, more than 80 distinct spastic gait genetic loci (*SPG1-80*) have been associated with HSP (Blackstone 2018; Mackay-Sim 2021; Meyyazhagan and Orlacchio 2022).

HSP is divided into two groups called "pure" and "complex" forms. The "pure" form of HSP, also known as Strümpell disease, is predominantly characterized by lower limb spasticity, clonus, gait disorder, hyperreflexia, and bladder dysfunction. In the "complex" form of HSP, additional

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manifestations, such as neuropathy, cognitive impairment, distal amyotrophy in the upper limbs, and optic atrophy may develop (Harding 1983; Lo Giudice et al. 2014; Blackstone 2018).

Myo1h protein encoded by the *MYO1H* gene is a member of the unconventional, non-muscle Myosin-1 family, which has a role in crosslinking of the cell membrane to the actin cytoskeleton (Adams and Pollard 1989; Cheney and Mooseker 1992; McConnell and Tyska 2010). Although the cellular role of Myo1h is not well known yet, the other members of the Myosin-1 family are the major proteins for cellular protrusion and migration, organelle transport, and endo/exocytosis (Nambiar et al. 2009; McConnell and Tyska 2010; Coudrier and Almeida 2011; Kittelberger et al. 2016).

In this report, we describe an HSP family harboring a deletion mutation in a novel putative causative gene *MYO1H*.

Methods

This study was approved by the Ethics Committee of Istanbul Technical University and Biruni University Hospital, and informed consent was obtained from all participants included in the study.

The index case (III:4, Fig. 1a) was a 52-year-old male HSP patient without parental consanguinity. The patient had 6 more affected relatives, including his mother, sister, brother, and niece/nephews (Fig. 1a). Neurological examination was done, cranial and whole spinal magnetic resonance imaging (MRI) and electromyography (EMG) were performed, and ophthalmologic and systemic metabolic consultation was provided for the members of this family.

Genomic DNA of all seven cases and seven other family members was extracted from peripheral blood using Roche Life Science DNA Isolation Kit for Mammalian Blood (Roche, Switzerland) according to the manufacturer's instructions. Exome sequencing was performed for six members (four cases [II:2, III:2, III:4, and IV:1] and two healthy individuals [I:1 and II:1]) through the commercial service of Yale University, Yale Center for Genome Analysis (YCGA, Connecticut, USA). Exome data were analyzed through our local bioinformatics workflow according to the GATK Best Practices (van der Auwera et al. 2013), and the called variants were annotated using the ANNOVAR software (Wang et al. 2010). First, variants of the known HSP-associated loci (Blackstone 2018; Mackay-Sim 2021) were analyzed to determine if the affected individuals of the family share a known pathogenic/likely pathogenic variant or a rare (minor allele frequency [MAF] < 0.5% in gnomAD) (Karczewski et al. 2020) variant with unknown significance in these loci in a fully penetrant manner. When we revealed that there was no such variant even though all HSP-associated loci were covered in exome sequencing, we further searched for candidate disease-causing variants by selecting heterozygous variants shared exclusively by the patients, considering the dominant inheritance pattern of the disease in this family. Variants with gnomAD MAF < 0.5% were included in the analysis to cover all potentially disease-causing variants

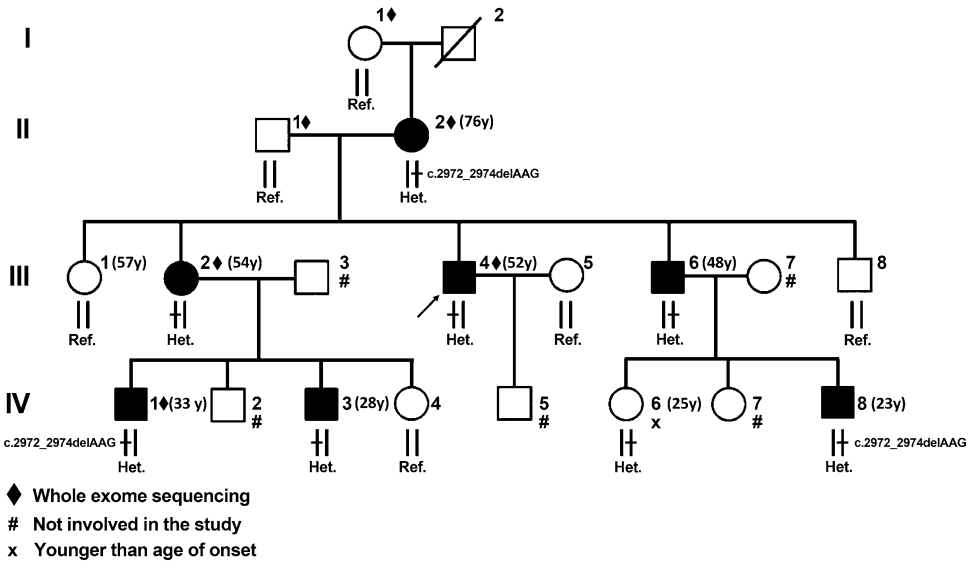


Fig. 1 Familial segregation of the *MYO1H* variant in the family. Affected family members are represented with a black circle (female) or square (male), and unaffected family members are represented with an empty circle or square symbol. The black arrow represents the proband (IC). Filled diamond Whole exome sequencing data were obtained. # Individuals were not involved in the study. X Individual is younger than the age of onset. The presence or absence of the *MYO1H* variant is represented as het. (heterozygote) and ref. (reference sequence), respectively. Ages of the individuals at the time of sample collection are presented at the right side of the symbols

since previously suggested HSP-associated variants have varying allelic frequencies as high as 0.9% (rs199659429, ClinVar Accession Number: VCV000241462.1) in gnomAD. The pathogenicity prediction tools SIFT (Kumar et al. 2009), PolyPhen2 (Adzhubei et al. 2010), Mutation Taster (Schwarz et al. 2010), and PROVEAN (Choi and Chan 2015) were used for variant prioritization of the remaining 14 variants on 13 genes. To evaluate the relevance of the variants and genes to HSP, Online Mendelian Inheritance in Man (OMIM), Mouse Genome Informatics (MGI), GeneCards, and UniProt databases were used, in addition to literature search for each gene, for further candidate disease-causing variant prioritization. Familial segregation analysis was performed for four variants in four genes, *MYO1H*, *ZFYVE27*, *ATAD3C*, and *VPS16*, by Sanger sequencing. The genomic regions including the variant sites were amplified by PCR using the following forward and reverse primers respectively: 5′-GACCAAAGGTTGAGAGAAGAA-3′ and 5′-AGTGTCTCATGTCAGCTTCTGG-3′ for *MYO1H*; 5′TTGACCTTCTGTGTTCCAGG-3′ and 5′-CACTAACTC CAGGGCAACA-3′ for *ZFYVE27*; 5′-GTCCACACCCAG GCATTCTC-3′ and 5′- CTACACGAGGGTGGGCAAAG-3′ for *ATAD3C;* 5′-TGCTGGACTGTGCTGTGCCAG-3′ and 5′-CTGAGGCAGAGCCAGGGTAG-3′ for *VPS16*. Sanger sequencing of the PCR products was performed through the commercial service provided by Macrogen (Korea)/BM Laboratory Systems (Turkey). The sequencing data were analyzed using the NCBI reference sequence NC_000012.12.

Results

Clinical findings

The pedigree of the family is given in Fig. 1. The index case (IC), (III.4) 52-year-old male, was admitted to the neurology clinic for complaints of gait problems (Table 1). He first stated that his complaints had started at the age of 33 when he felt stiffness in his feet while running. On eliciting further history, the patient reported that his walking was severely affected at the age of 40, a year after which he was only able to walk with a cane. He reported that cold and excitement also provoked the symptoms, and the spastic bladder started to manifest as 'urgency urine.' The patient's complaints were not based on any systemic conditions. In his neurological examination, he had full cognitive skills. Motor examination revealed 4/5 muscle strength in the lower extremities and a slightly increased spastic tonus in the ankles and knees. Bilateral Achilles clonus and bilateral Babinski sign were positive, and deep tendon reflexes (DTRs) were brisk in all four limbs. There were no superficial sensory defects, but vibration was detected for 17 s in the upper extremities, 5 s on the patella, and 0 s in the malleoli. The Romberg sign was positive. Extrapyramidal, cerebellar, and peripheral nervous system examinations were within normal limits. Cranial and whole spinal MRI examinations and the EMG investigation (needle and nerve conduction velocities) did not disclose any abnormalities, either.

Neurological examination of the 76-year-old mother (II.2) of the IC could not be performed; however, she reported that she had calf pain when she had long walks, and some of her relatives on the father's side had similar symptoms with the proband.

The eldest sister (III.1) of the IC was 57 years old and had no complaints. Neurological examination did not reveal any pathological findings except the brisk DTRs in the lower extremities. Vibration sense lasted for 16 s in all four extremities. Walking and running were normal.

The elder sister (III.2) of the IC was 54 years old and had no serious complaints apart from calf pain, which she experienced during long walks, as well as hypertension and migraine, from which she had been suffering for 10 years. Neurological examination revealed that DTRs were brisk in all four limbs, bilateral Babinski sign was positive, and there was increased bilateral spastic tonus in the Achilles. Cranial nerves, extrapyramidal, superficial and deep sensation, cerebellar system, posture and gait, MRI and EMG examinations were normal.

The younger brother (III.6) of the IC was 48 years old. He reported that running had easily provoked stiffness of his feet as of 34 years of age, and he developed the habit of refraining from running to avoid falls at the age of 36. At the age of 38, walking problems and numbness began in the front half of his feet. Neurological examination revealed increased spastic tonus, brisk DTRs, bilateral positive Babinski sign, and Achilles clonus in the lower extremities. He had subjective hypothesis and hypoalgesia in the form of half socks, including fingers. He was unable to run and had a spastic gait; however, he could walk without an assistant. Cranial nerves, extrapyramidal and deep sensation, as well as the cerebellar system were normal. EMG was not indicative of any abnormalities.

The nephew (IV.1) of the IC, who is the eldest son of III.2, was 33 years old. Neurological examination could not be performed; however, it was understood that he had been experiencing muscle contraction problems in his feet during walking and running for 5 years.

Another nephew (IV.3) of the IC, who is the younger son of III.2, was 28 years old and suffered from contractions in his feet when excited or performing motor activities. He also experienced hand/arm weakness after 15–20 min when he carried heavy objects. He reported that he had had these complaints for the past 6–7 years. On examination, his muscle strength was normal, DTRs were brisk in all four limbs, and Babinski sign was bilaterally positive. Hypothesis was detected on the sole of the right foot. EMG was normal.

Table 1 Clinical characteristics of affected family members

	(III.2)	(III.4, index case)	(III.6)	(IV.3)	(IV.8)
Age at examination (years)	54	52	48	28	23
Age at disease onset (years)	Asymptomatic	33	34	20	20
First complaint	Calf pain during long walks	Stiffness in his feet while running	Stiffness in his feet while running	Stiffness in his feet while walking, running, or excited. Arm weakness when carrying heavy objects	Stiffness in his legs while walking, running, any motor activities, or excited
Neurological presentation	Spastic paraparesis and pyramidal irritation in four limbs	Spastic paraparesis and vibration sensation impairment	Spastic paraparesis	Pyramidal irritation in four limbs	Spastic paraparesis
Cranial nerves	Normal	Normal	Normal	Normal	Normal
Muscle strength in the limbs	Normal	4/5 in the lower limbs	Normal	Normal	4/5 in the lower limbs
Spastic tonus	Increased in the ankles	Increased in the ankles and knees	Increased in the ankles	None	Slightly increased in the ankles
Deep tendon reflexes	Brisk in four limbs	Brisk in four limbs	Brisk in lower limbs	Brisk in four limbs	Brisk in lower limbs
Pathological reflexes	Bilateral Babinski sign (+)	Bilateral Achille clonus, Bilateral Babinski sign (+)	Bilateral Achille clonus, Bilateral Babinski sign (+)	Bilateral Babinski sign (+)	Bilateral Babinski sign (+)
Romberg sign	Negative	Positive	Negative	Negative	Negative
Extrapyramidal, cerebellar, peripheral nervous system examination	Normal	Normal	Normal	Hypothesis in the sole of the right foot	Normal
Gait	Normal	Spastic gait with stick. Unable to run	Spastic gait without assistant. Unable to run	Normal	Tiptoe-walking without assistance. Unable to run
Cognitive skills	Normal	Normal	Normal	Normal	Normal
MRI (cranial and whole spinal)	Normal	Normal	Normal	Normal	Normal
EMG investigation (nerve conduction velocities and needle EMG)	Normal	Normal	Normal	Normal	Normal
Additional features	Hypertension and migraine for 10 years	None	Sensorial complaints in half gloves and socks	Sensorial complaints in the soles of feet	None
Systemic examination	Normal	Normal	Normal	Normal	Normal
Ophthalmological examination	Normal	Normal	Normal	Normal	Normal

The niece (IV.6) of the IC, who is the daughter of III.6, was 25 years old. Neurological examination could not be performed; however, it was reported that she had no problems with gait and running.

The other nephew (IV.8) of the IC, who is the son of III.6, was 23 years old. He reported that he had been suffering from spasms in his legs for 1.5 years, which were provoked by motor effort or excitement. Neurological examination revealed 4/5 paraparesis, increased slight spastic tonus, positive bilateral Babinski sign, and brisk DTRs in the lower extremities. The patient had toe-walking but could walk unassisted. However, he could not run because of frequent falls and leg contractions.

Identification of the disease-causing deletion in *MYO1H*

The initial analysis of the exome sequencing data obtained for 4 affected (II.2, III.2, III.4, IV.1) and 2 unaffected (I.1, II.1) family members was restricted to the known 80 HSP-related loci. *ZFYVE27* (NM_001002261:c.244G > A) was first selected as the candidate causative gene due to its previous implication in a German HSP family (Mannan et al. 2006). Sanger sequencing results confirmed that all four cases carried the variant, while the two unaffected members did not. After that, other family members participated in our study, and three affected (III.6, IV.3, IV.8) and four unaffected (III.1, III.5, III.8, IV.4) family members, as well as one family member with clinically undetermined status (IV.6), were screened for this variant by Sanger sequencing to determine the familial segregation pattern. However, among the second group, the *ZFYVE27* variant was detected in only one affected individual (IV.3), and three unaffected family members (III.1, III.8, IV.4) carried the variant. The *ZFYVE27* variant was eliminated due to its incomplete penetrance, as well as the high population frequencies provided in the public databases.

Exome sequencing data were then re-analyzed in a way to include all other genes in addition to the known HSP-related genes. There were 14 rare, heterozygous variants exclusively shared by the affected family members. These variants and genes were prioritized based on their predicted damaging properties, cellular functions, expression data, previous disease relations, knock-out mouse phenotypes, and literature search (Supplementary Material 1). Three variants on three genes, *ATAD3C, VPS16,* and *MYO1H*, were selected as candidate causative variants for HSP in the studied family. Genotypes of all variants were screened for all participant members of the family by Sanger sequencing. The *ATAD3C* variant (NM_001039211.3:c.460C > T) and *VPS16* variant (NM_022575.4:c.616G > A) had incomplete penetrance due to the presence of these variants in an unaffected family member (III.8) and the absence of the variants in two affected family members (III.6 and IV.8). The *MYO1H* deletion (NM_001101421.3:c.2972_2974del (p.Glu992del, rs372231088), on the other hand, was identified in all 7 affected members and 1 clinically undetermined young family member (IV.6), and was not detected in any of the healthy individuals (Fig. 2). (According to the current version of *MYO1H* transcript NM_001101421.4, the detected deletion coincides at the c.3020_3022 nucleotides and p.Glu1008 amino acid residue; however, the mutation will be stated as c.2972_2974del and p.Glu992del throughout the remaining of the article to be compatible with the gnomAD database.) Pathogenicity of this variant was investigated in silico using SIFT, Mutation Taster, and PROVEAN bioinformatics tools and predicted as damaging, disease-causing, and deleterious, respectively. The allelic frequency of the variant is 0.1% and 0.07% in the overall exome and genome data available in gnomAD. In sequencing data of 3,362 unrelated individuals of Turkish origin (2589 exome and 773 genome sequencing), the frequency of the variant is 0.5% (Kars et al. 2021). Given that there were no rarer, fully penetrant variants segregated with the disease in the family and based on the frequencies of previously suggested HSP-associated variants varying from 0.000 to 0.9% (rs199659429) (Jia et al. 2018), c.2972_2974 deletion in *MYO1H* was determined to be the disease-causing mutation in this family, and *MYO1H* was proposed to be a novel causative gene for HSP.

Discussion

In this study, we clinically and genetically investigated an HSP-related family and identified a disease-causing variant in the *MYO1H* gene. We have designated *MYO1H* as a novel gene responsible for HSP.

The affected members show slowly progressive spasticity in the lower extremities, signs of corticospinal system involvement, decreased vibration sense, and varying degrees of hypertonic urinary symptoms, as in the general description of pure forms of HSP. In the cases, complicated type HSP findings, such as cerebellar and extrapyramidal symptoms, peripheral neuropathy, ophthalmological problems (i.e., cataract, chorioretinal dystrophy, optic atrophy), dysmorphic features, and mental retardation, (Harding 1983; Fink 2003; Salinas et al. 2008) were excluded by neurological examination, imaging, and special consultations.

While the inheritance pattern of the complex forms of HSP is usually autosomal recessive, the uncomplicated forms show dominant inheritance as in this family. Here, the disease is predominantly seen in males and started in the second and third decades, followed by a slow progression within 10 years, when symptoms became evident, but mobilization was preserved.

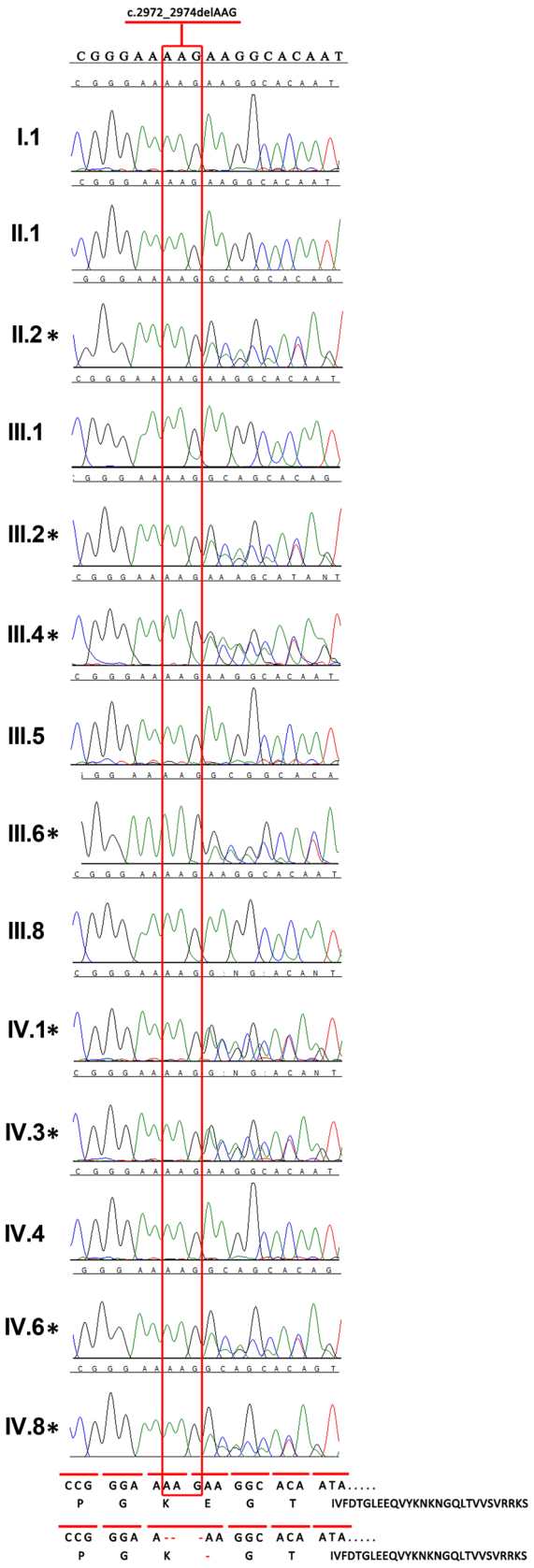


Fig. 2 Chromatograms of Sanger sequencing analysis of the identified *MYO1H* mutation in the family members. All unaffected individuals (I.1, II.1, III.1, III.5, III.8, IV.4) show a regular chromatogram pattern, while all affected (II.2, III.2, III.4, III.6, IV.1, IV.3, IV.8) and the clinically undetermined individual (IV.6) show messy sequence pattern after the deleted nucleotides because of their heterozygous state of the deletion. Individuals who carry the mutation are shown with an asterisk (*). The mutation is represented by the vertical red rectangle. Wild-type and mutant codons and corresponding amino acid sequences are given at the bottom of the chromatogram

The onset of the disease in two of the five affected men in the family was in their 30 s in the early generation (III) and in the 20 s in the remaining three affected men of the later generation (IV). The affected members IV.3 and IV.8, who are in their 20 s, expressed a more sedentary and non-sportive lifestyle compared to other male cases; however, this should not be the only possible factor for this minor clinical heterogeneity. This difference in the onset between the generations might be related to the awareness of the disease, as the proband is highly conscious of the disease and observes his relatives for the symptoms.

Of the female family members, the clinically examined patient (III.2) described a mild presentation after the fifth decade, and the clinically unexamined oldest woman (II.2) reported some mild symptoms, while the clinically unexamined youngest woman (IV.6) did not have any complaints and was asymptomatic. Roughly, the women in this family are more likely to have a later-onset and be nearly asymptomatic, albeit age related, and therefore, the youngest woman (IV.6) who has the *MYO1H* deletion has not presented any symptoms yet. It should be emphasized that her age (25 years old) is younger than the possible age of onset of the disease in women in this family. Given that the predicted age of onset is later and the disease manifests milder in the female cases in this family, the condition is likely to progress slowly and result in a definite HSP diagnosis for the clinically undetermined individual (IV.6) who did not report having any complaints yet, possibly due to her young age. We should also mention that the symptoms of the female cases did not reduce the quality of life and could only be detected by neurological examination. No significant sex differences were found in demographic and possible environmental conditions (i.e., climate, life in the city or rural area, psychological stress, eating habits), and it is not currently possible to determine the underlying reasons for this phenomenon.

Exome sequencing data were first analyzed specifically for the known HSP-associated genes, and c.244G > A variant in the *ZFYVE27 (SPG33)* (Mannan et al. 2006) gene was detected. Since the variant did not segregate in a fully penetrant manner with the disease in our family, we applied different filtering criteria on the exome sequencing data, considering non-HSP-associated genes as well. The filtering revealed 14 rare, heterozygous variants exclusively shared

by the affected members. Six deleterious variants were determined using in silico prediction tools, and three candidate disease-causing variants on the *ATAD3C, VPS16,* and *MYO1H* genes were selected based on their cellular functions. All three variants were Sanger sequenced in all family members, only the *MYO1H* variant being fully penetrant in the family. Although detecting deletion variants by Sanger sequencing is more challenging when they are present in the heterozygous state, the messy chromatogram appearances after the deletion site in the carriers confirmed that all seven cases and one clinically undetermined individual harbor the variant, while all six unaffected family members do not.

In a previous linkage study, chromosome 12q23-24 (LOD score > 3) was associated with autosomal dominant HSP, and this region was placed in the list of HSP-related genes as *SPG36* (Schüle et al. 2009). *SPG36* locus shows an overlap or a juxtaposition with genes that are associated with movement or neuromuscular disorders with clinical similarities to spastic paraplegia. A number of genes, *ATXN2, HSPB8, IFT81, UBE3B, VPS29*, as well as *MYO1H,* located in this region, were highlighted due to their cellular function related to axonal or intracellular transport or cytoskeleton reorganization. The authors of this study could not identify any disease-causing mutation in these genes, and further details of the study were not reported (Schüle et al. 2009).

To this date, the *MYO1H* gene has been associated with mandibular disorders (prognathism and retrognathism) (Tassopoulou-Fishell et al. 2012; da Fontoura et al. 2015; Arun et al. 2016) and congenital central hypoventilation (Spielmann et al. 2017). Moreover, it was shown that *MYO1H* NM_001101421.3:c.3002C > T (p.Pro1001Leu, rs3825393) missense variant was common among 199 cases with mandibular prognathism (Sun et al. 2018). The silencing of *Myo1h* homologs in zebrafish resulted in not only an underdeveloped jaw but also defects in tail structures, which draw attention in terms of its affiliation with locomotor functions. Myo1h protein is a member of the Myosin-1 family, of which main cellular functions are related to the regulation of cytoskeleton dynamics and construction of endosomal trafficking (McIntosh and Ostap 2016), which are the underlying mechanisms of HSP (Blackstone 2012, 2018; Fowler et al. 2019), further supporting the causative role of the *MYO1H* deletion in our family.

In addition, it was demonstrated that *Myo1a* knock-out mice did not apparently exhibit phenotypic differences compared to the wild-type mice; however, they had cellular perturbations and stress at the cellular level, such as decrement of cytoskeleton and membrane components and defects of the membrane structure of microvilli. Moreover, it was observed that the effect of Myo1a absence was recovered by changing the localization and expression levels of other Myosin-1 members, Myo1c and Myo1d (Tyska et al. 2005; Benesh et al. 2010). The cooperative function of Myosin-1 family members was proposed previously (Dai et al. 1999); however, the first evidence of compensation among Myo1 family members in vertebrates was shown through the recruitment of Myo1c in *Myo1a* knock-out cells (Tyska et al. 2005). Based on functional redundancy, we suggest that this compensation among Myosin-1 family members may lead to the late-onset mild phenotype in the cases of our family, despite the key roles of the Myosin-1 family of motor proteins in cells.

Myosin-1 family members interact with actin via the N-terminal head region as a motor protein and bind to the cellular membrane by mediating the C-terminal tail homology (TH1) domain. The TH1 domain includes the embedded pleckstrin-homology (PH) domain that shows low sequence homology but has highly conserved β-sheets and α-helix structures. It was identified that the crystal structure of the PH domain and other conserved regions locate in the TH1 domain of Myo1c (Brzeska et al. 2020). When these regions were aligned, it was found that numerous amino acids are also conserved outside the PH domain (Lu et al. 2015). Furthermore, it was put forward that the accurate folding of PH and TH1 domains requires all of the residues of the TH1 domain at the C-terminal. In addition, it was speculated that the frameshift mutation in *MYO1A* resulting in loss of the last β-strand of the TH1 domain (Mazzolini et al. 2012) may affect the correct folding of this domain (Lu et al. 2015). Myo1h displays the highest similarity (~60%) with Myo1c among the members of the Myosin-1 family (Bateman et al. 2021) (Fig. 3a). Since Myo1h is an important paralog of Myo1c (Hofmann et al. 2009), the determination of the TH1 domain of Myo1c allows us to estimate the Myo1h tail domain structure. When these two protein sequences are aligned, p.Glu992del in Myo1h coincides with the beginning site of the β10 sheet of Myo1c (Fig. 3b). Therefore, we hypothesize that the deletion of the glutamic acid residue at position 992 of Myo1h in our cases has a high possibility of causing a defect in the protein structure and hence functional consequences due to the loss of a negatively charged amino acid from the basic region. A mutation in *MYO1H* associated with mandibular disorders is located within the TH1 domain, and a *MYO1H* mutation related to hypoventilation leads to a frameshift in the coding sequence for the TH1 domain (Fig. 4a). Moreover, this residue is conspicuously conserved among different species (Fig. 4b). These findings strongly emphasize the important functional role of the TH1 domain.

It is reasonable to think of molecular transport dysfunction resulting from unsuitable construction of the ER structure, dysregulation of cytoskeleton dynamics, and ultimately axonal retraction as the major factors in the onset mechanism of HSP (Blackstone 2012, 2018; Fowler et al. 2019). The large proportion of HSP-related genes, such as the genes encoding Spastin, Atlastin-1, and Protrudin, are responsible for the establishment of ER morphology and

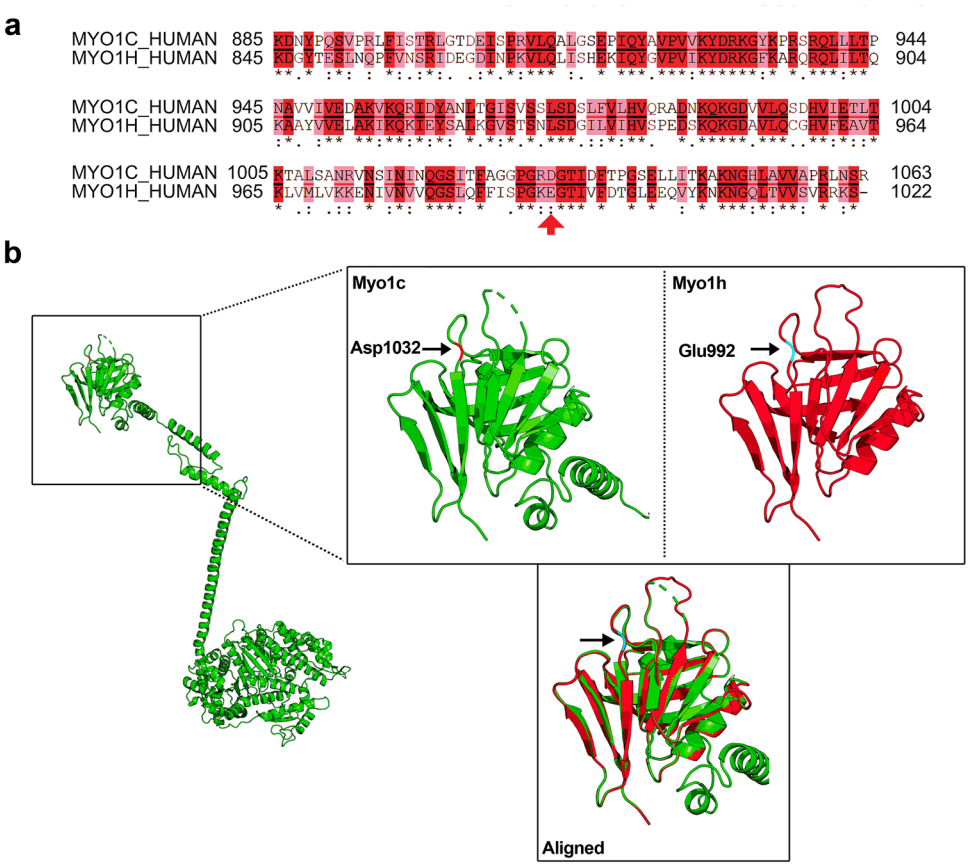


Fig. 3 Protein sequence homology of human Myo1c and human Myo1h. **a** Alignment of the amino acid sequence belonging to TH1 domain of Myo1c and Myo1h. The dark and light red highlighted residues represent the identical and chemically similar amino acids residues, respectively; **b** Crystal structure of Myo1c and homology model of the PH domain of Myo1h. Combined crystal structure of Myo1c tail (PDB ID: 4R8G) and motor (PDB ID: 4BYF) domains (left). Black squares show magnified images of the PH domain of Myo1c (green) and Myo1H (red). Superimposed structures of Myo1c and Myo1h are given at the bottom. The deleted amino acid in Myo1h is colored in cyan, and the corresponding amino acid in Myo1c is colored in red. 3D structure of the PH domain of Myo1h is generated using SPARKSX Fold Recognition software (Yang et al. 2011). The colored illustrations are created using PyMOL molecular visualization software

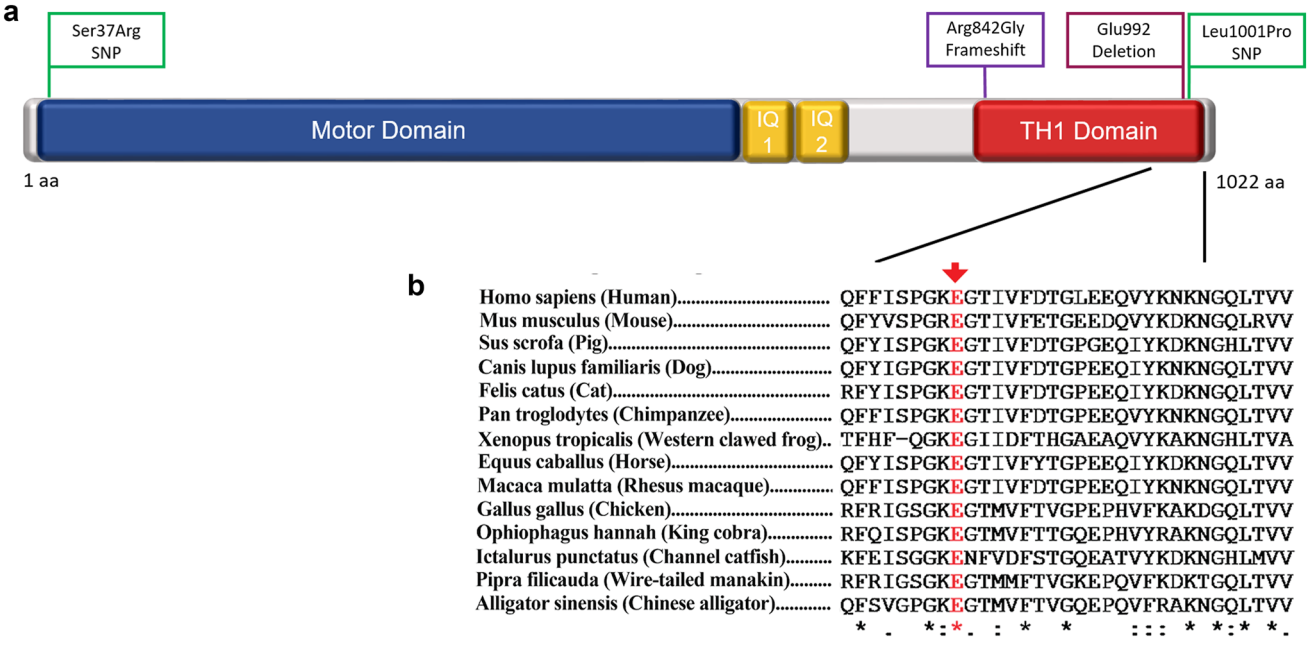


Fig. 4 The schematic illustration of Myo1h domains and disease-related mutations. **a** Mandibular disorder-associated variants are represented in green boxes, and the mutation related with hypoventilation is shown in the purple box. The Glu992del mutation identified in this study is also located in the TH1 domain and represented in the magenta box. **b** Protein sequence homology of the mutation-affected region among diverse species and the highly conserved Glu992 is denoted with a red arrow

cellular transport (Blackstone 2012, 2018). On the other side, current studies on Myo1h in the literature do not suggest a relationship to neurodegenerative disease or describe its cellular roles. Although previous studies have shown the association of *MYO1H* with mandibular dysfunction and congenital central hypoventilation by functional studies at the model organism or tissue level, its cellular function remains unknown. As mentioned above, Myo1h is a paralog of Myo1c, which has a role in the regulation of ER morphology (Joensuu et al. 2014) and interacts not only with the actin cytoskeleton but also with microtubules via its tail domain (Rump et al. 2011). Moreover, Myo1c has been indicated to regulate microtubule stabilization by inhibiting the kinesin motor protein activity during cell division (Rump et al. 2011). Another similar member with Myo1h in the Myosin-1 family is Myo1b, which has been shown to contribute to the formation of neuronal extensions (Iuliano et al. 2018). Based on the structural similarity of Myo1h with Myo1c, Myo1h might also have a role in ER morphogenesis. In this process, Myo1h might interact with Atlastin-1 and Spastin, both of which have crucial roles in maintaining ER morphology and vesicular trafficking between the ER and Golgi (Meyyazhagan and Orlacchio 2022). Therefore, it is necessary to investigate the cellular effects of the identified mutation in the *MYO1H* gene in ER shaping, ER stress, and neuronal morphology, especially in axon formation. Further functional studies will clarify the intracellular defects caused by the identified *MYO1H* mutation.

Together, we identified a novel candidate causative gene *MYO1H* in the *SPG36* region and determined a deletion (NM_001101421.3:c.2972_2974del, p.Glu992del) for late-onset autosomal dominant HSP. Since this mutation is in the TH1 domain of Myo1h, we hypothesize that the deletion of the glutamic acid residue from the basic TH1 domain prevents accurate protein structure formation. Furthermore, we suggest that it is worthwhile to investigate whether the loss of Myo1h function is compensated by other Myo1 members since the cases of the studied family have mild HSP symptoms. Lastly, our result will contribute to the understanding of the genetic basis of HSP and functional studies for the Myo1 family.

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Declarations

Conflict of interest Authors do not have any conflict of interest.

Ethical approval Ethical approval was obtained by the Ethics Committee of Istanbul Technical University and Biruni University Hospital.

Informed consent Informed consent was obtained from all participants included in the study.

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