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Variable phenotypic expression and onset in *MYH14* distal HMN phenotype in a large, multigenerational North American family

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Abstract

Introduction—Distal hereditary motor neuropathy (dHMN) causes distal-predominant weakness without prominent sensory loss. Myosin heavy chain disorders most commonly result in distal myopathy and cardiomyopathy with or without hearing loss, but a complex phenotype with dHMN, myopathy, hoarseness, and hearing loss was reported in a Korean family with a c.2822G>T mutation in *MYH14*.

Objective—To report phenotypic features in a North American family with the c.2822G>T in *MYH14*.

Methods—Clinical and molecular characterization was performed in a large, 6-generation, Caucasian family with *MYH14* dHMN.

Results—A total of 11 affected and 7 unaffected individuals were evaluated and showed varying age of onset and severity of weakness. Genotypic concordance was confirmed with molecular analysis. Electrophysiological studies demonstrated distal motor axonal degeneration without myopathy in all affected subjects tested.

Conclusions—Mutation of *MYH14* can result in a range of neuromuscular phenotypes that includes a dHMN and hearing loss phenotype with variable age of onset.

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Keywords

Myosin; distal hereditary motor neuropathy; hearing loss; autosomal dominant; myopathy

Introduction

Distal weakness and muscle atrophy with complete or marked sensory sparing is caused by several genetic neuromuscular conditions including the distal hereditary motor neuropathies (dHMN) and distal myopathies. Myosin heavy chain disorders can result in distal myopathies and cardiomyopathies with (for example, *MYH7*, *MYH14*, *MYH15* and *MYH9*) or without (for example, *MYH3* and *MYH8*)¹ hearing loss. Most of the myosin heavy chains associated with distal myopathies belong to the conventional or sarcomeric muscle myosin heavy chain or myosin complexes (including myosin light chains) (for example, *MYL2* and *MYL3*)². While conventional myosins contribute to the actin-myosin complex/actin-myosin crossbridge cycle in the sarcomere, the ‘unconventional’ (or non-muscle) myosins are important for many other cellular functions such as neuronal transport, cell division and ciliary motility, including the stereocilia of the ear^{3,4}. Several mutations in *MYH14* have been reported that result in autosomal dominant hearing loss^{5,6}. Recently, a Korean family with a complex phenotype of dHMN, myopathy, hoarseness, and hearing loss associated with a dominant mutation in *MYH14* (c.2822G>T) was described⁷. In this report, we describe the phenotype associated with the c.2822G>T mutation in the *MYH14* gene in a 6-generation, 71-member Caucasian family (See pedigree, Figure 1).

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the Institutional Review Board at The Ohio State University Wexner Medical Center, and written informed consent was obtained from all participants.

Subjects

The proband (Figure 1, III-3) presented with a 29 year history of progressive distal muscle weakness and atrophy associated with hearing loss. The family history was positive for a similar pattern of weakness in multiple family members. Eighteen clinically unaffected and affected family members (including the proband) were enrolled for clinical characterization and DNA linkage analysis. Clinical examinations were performed by a single examiner in 7 clinically affected and 5 clinically unaffected individuals prior to molecular analysis. Clinical, electrophysiological, and audiometric data were analyzed for phenotypic characterization. Genomic DNA was prepared from blood samples as previously described⁸.

Linkage analysis—DNA was hybridized to Human Mapping 500K SNP Set (Includes 250K Nsp Array and 250K Sty Array) Affymetrix™ GeneChips® to determine genotype at the loci. Genome wide genotyping calls were extracted using BRLMM algorithm⁹. The SNP call rate of each sample was >82% with an overall average call rate of 97%. Mendelian errors were identified (<1%) with the PEDSTAT program¹⁰. Parametric linkage analysis

with autosomal dominant inheritance was performed using MERLIN assuming a penetrance of 0.9 and disease allele frequency of 0.00001¹¹.

Whole exome sequencing

Exome sequencing was performed on 3 affected individuals (III-4, IV-1, and IV-2) and 1 unaffected individual (VI-1). DNA was prepared using the SOLiD exon capture kit, and SOLiD sequencing was performed (Thermo Fisher). Reads were aligned to the human hg19 genome using BWA¹². The Genome Analysis Tool Kit pipeline was used for calling variants¹³. This includes steps for removal of duplicate reads, recalibration of base quality scores, and local realignment of reads with insertions or deletions. Finally, pVAASST was used to determine which variants segregated with the affected individuals but not unaffected controls¹⁴. ANNOVAR was used to annotate the candidate variants¹⁵. At the time of these efforts, the study by Choi et al, reported the c.2822G>T *MYH14*⁷. We then searched for mutations of the *MYH14* gene in the exome sequences of affected individuals in our pedigree.

Variant validation

We developed a PCR assay followed by restriction digest to identify the c.2822G>T mutation in *MYH14*. This was used to identify the mutation in 11 affected individuals and the absence of the mutation in 7 unaffected individuals. PCR primers FP: 5'CAAAGCAAGTTACAAGGCAGTAG, RP: 5'GTTTGCATTTGACGGCTGCAC were used to amplify 50ng of genomic patient DNA. The *MYH14* mutation creates an SmlI site. PCR products were digested with SmlI (Catalogue Number: R0597S, NEB) for 2 hours at 37°C and run on a 2% agarose gel.

Results

The proband (III-3) initially presented at age 34 with progressive distal weakness and atrophy that began at age 5. Symptoms of progressive hearing loss began at age 20. She had no other pertinent medical history. Examination demonstrated bilateral hearing loss and distal muscle atrophy, greater in the legs than the arms. Strength testing (Medical Research Council grading) revealed shoulder abductors (5-/5, right and left respectively), elbow flexors (4/5), elbow extensors (4+/5), wrist extensors (4-/5), and hand intrinsic muscles (3/5). Neck flexors and extensors were 5/5. Lower extremity strength testing revealed hip flexors (4-/5), hip abductors (3-/5), knee extensors (5-/5), knee flexors (4/5), ankle dorsiflexors, evertors and invertors (0/5), and plantar flexors (1/5). Sensory examination and reflexes were normal. Electrodiagnostic testing (Table) demonstrated an absent fibular compound muscle action potential (CMAP), normal median motor and sural sensory nerve action potentials (SNAPs), and length-dependent active denervation (positive sharp waves and fibrillation potentials) associated with large-amplitude, long-duration motor unit action potentials with reduced recruitment patterns. Proximal limb or paraspinal muscle denervation and short-duration, small-amplitude motor unit action potentials were not noted. Audiometry confirmed sensorineural hearing loss that was preferentially reduced in the 1000 to 8000 Hz range.

The proband had a family history that included 25 family members spanning 6 generations who were affected with symptoms of distal muscle atrophy and weakness (Figure 2) and sensorineural hearing loss. Clinical examination of 7 affected individuals by a single examiner showed symmetric motor deficits that were most prominent in the distal upper and lower limbs (Supplemental Table S1, available online). Distal muscles of the upper and lower limbs were equally affected in 5 of 7 individuals. Proximal weakness was present in 2 of the older individuals. Sensory examinations were normal. Muscle stretch reflexes were generally reduced in proportion to weakness. Short hands and fingers were noted in affected individuals (Figure 2), but mildly short fingers were also noted in an unaffected individual (VI-1) who lacked the *MYH14* mutation. By history, and on examination as assessed by finger rub, all affected individuals had hearing loss. In addition to the proband, hearing loss was confirmed with formal audiometric testing in 2 other affected individuals. At age 7, IV-10 showed moderate to severe loss at 4–8 kHz frequencies, and IV-11 showed hearing loss at frequencies between 0.5–4 kHz frequencies at age 4. There was no history of cardiac abnormalities in any of the affected individuals enrolled in the study. There was no history of pulmonary abnormalities or ventilatory dysfunction in any affected family members except for the proband (III-3), who developed obstructive sleep apnea and chronic obstructive pulmonary disease later in life, which were felt to be related to obesity and smoking, respectively.

Variability of onset was noted in affected individuals (See Supplemental Table S1). Onset of symptoms was in late adolescence/early adulthood in 3 affected individuals and was pre-pubertal in 2 others. In 1 affected individual (III-1), late onset hearing loss (at age 67) was reported without complaints of weakness, but detailed examination demonstrated mild weakness of finger abduction. All other examined individuals had both motor and hearing symptoms, and the reported age of onset for motor weakness (mean: 14.0 ± 8.8 years, range 4 – 23, inter-quartile range 17.5) and hearing loss (mean: 13.7 ± 8.5 years, range 4 – 23, inter-quartile range 17.5) were strongly correlated such that individuals with earlier onset of weakness also experienced earlier onset of hearing loss (Pearson correlation coefficient $r=0.996$, $P<0.001$).

Electrodiagnostic studies were available in 4 affected individuals (Table). Fibular CMAPs were absent from the extensor digitorum brevis muscle in 3; 1 had a very low fibular CMAP amplitude from this muscle, with normal conduction velocity. Sural SNAPs were normal in all 4. Motor and sensory nerve conduction velocities were within reference ranges, excluding 2 patients with superimposed ulnar neuropathy at the elbow and 1 patient with bilateral median neuropathies at the wrist (not shown in Table). Needle electromyography demonstrated findings consistent with distal, chronic motor axonal loss in the upper and lower limbs in a length-dependent fashion. Fibrillation potentials, rare fasciculation potentials, and large-amplitude, long-duration motor unit action potentials with reduced recruitment were noted in distal muscles. Proximal muscles showed normal findings.

Parametric linkage analysis of the family members was performed which mapped the locus of interest to 19q13, specifically to a series of SNPs between SNP A-2141378-SNPA-2026352 which corresponds to the sequence position 45188471–53100377 on chromosome 19 using build 19 of the human genome. This position is 71.26–92.47 using the

Decode sex averaged recombination map¹⁶. A series of SNPs in this region showed an LOD score of 4, and the HLOD for these markers was also 4. No other regions showed significant LOD scores. We next performed exome analysis on individuals IV-2 (affected), III-4 (affected), IV-1 (affected), using pVAASST to determine which variants segregated with the affected individuals. This identified a mutation in the *MYH14* gene, a G-to-T change at position 2822, corresponding to a change in amino acid 941 (Arg941Leu). We extracted a list of all genes in the linkage region, and used Ingenuity Pathway Analysis to determine if any of these genes had a neurological phenotype¹⁷. Among the genes in the region were *APOE*, *RTN2*, *FKRP*, *MED25*, *MYH14*, and *CEACAM16*, all of which have been associated with a motor phenotype or hearing loss. However, only *MYH14* had a variant present in all 3 affected individuals on exome sequencing.

The c.2822G>T change in the *MYH14* gene was 100% concordant with the dHMN and hearing loss phenotype (present in 11 of 11 affected family members) and absent in 7 of 7 in unaffected family members (Supplementary figure S1).

Discussion

The pedigree presented here expands the dHMN and sensorineural hearing loss phenotype associated with *MYH14* (c.2822G>T) and is similar to the phenotype described previously by Choi and colleagues⁷. Nevertheless, the range of severity and extent of tissue involvement appears to differ. Whereas the Korean pedigree demonstrated a more complex phenotype of peripheral neuropathy, hearing loss, and myopathy, the phenotype in this Caucasian pedigree had a pure dHMN with sensorineural hearing loss without overt findings of myopathy⁷. No myopathic features were noted on electrodiagnostic testing in our study. It is possible that subtle electromyographic changes of myopathic involvement could have been obscured by changes of chronic denervation, and our study did not include muscle biopsies.

Onset of muscle weakness in this family was more variable [14.0 ± 8.8 years (range: 4–23)] compared with the previously reported family [10.6 ± 2.4 years (range: 5–14 years)]⁷. Detailed examinations of affected individuals demonstrated distal limb weakness, but interestingly, in some patients the distal upper limbs were similarly involved compared to the lower limbs. This pattern of weakness argues against a classic length-dependent distribution. Yet, in the 4 individuals who underwent electrophysiological assessment, the findings supported length-dependent axonal loss. A similar distribution was noted in 2 of the patients previously reported by Choi et al⁷. Additionally, 1 affected individual in our pedigree reported only late-onset hearing loss without weakness, although mild intrinsic hand weakness was noted on examination. Short hands have been noted in patients who carry mutations in the *MYH3* gene^{18–20}. Short hands and fingers were noted in affected individuals (Figure 2), but this was also noted in an unaffected individual who lacked the *MYH14* mutation. Thus, it is unclear whether this is an associated phenotypic characteristic.

The differences between the 2 pedigrees may be due to differences in genetic background, genetic modifiers, expression levels of the pathogenic protein in target tissues, or a combination of these factors. Individuals with this mutation may have long periods of subclinical disease given the wide range of age of symptom onset, thus diagnostic vigilance

for the *MYH14* mutation must be afforded to individuals with mild, chronic symptoms of hearing loss and/or muscle weakness and atrophy regardless of age of onset. When hearing loss and distal skeletal muscle weakness occur together, one should consider a *MYH14* gene disorder in the differential diagnosis.

The functional consequence of the *MYH14* (c.2822G>T, Arg941Leu) mutation is not known. *MYH14* is a member of the ‘unconventional’ non-muscle myosin II family of molecular motors. The human *MYH14* was cloned by RT-PCR strategies from sciatic nerve RNA²¹, and 2 splice variants were identified by Golomb and colleagues in 2004²². *MYH14* is expressed in skeletal muscle, brain, heart, colon, kidney, liver, small intestine, lung, and peripheral nerve²². *MYH14* encodes a 1995-amino acid protein containing an N-terminal myosin ‘motor’ domain, the myosin head domain, 2 IQ domains, and a C-terminal myosin tail. The myosin tail domain is important for attachment to various intracellular vesicular cargoes. Five mutations in the *MYH14* gene (Ser7Ter, Ser120Leu, Gly376Cys, Arg726Ser, and Leu976Phe) were found to be associated with an isolated phenotype of progressive hearing loss^{21,6}. The arginine to leucine transition (Arg941Leu) seen in our pedigree would likely disrupt the secondary structure of the C-terminal domain, which is the cargo-binding domain, and this could alter the interaction with client/adaptor/cargo attachment proteins. The particular cargo proteins affected could determine the most affected tissues. Further biochemical studies to understand the functional consequence of this mutation are warranted and could identify potential therapeutic targets.

In conclusion, we have described the phenotypic characteristics of a large family with a dominant mutation in the *MYH14* gene. The unique features of this familial condition are motor neuropathy and hearing loss without overt myopathy. Affected family members displayed a wide range of disease onset and severity. When hearing loss and distal skeletal muscle weakness occur together, one should consider a *MYH14* gene disorder in the differential diagnosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

CMAP	compound muscle action potential
dHMN	distal hereditary motor neuropathies
SNAP	sensory nerve action potential

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Figure 1.
Pedigree of the affected family. Filled symbols indicate affected family members.
Arrowhead indicates proband.

Figure 2.
Features of muscle atrophy in distal upper and lower limbs in 3 affected family members.

Table

Sensory and motor nerve conduction studies in affected family members.

ID	Age (years)	Median Motor		Peroneal Motor		Sural Sensory
		Amp (mV) (4 mV)	CV (m/s) (50 m/s)	Amp (mV) (2 mV)	CV (m/s) (40 m/s)	Amp (μV) (10μV)
III-3	40	7.9	58	NR	--	17
III-7	30	14	56	NR	--	16
IV-5	47	7.2	54	NR	--	31
IV-10	29	ND	ND	0.1	47	14

ID -- Pedigree identification, Amp - amplitude, CV - conduction velocity, mV - millivolts, μV - microvolts, ND - not done, NR - no response