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A Complex Phenotype of Peripheral Neuropathy, Myopathy, Hoarseness and Hearing Loss is Linked to an Autosomal Dominant Mutation in *MYH14*

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Abstract

Both peripheral neuropathy and distal myopathy are well-established inherited neuromuscular disorders characterized by progressive weakness and atrophy of the distal limb muscles. A complex phenotype of peripheral neuropathy, myopathy, hoarseness and hearing loss was diagnosed in a large autosomal dominant Korean family. A high density SNP-based linkage study mapped the underlying gene to a region on chromosome 19q13.3. The maximum multipoint LOD score was 3.794. Sequencing of 34 positional candidate genes in the segregating haplotype revealed a novel c.2822G>T (p.Arg941Leu) mutation in the gene *MYH14*, which encodes the nonmuscle myosin heavy chain 14. Clinically we observed a sequential pattern of the onset of muscle weakness starting from the anterior to the posterior leg muscle compartments followed by involvement of intrinsic hand and proximal muscles. The hearing loss and hoarseness followed the onset of distal muscle weakness. Histopathologic and electrodiagnostic studies revealed both chronic neuropathic and myopathic features in the affected patients. While mutations in *MYH14* have been shown to cause nonsyndromic autosomal dominant hearing loss (DFNA4), the peripheral neuropathy, myopathy, and hoarseness have not been associated with *MYH14*. Therefore, we suggest that the identified mutation in *MYH14* significantly expands the phenotypic spectrum of this gene.

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Keywords

hearing loss; hoarseness; genomewide scan; *MYH14*; myopathy; neuropathy

INTRODUCTION

Hereditary motor and sensory neuropathy, which is also called Charcot-Marie-Tooth disease (CMT), is a genetically and clinically heterogeneous disorders, and exhibits clinical symptoms of distal muscle weakness and atrophy, pes caus, and impaired sensation [Reilly and Shy, 2009]. Autosomal dominant CMT is classically divided into two types; the demyelinating form (CMT1) and the axonal form (CMT2) [Pareyson and Marchesi, 2009]. CMT1 has severely reduced median motor nerve conduction velocities (NCVs) (< 38 m/sec), whereas CMT2 has slightly reduced or normal NCVs with decreased amplitudes [Harding and Thomas, 1980]. To date, more than 50 causative genes or loci have been reported to be associated with CMT at the Inherited Peripheral Neuropathies Mutation Database (IPNMD) (<http://www.molgen.ua.ac.be/CMTMutations/Mutations/>).

Distal myopathies are also clinically and genetically heterogeneous inherited disorders with more than 10 genes and chromosomal loci identified to date [Udd, 2009]. A major symptom of distal myopathies is weakness of skeletal muscles in the lower legs and hands occasionally concurrent with cardiomyopathy and vocal dysfunction [Malicdan and Nonaka, 2008]. Autosomal dominant distal myopathies include tibial muscular dystrophy (TMD; MIM# 600334) caused by mutations in *TTN* (MIM# 188849) [Hackman et al., 2002], Laing distal myopathy due to changes in *MYH7* (MIM# 160760) [Meredith et al., 2004], distal caveolinopathy associated with *CAV3* (MIM# 6012530) mutations [Minetti et al., 1998], and myofibrillar myopathies (MFM) caused by mutations in *CRYAB* (MIM# 123590), *MYOT* (MIM# 6041030), and *LDB3/ZASP* (MIM# 605906) [Vicart et al., 1998; Selcen and Engel, 2004, 2005]. *MATR3* (MIM# 164015) have recently been reported as the underlying causes of dominant distal myopathy [Senderek et al., 2009]. Desminopathy and other forms of myofibrillar myopathy are caused by mutations in the *DES* gene (MIM# 125660) with both autosomal dominant and recessive inheritance [Goldfarb et al., 2004; Arias et al., 2006].

Several patients have been reported to have an unusual incidental combination of two neuromuscular diseases of CMT and muscle diseases, such as myotonic dystrophy, Becker muscular dystrophy (MIM# 300376) and facioscapulohumeral muscular dystrophy [Bütefisch et al., 1998; Bergmann et al., 2000; Hodapp et al., 2006; Kim et al., 2010]. Recently, a Dutch CMT neuropathy family that also showed complex phenotypes of myotonic dystrophy, encephalopathic attacks and hearing loss revealed an atypical complex mutations at the DM1 locus (MIM# 160900) [Spaans et al., 2009; Braida et al., 2010]. However, there has not been reported a mutation which causes autosomal dominant peripheral neuropathy, distal myopathy, hoarseness and hearing loss.

Although many causative genes have been reported to be associated with CMT or distal myopathies, lots of patients are still waiting to uncover their genetic causes. In the present study, we identified a large autosomal dominant family with a complex phenotype of peripheral neuropathy, distal myopathy, hoarseness, and hearing loss. Genomewide linkage analysis mapped on the underlying gene to chromosome 19q13.3 and sequencing of candidate genes revealed a c.2822G>T (p.Arg941Leu; NM_001077186.1) mutation in the nonmuscle myosin heavy chain 14 (*MYH14*) gene.

MATERIALS AND METHODS

Subjects

This study included a total of 33 members (15 affecteds and 18 unaffecteds) of a large Korean autosomal dominant family with a complex phenotype of peripheral neuropathy, distal myopathy, hoarseness, and hearing loss (family ID: FC317, Fig. 1), and 283 healthy controls, who had no clinical features and family history of neuromuscular disorders and hearing loss. Total DNA was extracted from peripheral blood samples using the QIAamp Blood DNA mini kit (Qiagen, Hilden, Germany). Informed consent was obtained from all participants and from parents of patients younger than 18 years of age according to the protocol approved by the Institutional Review Board for Ewha Womans University, Mokdong Hospital.

Genomewide SNP Linkage Scan

A genomewide SNP linkage scan was performed on 28 members of family FC317 applying the Infinium II Human Linkage-12 Panel (Illumina, San Diego, CA). The chip included 6,090 SNP markers that are uniformly distributed on every chromosome with an average gap of 441 kbp and 0.58 cM. Genotyping data were scanned on the Illumina BeadStation 500G array scanner and analyzed with the Merlin-1.1.2 software using an autosomal dominant parametric model.

Chromosomal Fine Mapping

Fine mapping of the chromosome 19q13 region was performed by genotyping 29 fluorescent-labeled microsatellites. PCR products were resolved on the automated genetic analyzer ABI3100, and data were analyzed using the Genotype program (Applied Biosystems, Foster city, CA). By applying the Mlink software, two-point LOD scores were obtained under an autosomal dominant model.

Determination of Copy Number Variation

Copy number variations (CNV) in the chromosome 19q13 region were determined by using a custom designed high-density comparative genomic hybridization (CGH) 135K microarray (Roche-NimbleGen, Madison, WI). The array covered a region on chromosome 19 between 36606560-60238000 bp (UCSC hg18, NCBI Build 36.1). The mean probe size and spacing length of the array were 60-mer and 215 bp, respectively. The CGH data were analyzed with NimbleScan (ver. 2.4) and SignalMap (ver. 1.9) softwares. The gain and loss threshold used in this study were $\log_2$ ratio >0.3 and <-0.3 , respectively.

Mutational Screening

Sequencing analysis of all coding exons and flanking intronic sequences was performed on 34 candidate genes in the linkage region at 19q13.3. In addition, mutational screening was performed on several previously reported genes with autosomal dominant CMT or distal myopathy. Particularly, tri-nucleotide extension was examined in the *DMPK* (MIM# 605377) in DM1 locus and *CNBP1* (*ZNF9*; MIM# 116955) in DM2 locus. Entire mitochondrial DNA (mtDNA) was also amplified and sequenced using the mitoSEQr resequencing system (Applied Biosystems). PCR primer sequences and conditions are available upon request. PCR products were sequenced on the automatic genetic analyzer ABI3100 using the BigDye terminator cycle sequencing kit (Applied Biosystems). Sequence variations were confirmed by analyzing both DNA strands. Nucleotides were counted by cDNA numbering with +1 corresponding to the A of the ATG initiation codon in GenBank RefSeq NM_001077186.1, according to the journal guidelines (www.hgvs.org/mutnomen).

Karyotyping and *MYH14* Expression in Muscle

Karyotyping was performed using cultured metaphase white blood cells obtained from proband (III-13) and her sibling (III-16). Metaphase chromosomes were visualized with Gimsa staining. *MYH14* expression in biopsied gastrocnemius muscle from a patient (III-16) was determined by quantitative real-time PCR using the QuantiTect Primer Assay (QT00080248, Qiagen).

Clinical Assessments

Clinical information was obtained through history taking, physical examinations, clinical observations, and electrophysiological investigations. Clinical observations included detailed neurological exams, including assessments of muscle weakness, sensory impairment, hearing loss, hoarseness, and reflexes. Electrocardiogram (ECG), echocardiography, and blood chemistry including creatine kinase (CK), lactic acid and pyruvic acid were done. The laryngeal study was performed in 4 patients (III-6, 11, 13, and IV-10) using a flexible fiberoptic laryngoscope. To determine hearing loss, pure-tone audiometry with air and bone conduction was performed in 17 individuals (11 affecteds and 6 unaffecteds) after otoscopic examination using an AC40 audiometry (Interacoustic, Denmark).

Electrophysiological Studies

Electrophysiological studies were carried out on 12 affected individuals (6 males and 6 females). Motor NCVs of the median, ulnar, peroneal, and tibial nerves were determined. Amplitudes of compound muscle action potential (CMAP) were measured from positive to negative peak values. Sensory NCVs were obtained from the median, ulnar, and sural nerves by an orthodromic scoring. Amplitudes of sensory nerve action potential (SNAP) were measured from positive peaks to negative peaks. An electromyography (EMG) was performed in the first dorsal interosseous, biceps brachii, tibialis anterior, medial gastrocnemius, and vastus lateralis muscles.

MRI studies of Lower Limbs

Fourteen individuals (12 affecteds and 2 unaffecteds) were studied with MRI of the lower limbs using a 1.5-T system (Siemens Vision, Siemens, Germany). Lower leg imaging was carried out in axial and coronal planes applying the following protocols: T1-weighted spin-echo (SE) (TR/TE 570–650/14–20, 512 matrixes), T2-weighted SE (TR/TE 2800–4000/96–99, 512 matrixes), and fat-suppressed T2-weighted SE (TR/TE 3090–4900/85–99, 512 matrixes). Muscles were graded on a five-point scale, as follows: 0 = no fat signal in muscle, 1 = some fatty streaks, 2 = fat occupying a minor part of muscle, 3 = similar amount of fat and muscle tissue, and 4 = fat occupying the greater part of muscle.

Histopathologic Studies

Muscle biopsies of left vastus lateralis and right lateral gastrocnemius were performed in patients III-6 and III-16, respectively. Serial frozen sections were stained with hematoxylin and eosin (H-E), NADH-tetrazolium reductase (NADH-TR), succinate dehydrogenase (SDH), modified Gomori trichrome (mGT), periodic acid-Schiff (PAS), Oil-red-O, and ATPase reaction with different pH preincubation. Immunostaining of myosin heavy chain (fast) (NCL-MHCf, monoclonal, 1:20) and myosin heavy chain (slow) (NCL-MHCs, monoclonal, 1:40: Vision Biosystems, Newcastle, UK), dystrophin, sarcoglycan, dysferlin, and titin was done. For the electron microscopic observation specimen were fixed in 2% glutaraldehyde in 25 mM cacodylate buffer at pH 7.4, and processed for semithin and ultrathin studies.

RESULTS

Clinical Manifestations

The clinical features of 15 affected members of the present family are shown in Table 1. Muscle weakness and atrophy started and predominated in distal portions of the legs and were noted to a lesser extent distally in the upper limbs. Distal muscle weakness of the lower limbs varied from asymptomatic to severe, and the progressive leg muscle atrophy was associated with disease duration (Supp. Fig. S1). Mean age at onset of distal weakness was 10.6 ± 2.4 yrs (range 5–14 yrs) and disease duration was 17.5 ± 15.3 yrs (range 1–41 yrs). All 15 affected patients showed distal leg muscle weakness, and three patients (III-4, 6 and 8) showed mild proximal thigh muscle weakness. The frequency of foot deformities was high (67%), and the decreased knee jerk reflexes were found in the majority of the patients. Serum CK levels were normal to slightly elevated, which was consistent with the other autosomal dominant distal myopathies [Saperstein et al., 2001]. ECG or echocardiography was normal.

A hoarse voice was present in 8 of 15 affected individuals (53%), albeit some affecteds had a hypophonic voice only. Six patients (III-4, 6, 8, 11, 13 and IV-3) experienced hoarseness when they were in their twenties, and in individuals IV-10 and IV-13, a hoarse voice was observed at ages 15 and 10, respectively. However, difficulty with swallowing, aspiration, dyspnea or ocular movement was not found, and flexible laryngoscope did not show paresis of vocal cord. Audiological studies showed bilateral sensorineural hearing loss in 5 of 11 affected patients (45%) (Supp. Table S1). Hearing loss was present in individual III-6 from about age 25 and in individuals III-8, III-11 and III-13 when they were in their thirties. High-frequency loss was observed in individual IV-13 at age 12 during a general physical examination. Unaffected family members noticed no symptoms of hearing loss.

Mapping of a New CMT Locus

Genomewide SNP linkage analysis revealed a maximum multipoint LOD score of 3.79 at SNP marker rs1058511 under an autosomal dominant inheritance model (0.0001, 0.1, 0.9, 0.99). A haplotype co-segregated among affecteds that spans a region from approximately 75–110 Mbp (rs2041975 to rs1051500) on chromosome 19q13.2–3 (NCBI Build 36.1) (Fig. 2A). No additional chromosomal region showed LOD scores higher than 2 under an autosomal dominant model.

Fine mapping of the chromosomal linkage region revealed a two-point maximum LOD score of 6.360 at D19S246 under an autosomal dominant model (Theta: 0.00). Haplotype and segregation analysis narrowed the chromosomal region at 19q13.3 to approximately 13 cM (D19S412 to D19S601) (Fig. 1 and 2B). The *DMPK* gene is close to this region, but could be excluded based on haplotype analysis (Fig. 2C). The linkage study also excluded main autosomal dominant CMT loci, such as 1p36.2 (*MFN2*; MIM# 608507), 1q23.3 (*MPZ*; MIM# 159440), 7p15 (*GARS*; MIM# 600287), 7q11.23 (*HSPB1*; MIM# 602195), 8p21.2 (*NEFL*; MIM# 162280), 8q21.1 (*GDAP1*; MIM# 606598), 9q34.13 (*ALS4*; MIM# 602433), 10q21.2 (*EGR2*; MIM# 129010), 12q24 (*HSPB8*; MIM# 608014), 16p13.13 (*LITAF*; MIM# 603795), 17p12 (*PMP22*; MIM# 601097) and 19p13.2 (*DNM2*; MIM# 602378).

Identification of a Missense Mutation in *MYH14*

Sequencing analysis was performed on all the coding exons of 34 candidate genes in the 19q13.3 linkage region (Fig. 2C). Particularly, 41 primer pairs were designed to amplify all exons and the promoter region of the *MYH14* gene. Because CMT4F (*PRX*; MIM# 605725) and CMT2B2 (*MED25*; MIM# 610197) loci are located near or within the disequilibrium region [Berghoff et al., 2004; Kabzinska et al., 2006; Leal et al., 2009], we carefully

examined both genes, but did not identify significant nucleotide changes. Similarly, no causative mutation was found in *EMP3* (MIM# 602335), which is located within the linkage region and shows highly conserved homology with *PMP22* myelin gene (*CMT1A*). Although the *DMPK* gene at the DM1 locus was excluded by the haplotype analysis, the careful examination of *DMPK* revealed neither abnormal CTG repeats nor a causative mutation in the coding regions. We also excluded chromosomal duplication and deletion events in the 19q13 region by applying a custom-designed high density CGH array to 3 individuals of the family (2 affecteds and 1 unaffected) (Supp. Fig. S2A). The karyotyping analysis in each affected male and female individual revealed no chromosomal abnormality (Supp. Fig. S2B).

In the screen of 34 candidate genes, the only functionally significant variant identified was a missense change in *MYH14*, which encodes the nonmuscle myosin heavy chain 14 (*MYH14*) protein [Leal et al., 2003]. The mutation was a G-to-T transversion at position 2282, c.2282G>T, which leads to substitution of an arginine residue with leucine, p.Arg941Leu (Fig. 2D). The c.2282G>T mutation has not been previously reported in the dbSNP database (<http://www.ncbi.nlm.nih.gov/snp/>). This mutation completely co-segregated with all affected members in the pedigree. Sequencing analysis confirmed the absence of this variant in unaffected members of the pedigree and in 566 ethnically matched healthy control chromosomes. The mutation is located in the tail domain of *MYH14* and it is highly conserved in other species (Fig. 2E). The amino acid corresponding to the mutation site in other human *MYH14* protein family members is lysine, which is a basic residue and thus similar to arginine. Additional polymorphic variants identified in *MYH14* are listed in Supp. Table S2.

Although *MYH14* expression in skeletal muscle, cochlea, brain and peripheral nerves has been reported [Leal et al., 2003; Donaudy et al., 2004; Golomb et al., 2004], we confirmed *MYH14* expression in biopsied gastrocnemius muscle with quantitative real-time PCR. The expression level in gastrocnemius muscle from an affected member (III-16) was slightly decreased compared to a normal male (Supp. Fig. S3).

Electrophysiological Findings

The electrophysiological findings of 29 nerves in 12 affected members (6 males and 6 females) are shown in Table 2. Nerve conduction studies demonstrated mildly reduced or normal median, ulnar and sural sensory NCVs, and reduced median and ulnar CMAPs were always associated with normal NCVs. Noteworthy, severely reduced CMAPs were observed in bilateral peroneal nerves. Needle EMG was performed in 12 patients, and they showed fibrillation potentials, but myotonic discharges were not found. Two affecteds (IV-8 and -11) did not show distal muscle atrophy, but they showed fibrillation potentials in the tibialis anterior muscles. In the same affected individuals, we observed both a large amplitude with long duration motor unit action potential (MUAP), which was usually seen in neuropathy (Fig. 3A), and also a small amplitude with short duration polyphasic MUAP seen in myopathy (Fig. 3B). These findings suggested that the affected patients have evidence of both neuropathy and myopathy.

Sequential Fatty Infiltration in Distal Muscle

All examined affected individuals showed abnormal fatty infiltrations on MRI (Table 1). Eight of 12 patients showed fatty replacements of muscle tissue only in their legs, and all of them had anterior compartment involvement. Four patients (III-6, 8, 11, and 13) showed both leg and thigh muscle involvement; in all these patients, the posterior compartment of the thighs was involved, and in two, the anterior and medial compartments were involved. We could observe a sequential pattern of onset of muscle involvement associated with

disease duration. In early stage of the disease, fatty infiltrations were present in the anterior and lateral compartments of the legs (Fig. 4A–B), and in later stages, posterior compartment leg muscles were also affected, and calf muscle atrophy was noticed (Fig. 4C–D).

Histopathologic Findings

The histopathologic features of muscle biopsies were similar in two patients (III-6 and III-16). Myofibers showed moderate to marked variation of fiber size and shape (Fig. 5A). Immunostaining with dystrophin, sarcoglycan, dysferin, and titin did not show abnormal findings, and no inflammatory infiltration was present. The grouping of the histochemical muscle fiber types were observed by ATPase with pH 9.4 preincubation and immunostaining with myosin heavy chain (fast), myosin heavy chain (slow), and myosin IIa (Fig. 5B). NADH-TR and SDH staining showed multifocal subsarcolemmal accumulation of mitochondria, although mGT staining did not show ragged-red fibers or rimmed vacuoles. Notably, the electron micrographs frequently revealed subsarcolemmal accumulation of enlarged mitochondria with variable sized rectangular or elongated rhomboidal paracrystalline inclusions (Fig. 5C).

DISCUSSION

We have mapped a new chromosomal locus for autosomal dominant peripheral neuropathy, myopathy, hoarseness, and hearing loss to 19q13.3. Sequencing analysis of 34 candidate genes revealed a novel c.2822G>T (Arg941Leu) mutation in the gene *MYH14*, that encodes the nonmuscle myosin heavy chain 14. *MYH14* consists of 41 exons (40 coding), which comprise 108 kbp of genomic sequence. It encodes 1995 amino acids and four conserved functional domains: the amino-terminal myosin domain, the myosin head, two IQ domains and the myosin tail. Northern blot analyses have revealed strong expression of the 7 kbp transcript in the skeletal muscle, small intestine, colon and cochlea, but it is also expressed in a wide range of tissue including brain and peripheral nerves [Leal et al., 2003; Donaudy et al., 2004; Golomb et al., 2004]. We also confirmed *MYH14* expression in the gastrocnemius muscle.

Our data support that the p.Arg941Leu mutation in the *MYH14* is responsible for the complex phenotype of peripheral neuropathy, myopathy, hoarseness, and hearing loss in family FC317 due to the following reasons: (1) co-segregation of the mutation with affected members in the pedigree, (2) no detection of the same mutation in 566 ethnicity matched control chromosomes, (3) high conservation of amino acids at the mutation site among different species, (4) previous involvement of *MYH14* in non-syndromic hearing loss, and (5) absence of alternative causative mutations in known CMT or distal myopathy genes. Moreover, CNV examination revealed no significant duplication or deletion in the linkage region. Karyotyping also showed no chromosomal abnormality. Recently complex mutations in *DMPK* were reported as the underlying cause of a sensorimotor neuropathy family with myotonic dystrophy, encephalopathic attacks and hearing loss [Spaans et al., 2009; Braida et al., 2010]. However, we excluded mutations in *DMPK*, which falls near 19q13.3 and also sequenced additional candidate genes, including *PRX* (CMT4F), *MED25* (CMT2B2), *MAG*, and *EMP3*. The complex phenotype of Dutch family is in some extent similar to the present Korean family, however, any myotonic discharges were not found.

Electrodiagnostic and histopathologic studies showed both chronic neuropathic and myopathic features in the affected patients. Needle EMG showed a small amplitude short duration myopathic MUAPs, and also revealed neuropathic MUAPs in the same affected individuals (Fig. 3). Histopathologic findings revealed marked variation of fiber size with many small round or angulated fibers, degenerating fibers, and endomyseal fibrosis, which were usually observed in myopathy, but also showed grouping of histochemical muscle fiber

types, which was one of well known features noted in neurogenic changes of skeletal muscle (Fig. 5). Additionally, variable sized rectangular or rhomboidal paracrystalline inclusions were frequently found in electron micrographs. Because the histopathologic abnormalities were reminiscent of cases with mitochondrial diseases, we completely sequenced the mtDNA from two biopsied patients (III-6 and III-13), but found no causative mutation (Supp. Table S2). The histological study suggested that the pathogenic mechanism of the disease may relate with abnormal translocation and dysfunction of mitochondria.

Myosins are a superfamily with a domain which interacts with actin in order to produce movement under hydrolysis of ATP. The three known nonmuscle myosins are encoded by the *MYH9* (MIM# 160775), *MYH10* (MIM# 160776), and *MYH14* genes located on chromosomes 22q11.2, 17p13.3, and 19q13.3, respectively (Simons et al. 1991; Leal et al. 2003). Myosin heavy chain genes underlie several forms of hereditary hearing loss. The hearing loss loci, DFNB2 (MIM# 600060) and DFNA17 (MIM# 600652), are associated with *MYH7A* (MIM# 160760) [Astuto et al., 2002] and *MYH9* mutations, respectively [Lalwani et al., 2000]. The nonsyndromic autosomal dominant form of hearing impairment, DFNA4 (MIM# 600652), is caused by mutations in *MYH14* [Mirghomizadeh et al., 2002; Donaudy et al., 2004]. In contrast, the studied Korean family exhibited a syndromic phenotype, dominated by peripheral neuropathy, myopathy, hoarseness, and hearing loss. Therefore, we suggest that this study significantly extends the known phenotype associated with *MYH14* mutations. Future molecular studies of similar patients should consider *MYH14* as the underlying cause.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Pedigree of family FC317. Haplotypes (indicated below each familial member) were determined using genotypes of microsatellite markers at chromosome 19q13.3 (genotypes in parentheses were inferred). Asterisks (*) indicate individuals whose DNA was used for linkage analysis. Black color indicates confirmed phenotypes by clinical exam, and grey indicates presumptive phenotypes based on history talking. Arrow (↖) indicates the proband.

Figure 2. Linkage analysis of family FC317 and identification of a missense mutation in *MYH14* gene. (A) Two-point LOD scores of chromosome 19. (B) Chromosomal fine mapping of the 19q13.2-3 region. Genotyping of 29 microsatellites revealed a ~13Mb linkage region flanked by markers D19S902 and D19S246. (C) Diagram of chromosome 19q13.2-3. Markers that co-segregate with the phenotype are indicated in bold. (D) Sequencing chromatograms of the c.2822G>T (Arg941Leu) mutation in the *MYH14* gene. The variant co-segregated with the affected family members. (E) High conservation of the Arg94 residue is illustrated by alignment of the amino acid sequences of MYH14 from different species and the MYH IIC family of proteins (ClustalX program, ver. 1.83) (*H. sapiens* MYH14-NP_001070654.1, MYH9-NP_002464.1, MYH10-NP_005955.1, MYH11-NP_002465.1; *M. musculus* Myh14-NP_082297.1, *R. norvegicus* Myh14- NP_001094160.1, *B. Taurus* Myh14- XM_882711.4).

Figure 3.

Motor unit action potential (MUAP). Recordings of the interference patterns in patient III-8 were made from the right vastus lateralis (A), and the first dorsal interosseous (B). The patient has evidence, clinically and electrophysiologically, of both neuropathy and myopathy. His electrophysiological studies revealed both a large amplitude with long duration (neuropathic, A) and a small amplitude with short duration polyphasic (myopathic) motor units (B).

Figure 4.

A sequential pattern of muscle involvement associated with disease duration (DD) in the T1-weighted axial MRI. (A) A 16-yr-old male patient (IV-10, DD=4 yrs) showed mild streaky fatty infiltrations of the anterior compartment muscles of the legs. (B) A 15-yr-old male patient (IV-13, DD=5 yrs) displayed more fatty infiltrations, including the anterior and the lateral compartments. (C) A 33-yr-old male (III-16, DD=24 yrs) showed prominent involvement of the anterior and lateral compartments of leg muscles with mild involvement of the posterior compartment. (D) A 41-yr-old female patient (III-13, DD=28 yrs) showed severe fatty involvement of all muscles compartments including a calf muscle atrophy (anterior compartment: blank arrowheads, lateral compartment: arrowheads, posterior compartment: arrow).

Figure 5.

Histopathologic findings of lateral gastrocnemius muscle in patient III-16. (A) A frozen section showing marked variation of fiber size and shape with many small rounded fibers, and degenerating fibers. Endomysial fibrosis was prominent on the background of degenerating myofibers without inflammatory cell infiltration by H&E stain. (B) Grouping of histochemical fiber types. ATPase reaction with different pH preincubation and immunostaining with myosin heavy chain (fast), myosin heavy chain (slow), and myosin IIa showed grouping of histochemical fiber types. (C) Subsarcolemmal accumulation of abnormal mitochondria. Electron micrographs revealed subsarcolemmal accumulation of frequent abnormal mitochondria including variable sized rectangular or elongated rhomboidal paracrystalline inclusions. Magnification: $\times 200$ (A), $\times 40$ (B), and $\times 30,000$ (C).

Clinical phenotypes of 15 affected individuals

Table 1

Patient	III-4	III-6	III-8	III-11	III-13	III-16	IV-3	IV-5	IV-7	IV-8	IV-9	IV-10	IV-11	IV-13	IV-14
Sex/current age (yrs)	M/52	M/52	M/48	F/45	F/41	M/33	M/31	F/18	M/15	F/11	F/18	M/16	F/15	M/15	F/11
Age of onset (yrs) ^a	12	11	10	12	13	9	10	12	10	9	13	12	14	5	7
Disease duration (yrs)	40	41	38	33	28	24	21	6	5	2	5	4	1	10	4
Muscle weakness	D>P	D>P	D>P	Distal	Distal	Distal	Distal	Distal	Distal	Distal	Distal	Distal	Distal	Distal	Distal
Distal muscle atrophy	Severe (L>U)	Severe (L>U)	Severe (L>U)	Severe (L>U)	Moderate (L>U)	Moderate (L>U)	Severe (L>U)	Mild (L>U)	Mild (L>U)	Absent	Mild (L>U)	Mild (L=U)	Absent	Mild (L>U)	Mild (L=U)
Sensory loss	–	–	±	–	–	–	–	–	–	–	–	–	–	–	–
Hearing loss	NA	+	+	+	+	–	NA	NA	–	–	–	–	–	+	NA
Hoarseness	+	+	+	+	+	–	+	–	–	–	–	+	–	+	–
Cardiac involvement	NA	–	–	–	–	–	NA	NA	–	–	–	–	–	–	–
Foot deformity	+	+	+	+	+	+	+	+	–	–	–	+	–	+	–
Knee jerk reflex	Areflex	Areflex	Areflex	Areflex	Areflex	Areflex	Areflex	Normal	Normal	Normal	Normal	Hyporeflex	Normal	Hyporeflex	Normal
Additional symptom	–	Seizure	Tremor	–	–	Tremor	Tremor	Arthritis	–	–	–	Seizure	–	–	–
Creatine kinase (IU/L) ^b	NA	WNL	2.5 fold	WNL	WNL	1.4 fold	NA	NA	WNL	WNL	WNL	1.3 fold	WNL	2.1 fold	NA
Lactic acid (mg/dl) ^c	NA	13.0	NA	19.0	9.0	11.0	NA	NA	NA	NA	10.0	13.0	7.0	12.0	NA
Pyruvic acid (mg/dl) ^d	NA	0.5	NA	1.0	0.7	0.7	NA	NA	NA	NA	0.7	1.1	0.6	0.7	NA
MRI of lower limb ^e															
Leg	NA	3.9±0.3	3.6±0.5	3.4±0.8	3.5±0.6	2.5±0.9	NA	NA	0.2±0.4	0.1±0.3	0.3±0.5	0.2±0.4	0.1±0.3	0.8±1.0	0.3±0.5
Thigh	NA	1.4±0.7	1.1±0.6	0.4±0.5	0.3±0.4	0	NA	NA	0	0	0	0	0	0	0
Muscle biopsy ^f	NA	Grouping ^g	NA	NA	NA	Grouping ^g	NA	NA	NA	NA	NA	NA	NA	NA	NA

Abbreviations: D = distal muscle; L = lower limb; NA = not available; P = proximal muscle; U = upper limb; WNL = within normal limit; +/- = positive or negative finding; ± = equivocal finding.

^a Age at onset of distal muscle weakness.

^b Creatine kinase values are listed in relations to the upper limits of normal for our laboratory.

^c Reference range: each 4.5–14.4 mg/dl

^d Reference range: each 0.3–0.9 mg/dl.

^e Degree of fatty infiltration was graded as five-point scale (mean±S.D.).

^f Muscle biopsy was done at vastus lateralis (III-6) and at gastrocnemius muscles (III-16).

^gGrouping of muscle fiber types and frequent paracrystalline inclusions.

Table 2

Nerve conduction velocities in 12 affected family members

Patient	Age (yrs)	R/L	Median motor		Ulnar motor		Peroneal motor		Tibial motor		Median sensory		Ulnar sensory		Sural sensory	
			Amp	CV	Amp	CV	Amp	CV	Amp	CV	Amp	CV	Amp	CV	Amp	CV
III-6	50	R	4.2	56.4	7.9	58.3	A	A	0.6	41.1	37.3	42.3	22.6	38.7	31.4	38.5
	50	L	6.3	57.4	6.4	70.0	A	A	0.6	49.3	24.2	44.1	22.3	39.1	20.4	38.7
	52	R	4.8	56.5	5.9	56.4	A	A	0.7	45.6	33.5	44.1	24.9	40.4	22.2	39.3
III-8	52	L	6.0	52.8	6.6	54.3	A	A	0.5	44.3	36.7	45.5	24.6	39.2	30.8	39.5
	48	R	2.9	53.5	6.1	54.1	A	A	12.5	36.1	39.8	35.7	31.7	34.2	23.4	30.3
	48	L	5.4	59.8	5.1	55.3	A	A	7.7	37.5	32.2	38.3	18.6	34.5	28.2	28.1
III-11	45	L	7.9	58.8	5.2	69.0	1.5	49.3	7.3	47.7	41.0	46.9	32.0	41.7	30.3	42.3
	40	R	10.3	56.1	9.9	56.4	A	A	16.5	45.6	47.8	42.3	23.6	41.7	24.4	39.5
	40	L	6.9	59.5	7.7	63.9	A	A	14.7	43.8	59.1	42.9	28.7	39.1	24.0	34.1
III-16	41	R	11.0	53.5	11.0	55.7	A	A	17.0	46.8	57.1	41.1	26.2	40.3	24.1	35.7
	41	L	7.3	53.7	7.3	57.5	A	A	16.8	43.3	49.0	41.7	22.6	39.1	22.3	36.1
	33	R	7.1	59.0	7.0	64.7	A	A	10.6	48.1	43.7	48.4	26.0	40.3	27.1	41.2
IV-7	33	L	8.2	57.3	4.8	64.7	A	A	7.5	48.6	57.8	46.9	33.9	43.8	29.4	42.2
	14	R	6.2	53.4	18.1	54.8	4.6	43.9	15.1	42.2	22.3	40.1	14.6	43.9	10.2	33.1
	14	L	7.3	54.5	16.2	60.5	5.9	37.7	12.1	38.6	22.0	43.4	15.9	37.7	12.9	34.8
IV-8	15	R	7.0	52.2	17.8	52.4	3.7	41.5	13.2	44.7	26.1	41.7	8.4	37.9	11.4	35.3
	15	L	8.4	55.3	17.8	53.7	5.1	37.5	14.1	38.4	19.2	40.5	13.4	38.6	11.9	38.2
	11	R	6.5	57.2	6.3	54.7	0.3	44.2	14.7	49.3	31.8	41.7	34.6	38.8	27.2	40.5
IV-9	11	L	8.1	56.1	4.5	58.1	0.2	46.9	10.8	48.0	45.1	41.7	32.4	41.7	28.6	41.7
	18	R	15.4	63.2	10.0	60.8	3.4	47.8	8.2	47.3	55.0	40.4	35.3	39.5	29.4	36.6
	18	L	11.5	58.3	14.0	63.6	4.6	45.1	8.0	47.9	53.7	43.0	26.1	40.3	31.6	33.4
IV-10	16	R	12.3	60.8	11.6	66.2	0.2	47.6	7.0	48.6	27.0	42.3	15.7	43.1	27.6	45.5
	16	L	14.7	64.7	10.6	67.5	0.6	48.6	7.1	47.2	41.2	43.5	32.4	41.7	30.5	47.8
	15	R	10.6	56.3	14.5	62.1	2.9	49.2	14.6	47.8	30.9	40.4	19.7	39.5	18.1	35.5
IV-13	15	L	10.5	64.3	13.9	62.9	3.3	45.7	12.7	45.7	30.0	41.7	20.7	39.5	21.5	37.2
	15	R	7.8	53.0	5.4	53.7	A	A	14.7	45.1	31.9	37.8	18.0	33.1	24.1	38.5
	15	L	5.2	55.3	4.1	57.9	A	A	10.5	46.2	35.0	36.9	17.9	35.7	26.7	38.2

Patient	Age (yrs)	R/L	Median motor		Ulnar motor		Peroneal motor		Tibial motor		Median sensory		Ulnar sensory		Sural sensory	
			Amp	CV	Amp	CV	Amp	CV	Amp	CV	Amp	CV	Amp	CV	Amp	CV
IV-14	11	R	9.1	52.9	11.4	56.1	2.1	42.9	12.5	43.8	31.5	40.4	22.4	39.4	14.3	36.0
	11	L	10.6	56.1	6.9	57.3	1.6	42.8	15.0	43.4	31.5	41.7	20.4	40.3	17.2	36.8

Boldface represents abnormal values. A = absent response; Age = age at examination; Amp = amplitude (motor: by mV; sensory: by μ V). CV = conduction velocity (m/s); R/L = right/left. Normal CVs: motor median $\geq$ 50.5, ulnar $\geq$ 51.1, peroneal $\geq$ 41.2, tibial $\geq$ 41.1, sensory median $\geq$ 39.3, ulnar $\geq$ 37.5, and sural $\geq$ 32.1. Normal amplitudes: motor median $\geq$ 6; ulnar $\geq$ 8, peroneal $\geq$ 6, tibial $\geq$ 6, sensory median $\geq$ 8, ulnar $\geq$ 7.9, and sural $\geq$ 6.0.