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Exome sequencing reveals *DNAJB6* mutations in dominantly-inherited myopathy

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Abstract

Objective—To identify the causative gene in an autosomal dominant limb-girdle muscular dystrophy (LGMD) with skeletal muscle vacuoles.

Methods—Exome sequencing was used to identify candidate mutations in the studied pedigree. Genome-wide linkage was then used to narrow the list of candidates to a single disease-associated mutation. Additional pedigrees with dominant or sporadic myopathy were screened for mutations in the same gene (*DNAJB6*) using exome sequencing. Skeletal muscle from affected patients was evaluated with histochemistry and immunohistochemical stains for dystrophy-related proteins, SMI-31, TDP43, and DNAJB6.

Results—Exome analysis in three affected individuals from a family with dominant limb-girdle muscular dystrophy and vacuolar pathology identified novel candidate mutations in 22 genes. Linkage analysis excluded all variants except a Phe93Leu mutation in the G/F domain of the *DNAJB6* gene, which resides within the LGMD 1E locus at 7q36. Analysis of exome sequencing data from other pedigrees with dominant myopathy identified a second G/F domain mutation (Pro96Arg) in DNAJB6. Affected muscle showed mild dystrophic changes, vacuoles, and abnormal aggregation of proteins, including TDP-43 and DNAJB6 itself.

Interpretation—Mutations within the G/F domain of *DNAJB6* are a novel cause of dominantly-inherited myopathy. *DNAJB6* is a member of the HSP40/DNAJ family of molecular co-chaperones tasked with protecting client proteins from irreversible aggregation during protein synthesis or during times of cellular stress. The abnormal accumulation of several proteins in patient muscle, including DNAJB6 itself, suggest that DNAJB6 function is compromised by the identified G/F domain mutations.

Introduction

The autosomal dominant limb-girdle muscular dystrophies (LGMD 1) are characterized by post-natal onset of progressive weakness and muscle atrophy affecting proximal muscles of the upper and lower extremities[1]. To date, seven loci have been associated with LGMD 1[2], but additional genetic heterogeneity is likely. Causative genes have been identified for three of the best described syndromes: *myotilin* in LGMD 1A, *lamin A/C* in LGMD 1B, and *caveolin-3* in LGMD 1C[3–5]. LGMD 1D (OMIM 602067) has been linked to 6q32 in a single family with significant cardiac involvement[6]. LGMD 1E (OMIM 603511) shows linkage to

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7q36 and is characterized by the onset of proximal limb weakness in mid-adulthood and vacuoles on skeletal muscle pathology[7, 8]. The LGMD 1F locus at 7q32 is close to, but not overlapping, the LGMD 1E locus[7, 9]. With the exception of age of onset and possible anticipation, LGMD 1F is clinically and pathologically similar to LGMD 1E [10]. The LGMD 1G locus resides on 4q21, and was described in a family with limb weakness accompanied by a striking limitation of finger and toe flexion[11]. Lastly, LGMD 1H is associated with 3p22 and characterized by mitochondrial abnormalities on biopsy, including ragged red fibers[12].

Although clinical features or muscle biopsy findings can point to one LGMD 1 over another (e.g. dysarthria in LGMD 1A, cardiac conduction defects in LGMD 1B, muscle rippling in LGMD 1C), there is enough phenotypic overlap that achieving a specific genetic diagnosis can be difficult. If no mutation is found with candidate gene sequencing, linkage analysis can identify known or novel loci in larger families, and lead to candidate gene sequencing in the interval. The recent advance of whole-exome sequencing allows for an alternative approach, beginning with the simultaneous identification of mutations across the entire coding region of the human genome[13]. Bioinformatic filtering of the identified mutations, including the integration of linkage data, pathogenicity predictions, gene expression patterns, or protein function, can be helpful in distinguishing between likely disease-causing mutations and probable polymorphisms[14].

We applied whole-exome sequencing to a small family resembling LGMD 1E in age of onset, pattern of weakness, and vacuolar pathology. Bioinformatic analysis of the variants integrated with linkage data identified a causative mutation in *DNAJB6*, a member of the HSP40 family of chaperone proteins. Exome sequencing in other pedigrees identified a second *DNAJB6* mutation in an adjacent residue in the G/F domain of the protein. Finally, we observed that DNAJB6 forms large protein aggregates in muscle from patients with *DNAJB6* mutations, analogous to those seen in other dominant myopathies with protein aggregates (such as myofibrillar myopathies). Together these findings provide strong evidence that *DNAJB6* mutations are a novel cause of autosomal dominant myopathy.

Subjects and Methods

Patient Selection and evaluation

Families with dominantly-inherited or sporadic myopathy with unknown genetic cause were identified from the Washington University Neuromuscular Clinic and Genetics Project. Charts, clinical records, and pathology slides were reviewed and individuals were re-examined. All participants gave written informed consent, and all study procedures were approved by the Human Studies Committee at Washington University.

Genetic Investigations

Exome sequencing: Indexed genomic DNA (gDNA) libraries were prepared from patient gDNA using the TruSeq DNA Preparation kit (Illumina) and exome-captured using the TruSeq Exome Enrichment kit (Illumina) according to manufacturer's protocol. Sequencing was performed with 100bp paired-end reads on a HiSeq2000 (Illumina). Reads were aligned to the human reference genome with NovoAlign (Novocraft Technologies). Variants were called with SAMtools[15] and annotated with SeattleSeq. Pathogenicity predictions for variants were obtained from SeattleSeq and SIFT[16]. Sequencing coverage depth was calculated using BEDTools[17] and genomic coordinates provided by Illumina. Control exomes were from patients undergoing exome analysis for diseases other than myopathy. All reported genomic locations are from GRCh37/hg19. Segregation of mutations was assessed with standard PCR-based sequencing using Primer3Plus

(http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) for primer design, an Applied Biosystems 3730 DNA Sequencer for sequencing, and LaserGene SeqMan Pro version 8.0.2 (DNAStar) for tracing analysis.

Linkage analysis: Genome-Wide Human SNP 6.0 arrays (Affymetrix, Santa Clara, CA) were run according to the manufacturer's protocols. Quality control and genotyping used Genotyping Console version 4.1.1.834. Linkage analyses used the easyLINKAGE Plus interface (v.5.08beta)[18,19] and assumed autosomal dominant inheritance with complete penetrance, a disease allele frequency of 0.01%, no phenocopies, and Affymetrix "Caucasian" allele frequencies. 2-point logarithms of the odds (LOD) scores were calculated using FastLink v4.1[20] while GeneHunter v2.1r5 was used for multipoint analysis and haplotype construction[21].

Histology and pathology of Muscle—Muscle tissue was processed as previously described[22]. In brief, cryostat sections of rapidly frozen muscle were processed for sections and fixed in acetone. For TDP-43 immunostaining, sections were incubated in 4% paraformaldehyde prior to acetone. Antibodies used were mouse anti-DNAJB6 (Sigma Chemicals St. Louis, MO), SMI-31 (Covance, Berkley, CA) and TDP-43 (BC001487, Rabbit, Protein Tech Group, Chicago IL).

Results

Exome sequencing identifies *DNAJB6* mutations in families with dominant myopathy

We identified a Caucasian family (Family 1, Fig 1A) with dominantly-inherited limb-girdle muscular dystrophy (i.e. LGMD 1) and prominent vacuoles on muscle pathology (see description below). DNA from three affected individuals underwent whole-exome capture followed by next-generation sequencing. An average of 10 Gb of sequence covering the target exome was generated per individual, resulting in an average coverage depth of 50× per targeted base (Supplementary Table 1). In each individual, ~14,000 single nucleotide polymorphisms (SNPs) and small insertions/deletions (indels) were identified in the targeted intervals. Based on the rarity of the disease and dominant inheritance pattern, we applied a filtering pathway (Table 1) to select heterozygous, autosomal, non-synonymous SNPs and coding indels that had not previously been reported to dbSNP131 or found in the 1000 Genomes Project (release August 2010).

Across the entire exome, a total of 22 variants fulfilling these criteria were shared by all three exomes (20 SNPs and 2 indels, Supplementary Table 2). The most intriguing candidate variation was a heterozygous T>C at chr7:157,160,108 (Supplementary Fig 1), producing a p.Phe93Leu substitution (c.277T>C in NM_058246) in a highly conserved residue within the G/F domain of *DNAJB6* (Fig 1C). The mutation was confirmed by PCR-based sequencing (Fig 1B) and segregates with disease in the broader pedigree (Fig 1A). This mutation was considered the most likely causative variant for two reasons. First, *DNAJB6* lies within the recently narrowed linkage interval for families with LGMD 1E[7], whose clinical and pathological phenotype shows considerable overlap with Family 1 (see Clinical Phenotypes below). Second, *DNAJB6* is a muscle-expressed member of the HSP40/DNAJ family of molecular chaperones[23] and mutations in three other chaperone proteins (*VCP, BAG3*, and *CRYAB)* cause dominantly-inherited myopathies with vacuoles on pathology[24–26].

To obtain additional genetic evidence for, or against, the *DNAJB6* variant, we performed genome-wide 2-point linkage analysis using 11 individuals (5 affected, 6 unaffected). The maximum LOD score (2.53 with theta=0.000) was found at SNP_A-2312119 on 7q36, and approached the maximum LOD score predicted for this pedigree by simulations (2.57 at

theta=0.000, Supplementary Fig 2A). This marker is within the minimal LGMD 1E locus and located in the gene immediately telomeric to *DNAJB6*. Multi-point analysis showed a LOD score of 3.00 across this region and negative LOD scores across the LGMD 1F locus at 7q32 (Supplementary Fig 2B, C). A disease-associated haplotype from 120 cM to the telomere was identified and a single recombination event narrowed the minimal interval to 2.1 Mb (chr7:157,090,296 to the telomere, Supplementary Fig 3). Although two other SNPs showed 2-point LOD scores >2.0 (SNP_A-8427187 at 1p36, LOD 2.31; SNP_A-8425136 at 20q13, LOD 2.05), they did not overlap with any of the 22 candidate variants identified by exome sequencing. This linkage data strongly supports the *DNAJB6* mutation as the disease-causing mutation in Family 1.

A strict criteria for whether a putative mutation causes a mendelian disorder is the identification of additional mutations in the same gene producing a similar phenotype. To obtain this level of certainty, we analyzed the exome sequencing data from an additional 13 individuals (8 with dominantly-inherited myopathy and 5 with sporadic vacuolar myopathy) for mutations in *DNAJB6*. The proband of an African American family (Family 2, Fig 1A) was found to have C>G at chr7:157,160,118 (Supplementary Fig 1), producing a p.Pro96Arg substitution (c.287C>G). Remarkably, this mutation is located only 2 amino acids away from the mutation found in Family 1 and is also in the G/F domain of the protein. This variant was confirmed with PCR-based sequencing and segregated with disease in participating individuals (Fig 1A and B).

Neither *DNAJB6* mutation was found in 150 control exomes performed at our institution, in which the relevant exon was covered >100×. They were also absent from 1,383 European-American and 1,081 African-American exomes from the NHLBI Exome Sequencing Project in which coverage of the relevant exon was >100× per individual[27]. Furthermore, neither was identified in the 1094 individuals of various ancestries sequenced as part of the 1000 Genomes Project [28].

Clinical Phenotypes of families with *DNAJB6* mutations

Five affected individuals from Family 1 were evaluated (Fig 1A and Table 2). Beginning in the 4th decade, all five individuals developed trouble climbing stairs or getting up from the floor. Examinations showed limb-girdle weakness proportional to disease duration, but there was no bulbar weakness or dysarthria. In two patients, the quadriceps group was relatively preserved compared to the hamstrings. Mild heel-cord contractures were seen only in II:3, but finger and toe flexion was normal. None had cardiac or pulmonary involvement, and none had muscle rippling or mounding. Creatine kinase (CK) levels were elevated in 4 of 5 subjects (2–5 times the upper limit of normal) and have remained elevated in those with serial testing. EMG/NCS had been performed in 2 patients, demonstrating clear myopathic changes with fibrillation potentials and positive sharp waves in one. Patients progressed at a similar rate, typically remaining capable of ambulation but requiring a wheelchair for community mobility within 20 years of symptom onset. Diagnostic muscle biopsies from three individuals (II:2, II:3, II:7) showed a chronic myopathy with vacuoles and are further described below.

Three affected individuals from Family 2 were evaluated and found to have a distal-predominant myopathy (Fig 1 and Table 2). Except for the variable ages of onset (18–35 years), the pattern of weakness and pace of progression have been similar across all three siblings. Weakness began in the distal lower extremities and presented with tripping. CK levels were elevated and EMG/NCS was consistent with myopathy. Weakness has gradually progressed to include the hands and proximal legs, with loss of ambulation after disease duration of 20–30 years. No cardiac or pulmonary involvement has been detected.

DNAJB6 immunohistochemistry in patient muscle

Deltoid biopsies from Family 1 (II:2, II:3, II:7) were obtained and reanalyzed. All three showed moderate variation in fiber sizes, myofibers with internal nuclei, and variable involvement of different fascicles (Fig 2A). Many of the small fibers were angular and some contained characteristic rimmed vacuoles on gomori trichrome and congo red staining (Fig 2B). The vacuoles were negative for non-specific esterase or acid phosphatase. No necrotic fibers or inflammation was present. Two of the three biopsies had scattered fibers with absent cytochrome oxidase staining. Immunostaining for dystrophin, α-, β-, γ-, and δ-sarcoglycan, α2-laminin, caveolin-3, dysferlin, and desmin were normal in all biopsies. Aggregates of SMI-31 and TDP-43 were present in all three biopsies (Fig 2C and F).

Mutations in other proteins involved in protein folding or degradation (e.g. VCP, CRYAB, and BAG3)[24,26,29] also cause dominant myopathies characterized by abnormal protein aggregates or vacuoles. Often, skeletal muscle shows accumulation of the mutated protein as inclusions[24,25,30,31]. We reasoned that DNAJB6 protein would similarly accumulate in patients harboring *DNAJB6* mutations. Indeed, immunostaining for DNAJB6 highlighted multiple fibers with subsarcolemmal accumulation and sarcoplasmic inclusions in all three patients heterozygous for the Phe93Leu mutation (Fig 2D and F). Some DNAJB6 positive inclusions were located around and within vacuoles. Inclusions of DNAJB6 were not seen in muscle biopsies from 4 control patients (two without neurologic disease and two with amyotrophic lateral sclerosis) (Fig 2G). Increased DNAJB6 immunostaining was seen also in small fibers from two patients with sporadic inclusion body myositis (Fig 2H), suggesting that DNAJB6 accumulation may play a role in other myopathies characterized by abnormal protein aggregation and vacuoles. No member of Family 2 consented to biopsy.

Discussion

Using exome sequencing, bioinformatic filtering, and linkage analysis, this study provides genetic and pathologic evidence that mutations in *DNAJB6* are a novel cause of dominantly-inherited myopathy. *DNAJB6* encodes a highly conserved member of the HSP40/DNAJ family of co-chaperones that function with HSP70 proteins to protect client proteins from irreversible aggregation during protein synthesis or during times of cellular stress[32]. DNAJ family members regulate the ATPase activity of HSP70 family members via their J-domain (Fig 1C) and are thought to help direct client protein specificity for HSP70 chaperone function[33]. DNAJB6 is a ubiquitously expressed HSP40 and is one of the more abundant DNAJB family members in skeletal muscle[23].

The functional role of DNAJB6 and the identity of its client proteins are not fully understood in mammalian cells. Genetic knockout of DNAJB6 leads to the accumulation of toxic keratin-18 containing inclusion bodies in embryonically derived chorionic cells [34]. Other studies have suggested that DNAJB6 facilitates ubiquitin-dependent degradation of select substrates such as β-catenin and even mitochondria via mitophagy [35, 36]. DNAJB6 is also present in the core of Lewy bodies and is upregulated in astrocytes of patients with Parkinson's disease [37].

Lastly, DNAJB6 plays an important role in modulating the aggregation and toxicity of polyglutamine (poly-Q) aggregates in both drosophila and mammalian cell lines [38–40]. Recent work has further delineated this role by identifying DNAJB6 as one of the most potent suppressors of poly-Q aggregation amongst the DNAJ proteins, a function that requires the C-terminal domain (amino acids 152–232)[40]. A differential role for the two isoforms of DNAJB6 has also been suggested by experiments demonstrating that DNAJB6a (the longer, nuclear-localized isoform) effectively suppresses nuclear poly-Q protein

aggregation but is ineffective with cytoplasmic aggregation, while DNAJB6b (the shorter, cytoplasmic isoform) is a potent suppressor of cytoplasmic poly-Q aggregation[40].

The mutations we have identified involve adjacent, highly conserved residues within the G/F domain of the DNAJB6 protein (Fig 1C), a domain shared by both isoforms and other DNAJB family members (Supplementary Fig 4). While the J-domain is essential for stimulating HSP70 ATPase activity and the C-terminal amino acids play a key anti-aggregation role, the precise role of the G/F domain is less understood. The mutations we have identified are likely to help clarify mammalian G/F domain function. Studies of this domain in lower organisms provide some initial information. In bacteria, the G/F domain is required for the recognition of partially unfolded client proteins and is important for the formation of stable DNAJB6/client protein complexes[41]. In *S. cerevisiae*, the G/F domain of *Sis1* (a DNAJB6 ortholog) is required for the propagation of specific yeast prions [42], a process that depends on the proper interaction of Sis1 with soluble and aggregated forms of Sup35 and Rnq1 proteins. Furthermore, deletion or mutation of a 13 amino-acid motif within the *Sis1* G/F domain blocks propagation of the Sup35 and Rnq1 prions[43]. This 13 amino acid motif is not present in DNAJA family members or other yeast HSP40 co-chaperones such as Ydj1 (Fig 1D, Supplementary Fig 4A). Yeast prions contain Q/N rich prion-like domains, a feature they share with TDP-43[44–46]. It is therefore notable that the human DNAJB6 mutations we have identified fall within this same 13 amino-acid motif (Fig 1D). This parallel is intriguing in light of the observation that TDP-43 aggregates were found in skeletal muscle from patients with *DNAJB6* mutations.

Because *DNAJB6* is ubiquitously expressed and implicated in other neurodegenerative diseases, it is interesting that patients with mutations show a selective disease of muscle. *In vitro* data suggests that the balance and availability of DNAJ family co-chaperones serve as the limiting factor on HSP70 chaperone activity[47]. Presumably therefore, small changes in the function of one chaperone (e.g. DNAJB6) could have global consequences on protein folding/handling within the cell. Post-mitotic differentiated skeletal muscle may be uniquely prone to such perturbations, as has been previously suggested for mutations in *VCP* which, like *DNAJB6*, is a ubiquitously expressed co-chaperone that can present with a phenotype limited to muscle[48]. The fact that DNAJB6 is a highly expressed DNAJB family member in skeletal muscle[23] could also contribute to the vulnerability of this tissue. Alternatively, client proteins requiring DNAJB6 for proper homeostasis might differ based on tissue or be more toxic to skeletal muscle in an aggregated state (i.e. TDP-43). It is even possible that mutant DNJAB6 aggregation itself is pathogenic.

Future studies of DNAJB6 and its mutations will be directed at a deeper understanding of the molecular pathogenesis of these myopathies. We hope to clarify how G/F domain mutations affect DNAJB6 function, examine the possibility of distinct roles for DNAJB6 isoforms, and investigate the mechanisms underlying the muscle specificity of the disease.

The clinical phenotypes of our two *DNAJB6* families share similarities and distinctions. Both families have onset of weakness in early adult-hood and gradual progression to severely impaired or lost ambulation in 20 or 30 years. In contrast to Family 1, where weakness occurs in a limb-girdle pattern, Family 2 has distal-predominant weakness. There is considerable precedent for this type of wide phenotypic spectrum among dominant myopathy disease genes. For example, mutations in myotilin, even identical ones, are associated with proximal-predominant (LGMD-1A), distal myopathy, and myofibrillar myopathy patterns[49, 50]. Similarly, mutations in *VCP* are known primarily for causing inclusion body myopathy with Paget's disease of the bone and fronto-temporal dementia[24–26]. Recently however, mutations have been described in patients with distal

myopathy[51]. Thus, it is not surprising to find *DNAJB6* mutations producing either a limb-girdle or distal myopathy phenotypes.

As noted above, *DNAJB6* resides within the 7q36 linkage interval previously reported for Finnish families with LGMD 1E[7,8]. These patients had proximal lower extremity weakness, a mean age of onset of 40 (range 28–60), mildly elevated CK and vacuolar pathology on muscle biopsy similar to the phenotype of Family 1. It will be interesting to see whether *DNAJB6* mutations explain these pedigrees as well.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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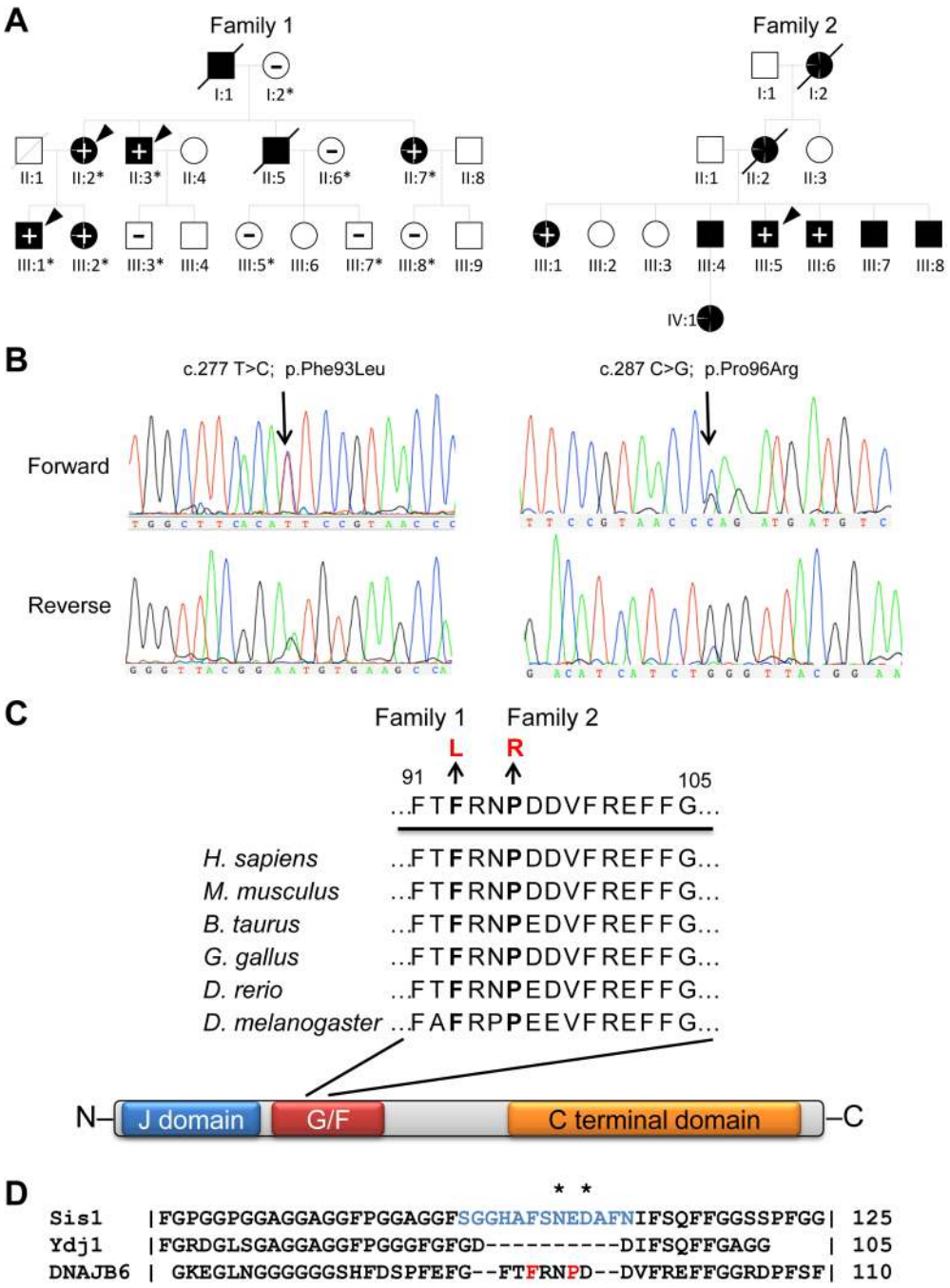

Figure 1. Exome analysis of families with dominantly-inherited myopathy

(A) Pedigree structures for dominant myopathy Families 1 and 2, with filled symbols for affected individuals. Subjects indicated by arrowheads were used for whole-exome sequencing. Linkage analysis used individuals indicated by "*". Genotypes for the c. 277T>C mutation were determined by direct sequencing and are indicated by "+" (mutation carrier) or "−" (non-carrier). (B) Sequence traces confirming the c.277T>C (p.Phe93Leu) and c.287C>G (p.Pro96Arg) variants. (C) Alignment of the DNAJB6 protein showing complete conservation of the mutated residues through *D. melanogaster*, with a domain diagram of the DNAJB6 protein below showing the position of the dominant myopathy mutations in the G/F domain, the highly conserved J domain and the C-terminal domain. (D)

Alignment of human DNAJB6 with *S. cerevisiae* orthologues performed using ClustalW2 show that the mutations identified in human myopathies overlap with the Sis1 domain required for yeast prion propagation.

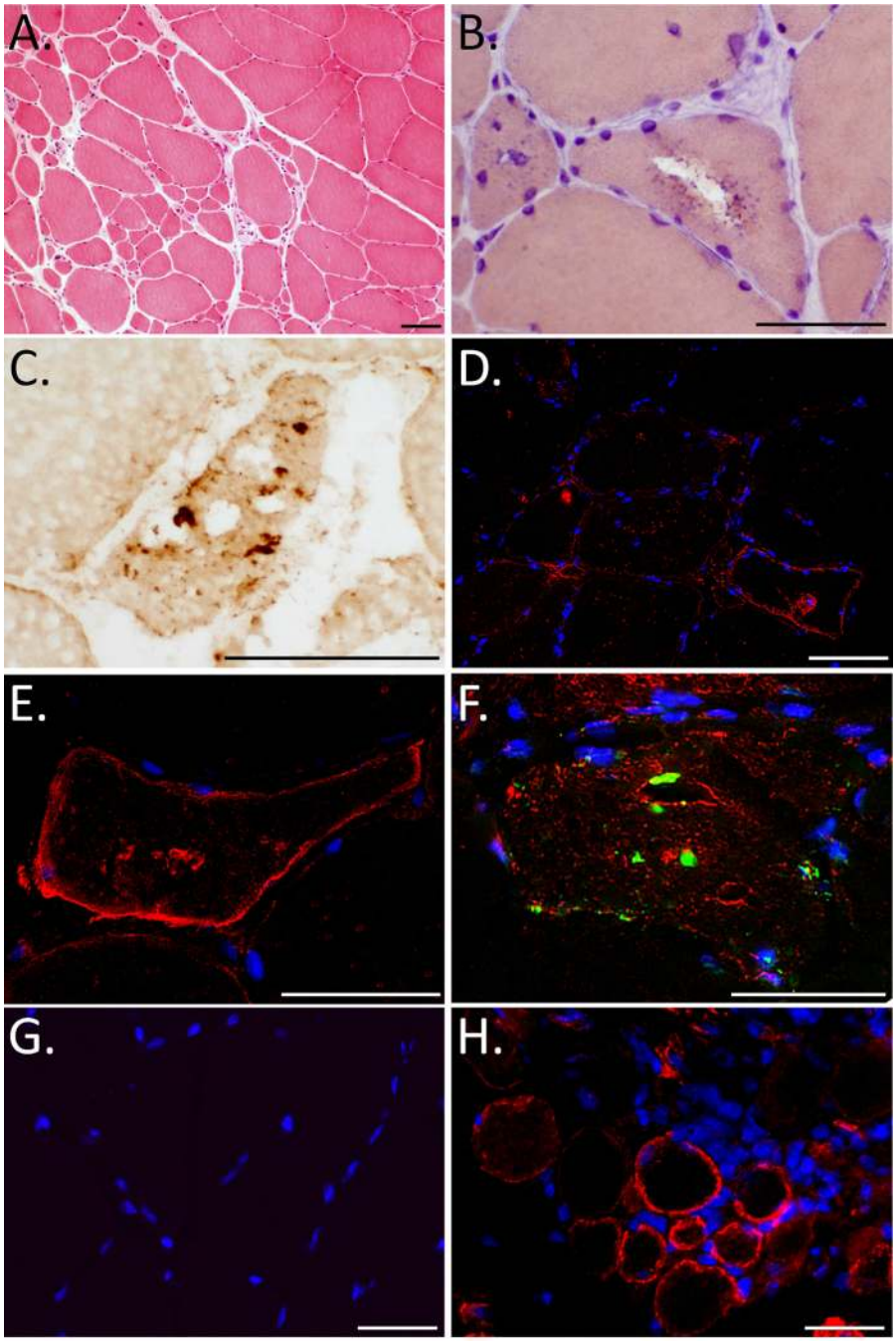

Figure 2. Pathologic characterization of skeletal muscle tissue from a subject with *DNAJB6* mutation

Clinically affected deltoid tissue from subject II:3 of family 1 (Phe93Leu mutation) was processed and histochemically stained with (A) hematoxylin and eosin or (B) congo red as previously described [22]. Additional immunohistochemistry with (C) anti-SMI-31 (brown), (D and E) anti-DNAJB6 (red) and (F) dual immunofluorescence with DNAJB6 (red) and TDP-43 (green). Anti-DNAJB6 (red) immunostaining was also performed in (G) normal control and (H) sporadic inclusion body myositis patient muscle tissue. DAPI blue nuclear stain is present in (D–H). Scale bar is 100uM.

Table 1

Bioinformatic analysis of whole-exome sequencing in Family 1

	Family 1		
Filtering Step:	**III:1**	**II:3**	**II:2**
SNPs in coding exons or at splice sites	14,630	13,659	14,049
…*and* heterozygous	9,410	8,649	8,903
…*and* not in dbSNP131 or 1000 Genomes	915	1,021	784
…*and* changes amino acid or splice site	628	740	534
…*and* not present in control exomes	438	563	367
Coding indels not present in control exomes	12	9	8
Total remaining candidate variants per individual	450	572	375
Candidate variants shared by all three individuals	22		
…*and located within the 7q36 linkage interval*	1 (*DNAJB6*)		

Filtering pipeline applied to Family 1 whole-exome sequencing data. Coding exons and splice sites were defined based on the NCBI annotation utilized by SeattleSeq 131. dbSNP131 was accessed through SeattleSeq as well. Here, 1000 Genomes refers to both the August 2010 release accessed through SeattleSeq and the May 2011 release of low-coverage SNP calls downloaded directly. Control exomes used for filtering at this stage included 30 individuals without myopathy.

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Table 2

Clinical characteristics of patients with *DNAJB6* mutations.

Family	PID	AOO	AE	Symptoms	Exam	CK	Electrophysiology
LGMD 7q36	NA	40* (28–60)	NA	Difficulties running or climbing stairs	Proximal legs more than arms	536*	Myopathic in all, rimmed vacuoles in all biopsies
Family 1 (F93L)	II:2	30	52	Difficulty climbing stairs, motorized wheelchair in community at age 56	Proximal predominant weakness in arms and legs, waddling gait	1118	N.D.
	II:3	33	56	Difficulty climbing stairs, motorized wheelchair in community at age 56	Proximal predominant weakness in arms and legs, Gower's sign, waddling gait	632	Short duration small amplitude polyphasic MUPs in the quadriceps and deltoid muscles
	II:7	32	37	Difficulty climbing stairs and rising from a sitting position, myalgias	Proximal predominant weakness in legs only, mildly waddling gait	543	N.D.
	III:1	35	41	Trouble climbing stairs and rising from a sitting position	Mild proximal predominant weakness in the lower extremities,	742	EMG: Fibs, PSW and increased insertional activity, with early recruitment of small amplitude narrow duration MUPs in iliopsoas and quadriceps muscles
	III:2	35	35	Fatigue, trouble climbing stairs and rising from a sitting position	Proximal leg weakness with waddling gait and Gower's maneuver	233	N.D.
Family 2 (P96R)	III:1	18	66	Initially developed tripping, then difficulty opening jars, progressed to require motorized wheelchair by age 62	Distal muscle wasting in legs and arms, severe distal weakness, mild-moderate proximal weakness	N.D.	N.D.
	III:5	25	45	Initially developed tripping, progressive distal weakness, wheelchair bound at 45	Distal muscle wasting in legs and arms, severe distal weakness, mild-moderate proximal weakness	278	NCS: small CMAPs, normal SNAPs EMG: fibs and PSW with narrow, short amplitude MUP and early recruitment
	III:6	35	56	Progressive gait difficulty, wheelchair bound at 53	Diffuse lower extremity weakness, severe distal and moderate proximal upper extremity	339	NCS: small CMAPs, normal SNAPs EMG: fibs and PSW with narrow, short amplitude MUP and early

Family	PID	AOO	AE	Symptoms	Exam	CK	Electrophysiology
					weakness		recruitment

Abbreviations: PID, individual identification from pedigree (see Figure 1A); AOO, age of onset; AE, age at last exam; CK, creatine kinase; N.D., no data; NCS, nerve conduction studies; EMG, electromyography; CMAP, compound muscle action potential; SNAP, sensory nerve action potential; Fibs, fibrillation potentials; PSW, positive sharp waves; MUP, motor unit potential.

*Indicates the average value derived from four published families with linkage to 7q36[7].