Exome Sequencing Identifies a *DYNC1H1* Mutation in a Large Pedigree with Dominant Axonal Charcot-Marie-Tooth Disease

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Charcot-Marie-Tooth disease is characterized by length-dependent axonal degeneration with distal sensory loss and weakness, deep-tendon-reflex abnormalities, and skeletal deformities. It is caused by mutations in more than 40 genes. We investigated a four-generation family with 23 members affected by the axonal form (type 2), for which the common causes had been excluded by Sanger sequencing. Exome sequencing of three affected individuals separated by eight meioses identified a single shared novel heterozygous variant, c.917A>G, in *DYNC1H1*, which encodes the cytoplasmic dynein heavy chain 1 (here, *novel* refers to a variant that has not been seen in dbSNP131or the August 2010 release of the 1000 Genomes project). Testing of six additional affected family members showed cosegregation and a maximum LOD score of 3.6. The shared *DYNC1H1* gene variant is a missense substitution, p.His306Arg, at a highly conserved residue within the homodimerization domain. Three mouse models with different mutations within this domain have previously been reported with age-related progressive loss of muscle bulk and locomotor ability. Cytoplasmic dynein is a large multisubunit motor protein complex and has a key role in retrograde axonal transport in neurons. Our results highlight the importance of dynein and retrograde axonal transport in neuronal function in humans.

Charcot-Marie-Tooth (CMT) disease is the most common inherited neuromuscular disorder and has an estimated prevalence of 1 in 2500. It is a chronic motor and sensory polyneuropathy characterized by distal muscle weakness and atrophy that might be associated with sensory loss, depressed tendon reflexes, and pes cavus. There are two main forms: the demyelinating type 1 that affects myelin (CMT1) and the axonal type 2 affecting the nerve axon (CMT2). Autosomal-dominant inheritance is most common, but autosomal-recessive and X-linked forms are also seen. There is considerable genetic heterogeneity, and more than 40 genes or loci have been identified.[1] We studied a four-generation family with 23 members affected (Figure 1) with CMT2, characterized by delayed motor milestones and/or an abnormal gait. Sanger sequencing excluded the genes commonly associated with CMT2; *MPZ* (MIM 159440), *NEFL* (MIM 162280), *MFN2* (MIM 608507), and *LMNA* (MIM 150330). Male to male transmission excluded *GJB1* (MIM 304040), and after sequencing of *PMP22* (MIM 601097) failed to identify a pathogenic mutation, we employed a whole-exome sequencing strategy.

Exonic sequences from three affected individuals (IV-2, -7, and -14) separated by eight meioses were enriched from genomic DNA with Agilent's SureSelect whole-exome kit (version 1) as recommended by the manufacturers except that gDNA fragmentation was carried out with a Diagenode Bioruptor. After hybridization reactions, captured DNA was amplified with 12 cycles of PCR then sequenced on an Illumina GAII sequencer with 76 bp paired-end reads. We used Novoalign (Novocraft Technologies) to align sequence reads to the hg19 reference genome and removed any duplicate reads from subsequent analyses. Seventy-six percent of targeted Consensus Coding Sequence project (CCDS) exonic bases were covered with at least 20 reads with an average coverage of 70× (Table S1, available online). We used Samtools[2] to call SNPs and indels. Annovar[3] was used for annotation of variants, and we filtered variants by using dbSNP131, 1000 Genomes (August 2010 release), and our in-house (exome data from 11 individuals of European descent) databases. Informed consent was obtained from all participants.

We assumed a rare autosomal-dominant model of inheritance, and after filtering, we found the three individuals had 177, 192, and 199 novel heterozygous variants (here, *novel* refers to a variant that has not been seen in dbSNP131or the August 2010 release of the 1000 Genomes project). annotated as missense, nonsense, frameshift, or splice site (Table 1 and Table S2). Four to six variants were shared by each pair, but only one variant in *DYNC1H1* (NM_001376.4 [MIM 600112]) was shared by all three (the number expected on the basis of the number of meioses separating these individuals). Testing of six additional affected family members by Sanger sequencing showed cosegregation with a maximum LOD score of 3.6 (see Figure 1). The variant was not present in 322

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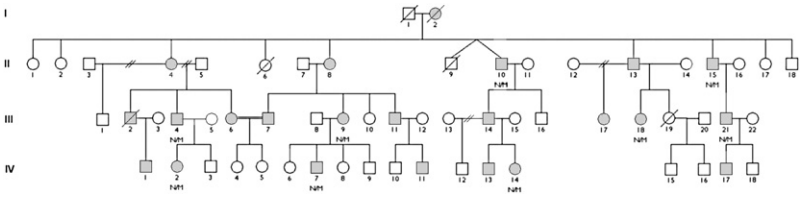

Figure 1. Partial Pedigree Showing Mutation Status

Open symbols represent unaffected individuals; filled squares represent affected males and filled circles affected females. Individual generations are numbered with roman numerals on the left. Individuals heterozygous for the p.His306Arg mutation are indicated as N/M. For the LOD-score calculation we assumed a rare autosomal-dominant model with a disease-allele frequency of 0.0001 and phenocopy rate of 0. The mutant *DYNC1H1* allele is rare (frequency <0.001); because we only tested affected individuals to avoid issues of predictive testing and variable penetrance and because there are 12 meioses separating the nine sequenced individuals, the LOD score for the variant can be approximated as $-\log10(0.5^{12}) = 3.6$.

ethnically matched control chromosomes. The shared *DYNC1H1* variant is a missense change, p.His306Arg (c.917A>G), at a highly conserved residue (Figure S1) within the homodimerization domain of cytoplasmic dynein heavy chain 1.

Cytoplasmic dynein is a large multisubunit motor protein complex that has a range of cellular functions. In particular, it is the primary motor protein responsible for retrograde axonal transport in neurons—the movement of cargo such as organelles from the cell periphery to the cell body along cytoskeletal microtubules. A homodimer of the 532 kDa cytoplasmic dynein heavy chain 1 forms the core of dynein and is responsible for the protein complex binding to and moving along microtubules.[4] Other subunits of dynein include the intermediate, light-intermediate, and light chains, thought to be responsible for interacting with cargo and maintaining the stability of the complex.[4]

Our results are corroborated by previous animal studies that have implicated disruption of *Dync1h1* in neuropathic disease. Legs at odd angles (*Loa*)[5], Cramping 1 (*Cra1*)[5] and Sprawling (*Swl*)[6] are mouse phenotypes that arose from N-ethyl-N-nitrosourea (ENU) or radiation-induced mutagenesis. Heterozygous mice have age-related progressive loss of muscle bulk and locomotor ability without a major reduction in life span.[5,6] Although there are differences between the mouse models, and indeed different pathogenesis suggested for the same mouse model by different researchers,[7,8] the mouse phenotype is broadly similar to that observed in our family. Affected patients typically have delayed motor milestones and/or an abnormal gait along with early-onset slowly progressive distal lower limb weakness and wasting with pes cavus deformity (see Figure S2). Clinical features of the best characterized family members are shown in Table 2. Upper limb involvement is less common, and ambulation is usually maintained through adulthood. Nerve conduction studies are within the normal range, sural nerve biopsies demonstrated minimal changes suggestive of increased axon degeneration, and muscle biopsies show findings consistent with secondary muscle involvement due to denervation (see Table 3). Transient paresthesia and neuropathic lower limb pains are also reported by several family members, typically those more severely affected. Although the core features consistent with CMT2 are seen across the family, there is significant variability in other findings: reflexes might be lost or preserved; fine touch, vibration sense, and proprioception are retained in many family members but lost in some; atypical features, such as predominant proximal muscle involvement, periscapular wasting and weakness, spinal and hip problems, and a broad-based waddling gait, are also found in a few.

Table 1. Novel Heterozygous Missense, Nonsense, Frameshift, or Splice-Site Variants

Individuals			Shared			
IV-2	IV-7	IV-14	IV-2 and IV-7	IV-2 and IV-14	IV-7 and IV-14	All
177	192	199	6	5	4	1

Number of novel heterozygous variants in the exome of each sequenced individual and the number shared across the individuals. Here, "novel" refers to a variant that has not been seen in dbSNP131, the August 2010 release of the 1000 Genomes project, or 11 in-house control exomes processed with the same laboratory and analysis pipeline.

The mouse mutations of *Dync1h1* (NM_030238.2; c.1739T>A [p.Phe580Tyr] in Loa; c.3164A>G [p.Tyr1055Cys] in Cra; and a 9 bp deletion, c.3119_3127delGCATAGTGA [p.(Gly1040_Thr1043delinsAla)], in Swl) also affect residues within the homodimerization region of the dynein stem domain (Figure 2). The embryonic lethality of the homozygous *Dync1h1* null mouse but normal phenotype of heterozygous null mice[9] raises the possibility of a dominant-negative effect for the neuropathic mutations. Given the multiple functions of dynein, the exact mechanism by which these mutations cause disease is still unclear. One recent study of the *Cra* mouse suggested an impact on synapse structure at neuromuscular junctions,[7] whereas another proposed an effect on striatal neurons.[8] However, a recent report with single-molecule and live-imaging techniques in the *Loa* mouse strongly suggested that defects in dynein motor processivity play a key role in disease causation.[10] Measurement of retrograde run-lengths (periods of uninterrupted motion) showed a marked reduction in the ability of mutant dynein to move along microtubules both in vitro and in vivo.[10] There was also evidence of altered interaction between the dynein motor and stem domains, and this interaction raises the possibility of motor domain miscoordination.[10] This reduction in processivity could explain the observed neuropathy phenotype because the neurons most affected will be those with long axons such as the motor and sensory neurons.

Mutations in other axonal transport genes cause related neuronal diseases. Autosomal-dominant mutations in the

Table 2. Clinical Features of Selected Family Members

Patient	Presentation	Features
II 10	delayed motor milestones; recurrent surgery to feet and ankles in childhood	reduced power and wasting in distal upper and lower limbs; pes cavus; reduced proprioception, normal vibration sense; reflexes normal
II 13	pes cavus at birth; recurrent surgery to feet and ankles in childhood	distal lower limb weakness and wasting; pes cavus; reduced proprioception, pin-prick, fine touch and vibration sense; reflexes normal; significant neuropathic pain; depression and paraphrenia; extrapyramidal features probably due to antipsychotics
III 4	delayed motor milestones and recurrent falls	mild distal lower limb weakness and wasting; pes cavus; reduced fine touch, other sensory modalities normal; reflexes normal
III 7	presented at age 11 with difficulties running	mild distal lower limb weakness and paresthesia; pes cavus; all sensory modalities reduced; reduced reflexes
III 9	presented at age 14 with frequent falls	distal upper and lower limb weakness and wasting (LL > UL); pes cavus; all sensory modalities reduced; reduced ankle reflexes
III 11	talipes at birth; delayed motor milestones	distal lower limb weakness and wasting; pes cavus; retained reflexes and sensation; retinoblastoma
III 14	delayed motor milestones	broad-based gait; distal lower limb weakness and wasting; reduced reflexes; sensory modalities retained; mild intention tremor; strabismus
III 17	abnormal gait in early childhood	minimally reduced power and wasting in distal lower limbs; normal reflexes and sensation; no pes cavus
III 18	delayed motor milestones; speech delay	proximal and distal lower limbs weakness and wasting and scapula wasting; pes cavus and bilateral foot drop; reflexes reduced; paraesthesia but objectively normal sensation; significant neuropathic pain and back pain
III 21	delayed motor milestones and learning difficulties	proximal and distal lower limb weakness and wasting; reduced reflexes; normal sensory modality testing; lumbar lordosis and reduced hip movements; behavioral problems including school exclusions
IV 2	abnormal gait and falls in early childhood; global developmental delay	distal lower limb weakness; no pes cavus; normal reflexes and sensation
IV 7	delayed motor milestones; speech delay and learning difficulties	broad-based gait; distal lower limb weakness and wasting; no pes cavus; reduced reflexes; reduced proprioception, other sensory modalities normal; lumbar lordosis and reduced hip movements; Achilles-tendon-lengthening operations
IV 14	delayed motor milestones	Waddling gait; proximal lower limb weakness more dominant than distal lower limb weakness; no pes cavus; reduced knee reflexes; sensory modalities normal; lumbar lordosis

p150 subunit of dynactin (also known as dynein activator) cause a form of motor neuron disease (MIM 601143)[11] and a heterozygous *KIF1B* (MIM 605995) mutation results in CMT2A (MIM 118210).[12] KIF1B is a member of the kinesin superfamily of motor proteins that are involved in anterograde axonal transport (taking cargo from the cell body to the periphery of the neurons). Mutations in other kinesin genes have been shown to cause neuronal disease, for example *KIF5A* (MIM 602821) mutations cause hereditary spastic paraplegia (MIM 604187).[13] Our findings provide further evidence of the importance of motor proteins and in particular retrograde axonal transport in the normal functioning of neurons.

Our work has used exome sequencing in selected individuals from a large pedigree to identify a disease-causing mutation. Charcot-Marie-Tooth disease can be caused by mutations in over 40 genes, and screening with Sanger sequencing is costly and time consuming. *DYNC1H1* (14q32.31) is not within a known region of linkage for CMT. An alternative approach,[14,15] linkage analysis followed by candidate gene Sanger sequencing, utilized in other studies would still have required the sequencing of at least the 78 exons of *DYNC1H1*.

In summary, we have identified a mutation in *DYNC1H1* that causes Charcot-Marie-Tooth disease by using exome sequencing in a large dominant pedigree. The mouse models also harboring mutations in the homodimerization domain of cytoplasmic dynein heavy chain 1 support the pathogenicity of the human *DYNC1H1* mutation and give valuable insights to pathophysiology. These results highlight the importance of dynein and retrograde axonal transport in neuronal function in humans.

Supplemental Data

Supplemental Data include two figures and two tables and can be found with this article online at http://www.cell.com/AJHG/.

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Table 3. Clinical Investigation Results

		Motor				Sensory						
		Right Peroneal		Right Tibial		Right Sural		Right Sup Peroneal				
Patient	Age at Study	Amplitude (mV)	Velocity (m/s)	Amplitude (mV)	Velocity (m/s)	Pk-Pk (μV)	Velocity (m/s)	Pk-Pk (μV)	Velocity (m/s)	Electromyography	Sural Nerve Biopsy	Muscle Biopsy
II 10	29	–	–	3.85	46	20	39	–	–	chronic denervation	–	–
II 13	31	–	–	4.3	51	12	41	–	–	central neuronal atrophy	–	severe connective tissue replacement
II 15	36	normal	normal	normal	normal	normal	normal	normal	normal	–	indolent axon degenerative process with some evidence of fiber regeneration consistent with a diagnosis of HMSN type 2	–
III 4	25	2.0	56.6	8.9	–	20.3	42.9	13.3	45.5	normal	–	–
III 14	28	5.4	44.2	7.1	39.6	8.6	37.7	7.8	40	vastus medialis MUPs enlarged otherwise normal	–	–
III 17	20	normal	normal	normal	normal	normal	normal	normal	normal	–	–	–
III 18	22	2.9	44	11.5	–	7	28	15	35	mild degree of large fiber axonal sensory motor peripheral neuropathy	–	–
III 21	2	normal	normal	normal	normal	normal	normal	normal	normal	interference pattern nonspecifically abnormal, occasional units up to 6 mV	–	–
IV 7	5	not performed	not performed	not performed	not performed	not performed	not performed	not performed	not performed	–	–	atrophic fibers consistent with chronic partial denervation

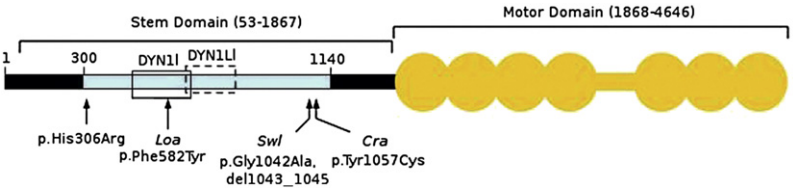

Figure 2. Schematic Representation of Human DYNC1H1

The N-terminal region of DYNC1H1 is represented by a horizontal black bar, and the stem domain (amino acids 53–1867) is indicated by a bracket above. Residues involved in DYNC1H1 dimerization (300–1140) are shown by a light blue bar; open boxes represent binding regions for intermediate (DYN1I; residues 448–703, solid line) and light-intermediate (DYN1LI; residues 651–802, broken line) chains, respectively. The motor domain (amino acids 1868–4646) is indicated by the orange shaded area; the seven ATPase domains are represented by circles, whereas the horizontal bar indicates the stalk region. The equivalent positions of mutations in three mouse models, *Loa*, *Swl*, and *Cra1*, are shown below the figure along with the p.His306Arg mutation identified in the family reported here. Note that the human protein contains two additional glycine residues at position 7 relative to mouse Dync1h1, that is numbering of equivalent residues in human DYNC1H1 is 2 higher than in mouse models. Phe582 is within a highly conserved domain responsible for binding the dynein intermediate chains as well as homodimerization.[4] The 9 bp *Swl* deletion and *Cra* p.Tyr1057Cys mutation are outside the dynein intermediate-chain binding region but within the putative homodimerization domain.[4] Numbering of amino acids is taken from Tynan et al.[16] and the UniProtKB entry for human DYNC1H1 (identifier Q14204).

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Web Resources

The URLs for data presented herein are as follows:

1000 Genomes, http://www.1000genomes.org/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org

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