



Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A

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We report missense mutations in the mitochondrial fusion protein mitofusin 2 (MFN2) in seven large pedigrees affected with Charcot-Marie-Tooth neuropathy type 2A (CMT2A). Although a mutation in kinesin family member 1B- β (KIF1B) was associated with CMT2A in a single Japanese family, we found no mutations in KIF1B in these seven families. Because these families include all published pedigrees with CMT2A and are ethnically diverse, we conclude that the primary gene mutated in CMT2A is MFN2.

Charcot-Marie-Tooth disease (CMT) comprises a frequently occurring, genetically heterogeneous group of peripheral neuropathies, although the clinical picture is rather uniform¹. Following electrophysiological criteria, CMT falls into two main forms: the demyelinating CMT type 1 with decreased nerve conduction velocities and the axonal form, CMT type 2. In contrast to the well-known molecular genetic defects causing the CMT1 phenotype, the genes associated with CMT2A, CMT2B, CMT2D and CMT2E have only recently been identified. A mutation in *KIF1B* was previously reported to be associated with CMT2A², but no further mutations in *KIF1B* have been identified, although several families with different ethnic origins with linkage to the CMT2A locus on 1p36.2 have been reported^{3–6}.

We included seven families with CMT2A with the classic clinical phenotype and different ethnic backgrounds in the present study: families DUK662 (ref. 3), DUK1241 and DUK170 (ref. 5), CMT156 (ref. 6), RU45 (identified by I.V.M, E.L.D. and O.E), CMT166 (identified by N.B-T and E.N.), and J693 (ref. 4). We found lod scores between 1.9 and 5.88, indicating linkage to the CMT2A locus (Table 1). Direct sequencing of the entire *KIF1B* gene, encompassing the two splice variants α and β , identified no mutation in the coding exons of the affected individuals in all families but identified several intronic and synonymous single-nucleotide polymorphisms distributed over the entire gene (Supplementary Table 1 online). We found no mutation in *KIF1B* cDNA of families CMT156 and CMT166.

Table 1 Clinical, ethnic and genetic characterization of families with CMT2A

Family	DUK662 (ref. 3)	DUK1706 (ref. 5)	DUK1241 (ref. 5)	CMT156 (ref. 6)	RU45	CMT166	J693 (ref. 4)
Ethnic origin	European descent	European descent	European descent	Italy	Russia	Turkey	Japan
lod score (TP/MP), STR marker	3.40/5.82, <i>DIS228</i>	2.27/2.20, <i>DIS228</i>	2.20/2.53, <i>DIS228</i>	3.11/3.29, <i>DIS2667</i>	3.55/–, <i>DIS228</i>	5.88/–, <i>DIS450</i>	1.50/1.93, <i>DIS244</i>
Mutation in <i>MFN2</i> , amino acid substitution	2219G→C, W740S	227T→C, L76P	839G→A, R280H	751C→G, P251A	281G→A, R94Q	205G→T, V69F	281G→A, R94Q
Age at onset (y)	5–52	7–44	11–35	8–50	6–17	5–15	3–15
Distal weakness and atrophy (UL/LL)	+++	+++	+++	+++	+++	+++	+++
Distal sensory loss	+	+	+	+	+	+, ++	+
Proximal muscle strength	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Other symptoms	–	–	–	Tremor	Tremor	Tremor, fatigue	–
Achilles tendon reflex	Absent	Absent	Absent	Absent	Absent	Absent	Absent
MNCV at median nerve (m/s)	46–54	40–50	42–52	40–59	52–62	53	52

TP, two-point lod score; MP, multipoint lod score; +, mild; ++, moderate; +++, severe; UL, upper limbs; LL, lower limbs; MNCV, motor nerve conduction velocity.

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This led us to investigate additional genes in the 9.6-cM chromosomal region associated with CMT2A. We excluded mutations in 14 candidate genes with known expression in the nervous system (*UBE4B*, *PEX14*, *TARDBP*, *PMSCL2*, *FRAP1*, *KIAA1337*, *FBXO2*, *FBX30*, *FBXO6*, *CLCN6*, *NPPA*, *NPPB*, *TNFRSF8* and *VPS13D*). But all seven families with CMT2A had missense mutations in the gene encoding mitofusin 2 (*MFN2*), which is located 1.65 Mb centromeric of *KIF1B* (Fig. 1 and Table 1). *MFN2* is ubiquitously expressed^{7–9}, and we identified mRNA transcripts in spinal cord and peripheral nerve (Supplementary Fig. 1 online). *MFN2* is localized to the outer mitochondrial membrane and regulates the mitochondrial network architecture by fusion of mitochondria^{7–11}. All the mutations that we detected in *MFN2* cosegregated with the disease phenotype in the respective families. None of the resulting amino acid changes were detected in 250 healthy control samples (500 chromosomes) of European descent or in 70 additional Japanese controls (140 chromosomes). Moreover, in 36 families with axonal CMT, each too small for linkage analysis, we identified seven individuals (19%) with additional *MFN2* mutations. Most of these individuals had childhood-onset CMT, including one who was diagnosed at the age of one year (Supplementary Table 2 online).

The amino acids affected by the *MFN2* mutations in the seven families with CMT2A are highly conserved in different species, including *Caenorhabditis elegans* and *Drosophila melanogaster* (Fig. 1d; the *D. melanogaster* homolog is called fuzzy onions, *fzo*). Six of seven identified mutations are within or immediately upstream of

the GTPase domain of *MFN2* (Fig. 1b,d). Four mutations were clustered in exon 4. Both the Russian family RU45 and the Japanese kindred J693 carried the mutation 281G→A, resulting in the amino acid substitution R94Q. Arg94 marks the predicted beginning of the GTPase domain and is conserved in the GTPase domain of mitofusin 1, the only human homolog with an *fzo_mitofusin* domain (Fig. 1d). An intact GTPase domain is essential for the function of mitofusins^{7,10,11}. In family DUK662, the 2219G→C transversion led to a substitution of the aromatic tryptophan to the small polar serine (W740S). This exchange was predicted to extend the coiled-coil domain at the end of the *fzo_mitofusin* domain (Supplementary Fig. 2 online).

Mitochondria undergo a dynamically regulated balance between fusion and fission reactions and have a tubular and branched membrane network¹². An efficient mitochondrial network is required for fundamental cell functions, such as equilibrating mitochondrial gene products to overcome acquired somatic mutations of mitochondrial DNA¹³ and establishing a uniform membrane potential at the mitochondrial double membrane for even energy supply throughout the cell¹⁴. In addition, studies have implicated mitochondrial dynamics in the regulation of apoptosis. Mitofusin 2 might be involved in this process, as it colocalizes with the proapoptotic protein Bax¹⁵.

Homozygous *Mfn2* knockout mice die in midgestation owing to placental defects¹¹. Although heterozygotes were reported to have a normal phenotype, mouse embryonic fibroblast cultures from

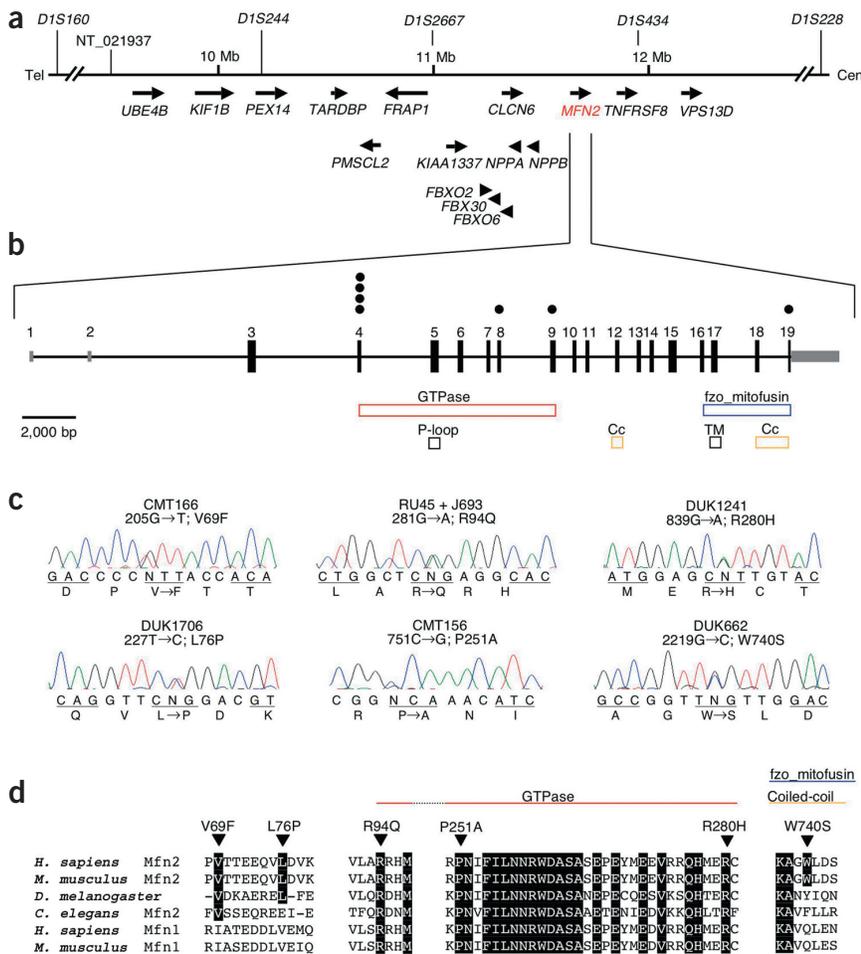


Figure 1 Overview of chromosomal region 1p36.2, the genomic organization of *MFN2*, the detected mutations and their conservation in different species. (a) Transcript map of the region on chromosome 1p36.2 associated with CMT2A. The physical map of contig NT_021937 contains *KIF1B*, typical STR markers and the screened genes including *MFN2*. The markers *D1S160* and *D1S434* define the CMT2A-linked locus. The exons of the entire *KIF1B* gene as well as cDNA transcripts were amplified and directly sequenced following standard procedures. Primer sequences are available on request. Tel, telomeric; Cen, centromeric. (b) Genomic structure of *MFN2* with six detected missense mutations in seven families (filled circles). Functional domains (colored bars), translated mRNAs (black bars) and untranslated mRNAs (gray bars) are indicated. TM, transmembrane domain; Cc, coiled-coil domain. (c) Direct sequencing of the amplified coding exons identified six different missense mutations in seven families with CMT2A. Table 1 presents details of these mutations and the affected families. We amplified DNA of family members in standard PCR reactions (primer sequences available on request), and sequenced it using the ABI system (Applied Biosystems). (d) The affected amino acids are highly conserved between different species and within the homologous protein mitofusin 1 as indicated by triangles and black background. Most mutations fell into the GTPase domain, but one was found in the *fzo_mitofusin* domain. The *D. melanogaster* homolog is called mitochondrial assembly regulatory factor.

Mfn2-deficient mice had markedly lower mitochondrial mobility¹¹. Mobility and transport of mitochondria are key elements to the functional health of the extended neuronal axons, particularly in peripheral nerves. This could be a clue to a possible mechanism of action in CMT2A. Further study may be needed to see if *Mfn2*^{+/-} mice develop an abnormal phenotype with age.

Introducing a virally transported mitofusin 2 construct in a *Mfn2*-deficient mouse cell line rescued the normal phenotype by correcting the fusion-fission imbalance¹¹. This raises the possibility that CMT2A may be amenable to some similar type of intervention in the future.

We conclude that mutations in *MFN2* are the primary cause underlying CMT2A. The present study demonstrates a new mechanism for axonal neuropathies and should provide insight into the pathophysiology of neuropathic disease, both hereditary and acquired.

Accession numbers. Entrez Protein: *Homo sapiens* mitofusin 2, AAH17061; *Mus musculus* mitofusin 2, AAM88577, *D. melanogaster* mitochondrial assembly regulatory factor, AAM00196; *C. elegans* mitofusin 2, NP_495161; *H. sapiens* mitofusin 1, AAH40557; *M. musculus* mitofusin 1, NP-077162.

GenBank: *H. sapiens* chromosome 1 genomic contig, NT_021937; *UBE4B*, NM_006048; *PEX14*, NM_004565; *TARDBP*, NM_007375; *PMSCL2*, NM_002685; *FRAP1*, NM_004958; *KIAA1337*, XM_052561; *FBXO2*, NM_012168; *FBX30*, NM_033182; *FBXO6*, NM_018438; *CLCN6*, NM_001286; *NPPA*, NM_006172; *NPPB*, NM_002521; *TNFRSF8*, NM_001243; *VPS13D*, XM_044546; *KIF1B*, NM_015074; *MFN2*, NM_014874; *MFN1*, NM_033540.

Note: Supplementary information is available on the Nature Genetics website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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