Recessive axonal Charcot-Marie-Tooth disease due to compound heterozygous mitofusin 2 mutations

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ABSTRACT

Objective: Mutations in mitofusin 2 (*MFN2*) are the most common cause of axonal Charcot-Marie-Tooth disease (CMT2). Over 50 mutations have been reported, mainly causing autosomal dominant disease, though families with homozygous or compound heterozygous mutations have been described. We present 3 families with early-onset CMT2 associated with compound heterozygous *MFN2* mutations. Transcriptional analysis was performed to investigate the effects of the mutations.

Methods: Patients were examined clinically and electrophysiologically; parents were also examined where available. Genetic investigations included *MFN2* DNA sequencing and dosage analysis by multiplex ligation-dependent probe amplification. *MFN2* mRNA transcripts from blood lymphocytes were analyzed in 2 families.

Results: Compound heterozygosity for *MFN2* mutations was associated with early-onset CMT2 of varying severity between pedigrees. Parents, where examined, were unaffected and were heterozygous for the expected mutations. Four novel mutations were detected (one missense, one nonsense, an intragenic deletion of exons 7 + 8, and a 3–base pair deletion), as well as 2 previously reported missense mutations. Transcriptional analysis demonstrated aberrant splicing of the exonic deletion and indicated nonsense-mediated decay of mutant alleles with premature truncating mutations.

Conclusions: Our findings confirm that *MFN2* mutations can cause early-onset CMT2 with apparent recessive inheritance. Novel genetic findings include an intragenic *MFN2* deletion and nonsense-mediated decay. Carrier parents were asymptomatic, suggesting that *MFN2* null alleles can be nonpathogenic unless coinherited with another mutation. **Neurology® 2011;77:168–173**

GLOSSARY

CMT = Charcot-Marie-Tooth disease; **MLPA** = multiplex ligation probe amplification; **SEOAN** = severe early onset axonal neuropathy.

Mitofusin 2 (*MFN2*) mutations are the most prevalent cause of axonal Charcot-Marie-Tooth disease (CMT).[1-3] Over 50 mutations have been reported in *MFN2*,[4] the majority causing autosomal dominant CMT2; however, homozygous and compound heterozygous *MFN2* mutations have also been reported in early-onset CMT2.[2,5-7] In some reported cases, the phenotype of patients with 2 *MFN2* mutations in *trans* have severe early onset axonal neuropathy (SEOAN),[8] though patients with mild and moderate phenotypes have been reported with either homozygous or compound heterozygous mutations.[6,7] We describe 3 families with compound heterozygous *MFN2* mutations causing early-onset CMT2. The severity of the disease varied between pedigrees.

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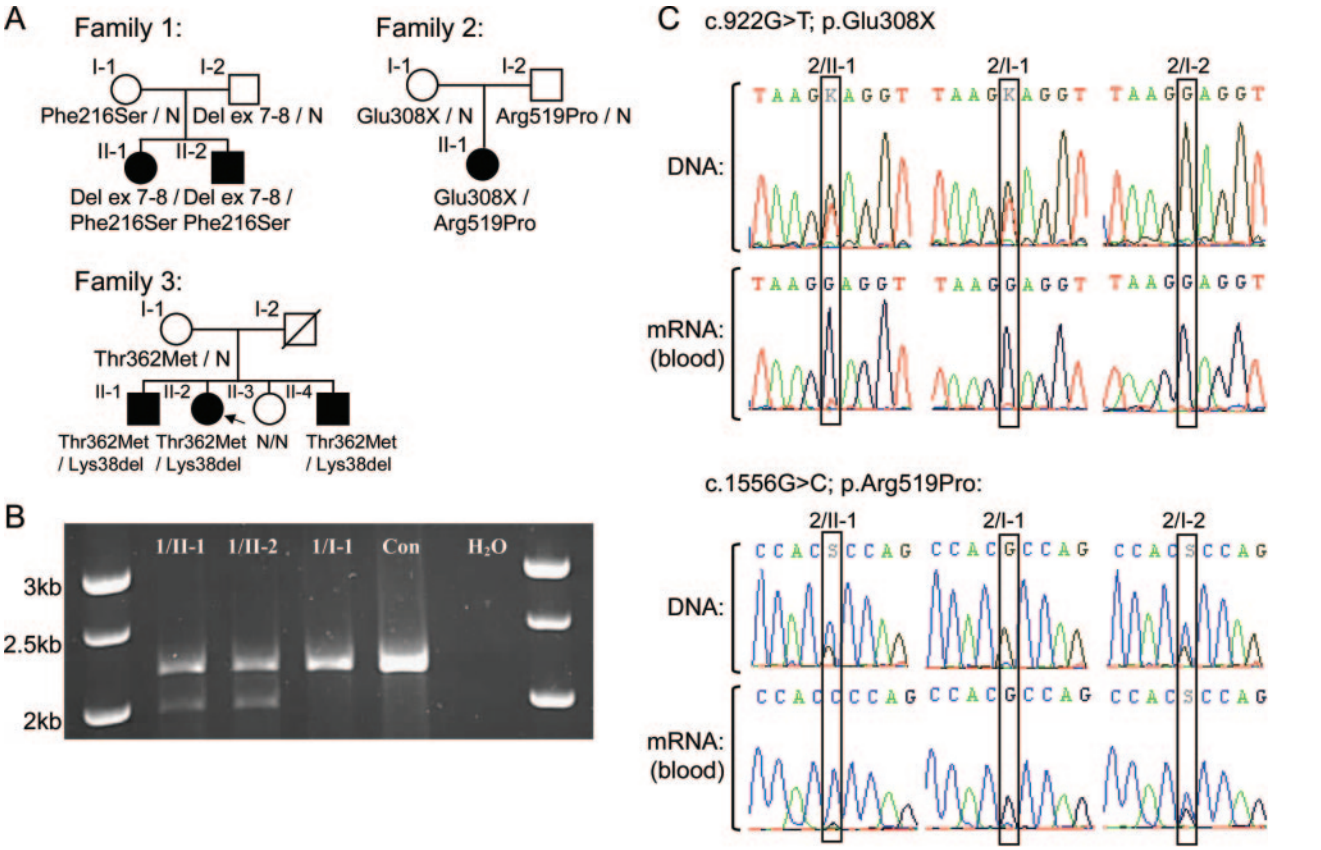
Figure Pedigrees and transcript analysis



(A) Pedigrees. Arrow = index case; N = normal genotype. (B) Family 1 *MFN2* mRNA PCR from exons 4 to 19. All bands were sequenced. Two transcripts were separated by agarose gel electrophoresis in 1/II-1 and 1/II-2; the upper band encodes the maternal c.647T>C mutation, the lower paternal band, encodes a shortened transcript in which exon 6 is spliced to exon 9, confirmed by sequencing. 1/I-1 was confirmed to heterozygously express c.647T>C. Con = normal control PCR; H_2O = water control PCR. (C) Family 2 *MFN2* DNA and mRNA sequencing. The thymine allele at base 922 was not evident in 2/II-1 and 2/I-1 in the mRNA sequencing. This allele is likely subjected to nonsense-mediated decay. Because of the nonsense-mediated decay of her maternal allele, 2/II-1 only expresses a cytosine at base c.1556, while it is heterozygously expressed in 2/I-2.

METHODS

Patients. We examined 5 affected patients belonging to 2 British families (families 1 and 2) and one Italian family (family 3) (figure, A). The parents were reviewed, when available, clinically and neurophysiologically, and tested for *MFN2* mutations.

Ethical approval and patient consent. The study was approved by the ethics committees of The National Hospital for Neurology and Neurosurgery and the Institutional Review Board of the Carlo Besta Neurological Institute IRCCS Foundation and all patients or guardians of patients provided written consent.

Genetic analysis. *MFN2* sequencing was performed with the BigDye Terminator kit v1.1 and a 3730XL genetic analyzer (Applied Biosystems). A total of 350 British and 200 Italian control chromosomes were screened for the novel *MFN2* sequence mutations. A multiplex ligation probe amplification (MLPA) assay (MRC Holland kit P143) was used for *MFN2* copy-number analysis in families 1 and 2. For transcriptional analysis, blood was collected and extracted using the Qiagen/PreAnalytix blood RNA system. cDNA was synthesized using the Applied Biosystems high capacity cDNA reverse transcription kit.

RESULTS

Clinical and neurophysiologic findings. Detailed clinical features and nerve conduction studies are summarized in tables 1 and 2. The affected individuals in families 1 and 3 had SEOAN; the proband in family 2 had early onset moderate axonal neuropathy. The parents of families 1 and 2 were unaffected on clinical examination. Only the mother in family 2 had neurophysiology that was normal. The mother in family 3 was unavailable but reportedly unaffected; the deceased father was also thought to be unaffected.

Genetic results. ***Family 1.*** *MFN2* sequencing identified a maternally inherited missense mutation, c.647T>C; p.Phe216Ser in exon 7 in both children. MLPA analysis detected a deletion of *MFN2* exons 7 and 8 in the children and their father, confirmed by long PCR (data not shown). MLPA on 306 further patients with CMT2 (with and without *MFN2* sequence mutations) detected no other rearrangements. The *MFN2* transcript was amplified in the children and

Table 1 Clinical features

	Family 1/patient II-1	Family 1/patient II-2	Family 2/patient II-1	Family 3/patient II-2	Family 3/patient II-1
Mutations	Del ex 7–8 + Phe216Ser	Del ex 7–8 + Phe216Ser	Glu308X + Arg519Pro	Thr362Met + Lys38del	Thr362Met + Lys38del
Sex/age, y	F/15	M/10	F/17	F/39	M/42
Age at onset	18 mo	12 mo	14 mo	3 y	3 y
Presenting symptom	Foot drop	Foot drop	Difficulty walking	Foot drop	Foot drop
Weakness[a]					
UL	+++	+++	++	+++	+++
LL	+++	+++	++	+++	+++
Pinprick[b]					
UL	++	−	−	+	+
LL	++	−	−	++	++
Vibration[b]					
UL	−	−	−	+	+
LL	+	−	−	++	++
Skeletal deformities	No	No	Scoliosis, pes cavus	Kyphoscoliosis, pes cavus	Kyphosis, lumbar hyperlordosis, small foot
Fundi	Bilateral optic atrophy	R pale optic disc	N	Bilateral pale optic disc	Bilateral pale optic disc
Other findings	No	No	Hypermobile joints	Visual loss, severe facial weakness, hearing loss, vocal cord palsy, respiratory muscle weakness	Visual loss, severe facial weakness, respiratory muscle weakness
Brain MRI	ND	N	N	N	ND
Other investigations	Abnormal VEP	ND	N CMCT	Abnormal VEP/BAEP	ND
CMTES	18	14	8	26	24
CMTNS	ND	ND	9	34	ND
Aid for walking	Wheelchair	Wheelchair	No	Wheelchair	Wheelchair

Abbreviations: BAEP = brainstem auditory evoked potential; CMCT = central motor conduction time; CMTES = Charcot-Marie-Tooth evaluation score (max score 28/28); CMTNS = Charcot-Marie-Tooth Neuropathy Score (max score 36/36); F = family; LL = lower limbs; N = normal; ND = not done; UL = upper limbs; VEP = visual evoked potential.

[a] Weakness: − normal, + ≥4 in distal muscles, ++ <4 in distal muscles, +++ proximal weakness (knee flexion and extension, elbow flexion and extension, or above).

[b] Pinprick and vibration sense: − normal, + reduced below wrist/ankle, ++ reduced below elbow/knee, +++ reduced at or above elbow/knee.

the mother (figure, B). Translation of the mutant paternal transcript would result in a truncated protein of 199 in-frame amino acids, followed by 61 out-of-frame amino acids before a premature stop codon.

Family 2. *MFN2* sequencing identified 2 novel mutations in the proband, a maternally inherited nonsense mutation in exon 9 (c.922G>T; p.Glu308X), and a paternally inherited missense mutation in exon 15 (c.1556G>C; p.Arg519Pro). Neither mutation was detected in 350 British control chromosomes. MLPA analysis of the proband detected no *MFN2* rearrangements. Sequencing of *MFN2* transcripts demonstrated nonsense-mediated decay of the maternal allele (figure, C).

Family 3. Two *MFN2* mutations were detected in the proband (3/II-2), c.1085C>T; p.Thr362Met and c.113_115delAGA; p.Lys38del, and her 2 affected brothers; neither were detected in her unaffected sister. c.1085C>T was maternally inherited; no DNA was available from the deceased father. The novel c.113_115delAGA; p.Lys38del mutation was not detected in 200 Italian and 350 British control chromosomes.

DISCUSSION CMT2 caused by *MFN2* mutations is typically an autosomal dominant disease, with the assumption that mutations act via a dominant-negative or toxic gain-of-function mechanism.[9] There are previous reports of patients with CMT2 with 2 *MFN2* mutations in *trans*[2,5-7]; in most cases the inheritance was recessive, though semidominant inheritance has also been described, where the parents were slightly affected.[5] *MFN2* is not unique among CMT-related genes in causing disease via multiple inheritance patterns. In most cases recessive forms are more severe, though exceptions are known.[10,11]

In our series, SEOAN was associated with compound heterozygosity for a deletion of *MFN2* exons

7 and 8 and the missense mutation c.647T>C; p.Phe216Ser in family 1. The mechanism of the pathogenic effect of the exon 7 + 8 deletion, carried by the unaffected father, is uncertain. This transcript is likely to be subject to nonsense-mediated decay, as would be expected for any stop codon located >55 bp from a downstream intron/exon boundary.[12] This is suggested by the reduced intensity of the lower band in the figure, C. Although the PCR performed here was not a strictly quantitative assay, preferential amplification of a shorter template would be expected to generate a more intense shorter band if template concentrations were equal. If translated, the deletion transcript would result in a truncated, out-of-frame protein lacking nearly 50% of the GTPase domain, both coiled-coil domains and the transmembrane domain. Depending on the amount of protein synthesized and its activity, this may therefore be a null mutation or have a dominant-negative or toxic gain-of-function effect with reduced penetrance (unless coexpressed with a second mutation). Interestingly, homozygosity for the Phe216Ser mutation caused early-onset mild CMT2 in another patient.[7]

We detected nonsense-mediated decay of the maternal transcript carrying the Glu308X mutation in family 2, who has a milder phenotype. As a result, the proband in family 2 expresses only her unaffected father's Arg519Pro allele. Despite only expressing a single *MFN2* allele the mother remains unaffected. This suggests that MFN2 is not sensitive to haploinsufficiency, confirming that the pathogenic dominant missense mutations described to date are likely to have a dominant-negative or a toxic gain-of-function effect and highlighting that nonsense mutations in *MFN2* should not necessarily be assumed to be pathogenic without considering the likelihood of nonsense-mediated decay and the presence of a mutation on the other allele. This is supported by experiments showing that dominant *MFN2* mutations do not affect the stability or targeting of the protein.[9] In addition, heterozygous *MFN2*-knockout mice do not develop peripheral neuropathy.[13] Thus the pathogenic mechanism in family 2 may be hemizygous expression

Table 2 Nerve conduction studies

	Family 1/ patient II-1	Family 2/ patient II-1	Family 3/ patient II-2[a]
Age, y[b]	7	17	39
Median nerve			
Motor DML	NR	4.0	Abs
Motor amp	NR	4.8	Abs
Motor CV	NR	53	Abs
Sensory amp	NR	11	Abs
Sensory CV	NR	57.5	Abs
Ulnar nerve			
Motor DML	3.4	3.2	Abs
Motor amp	0.25	7.4	Abs
Motor CV	58	NR	Abs
Sensory amp	NR	8	Abs
Sensory CV	NR	55	Abs
Peroneal nerve			
Motor DML	N	Abs	Abs
Motor amp	NR	Abs	Abs
Motor CV	NR	Abs	Abs
Radial nerve			
Sensory amp	5.8	NR	Abs
Sensory CV	47	NR	Abs
Radial nerve			
Sensory amp	NR	5	Abs
Sensory CV	NR	38.5	Abs

Abbreviations: Abs = absent; CV = conduction velocity (m/s); DML = distal motor latency; motor amp = motor amplitude (mV); NR = not recorded; sensory amp = sensory amplitude (μV).
[a] Axillary and musculocutaneous motor responses were recorded (axillary: amp = 1.9, DML = 4.2; musculocutaneous: amp = 1.5, DML = 4.7).
[b] Age when the test was performed.

Comment: The increasing complexity of phenotype–genotype correlations in CMT

*Mutations in the mitochondrial GTPase mitofusin 2 (*MFN2*) account for about 20% of patients with axonal forms of Charcot-Marie-Tooth neuropathy.[1] When considering diagnostic testing for these patients one typically looks for the pattern of autosomal dominant inheritance, as has been reported for* MFN2 *CMT families (CMT2A). However, we now see that* MFN2 *mutations causing disease can occur with an autosomal recessive pattern of inheritance,[2] adding* MFN2 *to the list of genes included in the category of CMT4. Although the true mechanism of disease pathogenesis is unknown, the absence of disease in the parents supports the hypothesis that either haploinsufficiency, or the presence of these specific mutations in the context of a normal* MFN2 *allele, is not enough to cause disease.*

In terms of the clinical approach to patients with inherited neuropathies, these 3 cases are particularly informative. Families 1 and 3 have 2 or more children with severe neuropathies, compelling the clinician to search for genetic causes for the disease. Given the recessive pattern of inheritance, the causative gene would not have been expected to be MFN2*. Family 2, however, presents with a single affected individual with relatively mild neuropathy. Again,* MFN2 *mutations may not have been suspected because of the lack of dominant inheritance, and spontaneous* MFN2 *mutations are frequently reported as severe early-onset disease.*

Charcot, Marie, and Tooth described the essential clinical features of inherited neuropathies in 1886. It took about 80 years to separate CMT types 1 and 2 based on physiology. But in just the past 20 years molecular genetics has advanced our knowledge of these diseases such that we are likely to see new treatments based on an understanding of disease mechanisms at the level of individual proteins.[3] Importantly, though, it will continue to be the careful investigation of individual patients and families that will drive the science forward.

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of a missense mutation that is not sufficient to cause disease when coexpressed with normal protein.

The inheritance patterns in families 1 and 2 appear recessive though one or more of the parents (age range 36–46) may yet develop symptoms.

In family 3, coinheritance of c.1085C>T; p.Thr362Met and c.113_115delAGA; p.Lys38del caused SEOAN. The mode of inheritance is unknown; neither parent was known to be affected. The disease severity might be caused by the presence of 2 abnormal MFN2 proteins which in the heterozygous state can be compensated by normal MFN2. Given that the c.1085C>T; p.Thr362Met mutation has been reported in autosomal dominant[3] and semidominant[5] pedigrees, it is likely to have a dominant-negative effect. The pathogenic effect of the c.113_115delAGA; p.Lys38del mutation is unknown at present.

We have presented 3 pedigrees supporting the hypothesis that coinheritance of 2 non- or minimally pathogenic *MFN2* mutations causes early-onset CMT2. Similar to earlier reports, the disease was very severe in 2 families, but moderate in one (family 2). The lack of rearrangements in 306 additional patients with CMT2 indicates that exonic rearrangements are rare in *MFN2* but should be considered, particularly in cases of early-onset neuropathy where one mutation has been identified by sequencing. Thorough genetic analysis is absolutely required for accurate prognosis and prediction of recurrence risk. Further work is required to understand the underlying molecular mechanisms of *MFN2* mutations in dominant and apparently recessive families; long-term follow-up of carrier parents will help to clarify the inheritance patterns involved.

AUTHOR CONTRIBUTIONS

Dr. Polke: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, study supervision. Dr. Laurá: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, study supervision. Dr. Pareyson: drafting/revising the manuscript, analysis or interpretation of data, acquisition of data. Dr. Taroni: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, contribution of vital reagents/tools/patients, acquisition of data, obtaining funding. M. Milani: analysis or interpretation of data, acquisition of data. G. Bergamin: analysis or interpretation of data, acquisition of data. Dr. Gibbons: analysis or interpretation of data, acquisition of data. Prof. Houlden: drafting/revising the manuscript, analysis or interpretation of data, acquisition of data. S.C. Chamley: analysis or interpretation of data, acquisition of data. Dr. Blake: drafting/revising the manuscript, analysis or interpretation of data, acquisition of data. Dr. DeVile: drafting/revising the manuscript, acquisition of data. Dr. Sandford: drafting/revising the manuscript, acquisition of data. M.G. Sweeney: drafting/revising the manuscript, analysis or interpretation of data, acquisition of data, study supervision. Dr. Davis: drafting/revising the manuscript, analysis or interpretation of data, study supervision. Prof. Reilly: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, study supervision.

DISCLOSURE

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