Genotype-Phenotype Correlations in Charcot-Marie-Tooth Disease Type 2 Caused by Mitofusin 2 Mutations

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Background: Mutations in the gene encoding mitofusin 2 (MFN2) cause Charcot-Marie-Tooth disease type 2 (CMT2), with heterogeneity concerning severity and associated clinical features.

Objective: To describe MFN2 mutations and associated phenotypes in patients with hereditary motor and sensory neuropathy (HMSN).

Design: Direct sequencing of the MFN2 gene and clinical investigations of patients with MFN2 mutations.

Setting: Molecular genetics laboratory of a university hospital and the Limoges National Referral Center for Rare Peripheral Neuropathies.

Patients: One hundred fifty index patients with HMSN and a median motor nerve conduction velocity of 25 m/s or greater and without mutations in the genes encoding connexin 32 and myelin protein zero.

Main Outcome Measures: Results of genetic analyses and phenotypic observations.

Results: Twenty different missense mutations were identified in 20 index patients. Mutation frequency was 19 of 107 (17.8%) in patients with CMT2 and 1 of 43 (2.3%) in patients with a median motor nerve conduction velocity less than 38 m/s. Four patients had proven de novo mutations, 8 families had autosomal dominant inheritance, and 3 had autosomal recessive inheritance. The remaining 5 patients were sporadic cases with heterozygous mutations. Phenotypes varied from mild forms to early-onset severe forms. Additional features were encountered in 8 patients (32%). Six patients underwent sural nerve biopsy: electronic microscopy showed prominent mitochondrial abnormalities on longitudinal sections.

Conclusions: MFN2 mutations are a frequent cause of CMT2, with variable severity and either dominant or recessive inheritance. MFN2 gene testing must be a first-line analysis in axonal HMSN irrespective of the mode of inheritance or the severity of the peripheral neuropathy.

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Charcot-Marie-Tooth disease (CMT) represents a subset of hereditary motor and sensory neuropathy (HMSN) and is characterized by the presence of an isolated polyneuropathy. The genetically determined peripheral neuropathy may be classified as demyelinating (CMT1 or HMSN I), with reduced motor nerve conduction velocities (MNCVs) (median MNCV, <38 m/s), or as axonal (CMT2 or HMSN II), with median MNCV of 38 m/s or greater. Interimmediate forms in which different family members have median MNCVs greater than and less than 38 m/s have subsequently been recognized. In each group, various modes of transmission are possible (autosomal dominant, autosomal recessive, or X-linked). Mutations in the gene encoding mitofusin 2 (MFN2), a mitochondrial GTPase protein involved in mitochondrial dynamics, seem to account for many cases of autosomal-dominant CMT2. In subsequent series of patients with CMT2, the frequency of MFN2 mutations was 10% to 20%. Some particular phenotypes have been associated with MFN2 mutations, such as early-onset HMSN of axonal type, HMSN V (axonal HMSN with pyramidal signs), and HMSN VI (severe peripheral neuropathy with optic atrophy). We describe a large series of patients with MFN2...
mutations diagnosed in the molecular genetics resource of the Limoges National Referral Center for Rare Peripheral Neuropathies.

METHODS

Between February 1, 2006, and October 31, 2008, 107 index patients with CMT2 and 43 patients with a median MNCV of 25 to 38 m/s (in the range of “intermediate” CMT) were screened for MFN2 mutations. None of the patients carried mutations in the genes encoding connexin 32 (GJB1) or myelin protein zero (MPZ). Written informed consent for genetic analyses had been obtained for all patients. DNA was extracted from blood leukocytes using standard techniques, and mutational analysis of MFN2 was performed by direct sequencing of all 19 exons and exon-intron boundaries, including the first 2 noncoding exons (primer sequences available on request). Both DNA strands were sequenced using BigDye Terminator Cycle Sequencing Kit v.1.1 (Applied Biosystems, Foster City, California). The products of sequencing reactions were separated using a genetic analyzer (model ABI3130xl; Applied Biosystems). The mutation search was then performed using Sequence Navigator version 1.0.1 (Applied Biosystems).

In 14 families, electron microscopy of the nerve biopsy showed a de novo origin. Two other patients (patients 11 and 12) carried compound heterozygous mutations of the MFN2 gene (previously reported as cases 1/CMT742 and 2/CMT231 by Nicholson et al[13]). In both cases, parents carried the relevant single heterozygous mutation and had normal electrophysiologic explorations without objective clinical signs of neuropathy, confirming a recessive pattern of transmission. Another family with 3 affected children displayed a typical recessive inheritance pattern: patients 6, 7, and 8 were siblings with a moderate form of CMT2. Their parents had no signs or symptoms of peripheral neuropathy and had normal electroneuromyographic findings. Each parent carried a single heterozygous mutation.

Age at onset of symptoms was before 10 years in 21 patients (84%). Eight patients (32%) experienced their first symptoms before age 5 years. Regarding disease severity status, 3 groups of patients were delineated: 3 patients (12%) had a CMT neuropathy score of 0 to 12, corresponding to a mild form of the disease; 12 (48%) had a score of 13 to 24 (moderate severity); and 10 (40%) were severely affected, with a score greater than 24. The mean ages of the severity groups were 39.3, 34.4, and 19.5 years, respectively. Seven of the 8 patients who experienced their first symptoms before age 5 years had severe neuropathy. Of the 4 patients with an age at onset older than 10 years, 2 had a moderate form of disease and 2 had a mild form.

Of all the patients had distal motor and sensory neuropathy that affected the 4 limbs, consistent with the diagnosis of CMT. However, some patients had other features: 3 of 25 patients (12%) had pyramidal signs, 3 (12%) had asymmetrical neuropathy, 3 (12%) had important vasomotor troubles, 2 (8%) had abnormal respiratory function (1 had clinical respiratory failure and 1 had reduced vital capacity: 66% of theoretical value), 2 (8%) had hearing loss, 1 (4%) had optic atrophy, and 1 (4%) had a distal intention tremor.

Median MNCVs were available for 21 patients. In patient 1, the absence of compound motor action potential did not allow MNCV measurement; in patient 8, electroneuromyography had not been performed (2 siblings with an identical phenotype, one of whom was the index case, had median MNCVs of 50 and 55 m/s); and in patients 18 and 24, median MNCV had not been recorded. All other patients had median MNCVs greater than 38 m/s except patient 12, whose MNCV was 34.8 m/s. Four patients underwent brain magnetic resonance imaging: patient 22 had normal findings, patient 21 showed diffuse brain atrophy at age 46 years, patient 19 had several subcortical white matter hyperintense signals on T2-weighted images but had a personal history of chronic hypertension, and patient 5 showed a tumor localized in the left temporal-insular region that was diagnosed as an epidermoid cyst.

Six patients underwent a sural nerve biopsy (patients 3, 4, 9-12). Histopathologic findings in patients 3, 4, 11, and 12 have already been reported.11,12 Patient 9 was affected by severe early-onset neuropathy, whereas patient 10 had a moderate form of CMT2. In both patients, electron microscopy of the nerve biopsy showed
ultrastructural abnormalities similar to those observed in previously reported early-onset cases, \(^{13}\) with a marked decrease in the density of myelinated fibers and abnormally aggregated on longitudinal sections of the sural nerve (Figure 2).

In the present series, MFN2 mutations were found in 19 of 107 index patients with CMT2 (17.8%). Only 1 of 43 patients (2.3%) with median MNCVs of 25 to 38 m/s (34.8...
Six patients in the present series underwent sural nerve biopsy, in all cases before MFN2 was identified as a major gene for CMT2. In all of them, prominent mitochondrial abnormalities were found in myelinated and unmyelinated axons. Peripheral nerve biopsy is an invasive investigation and should not be performed before MFN2 screening in patients with typical CMT2. However, in patients with atypical features who have undergone sural nerve biopsy, careful examination of longitudinal sections using electron microscopy should be systematic to identify abnormal mitochondria suggestive of a defect in mitochondrial dynamics. From our experience, we recommend screening of patients with such abnormalities for MFN2 mutations.

In line with previous study findings, the mutations identified in the present series are localized in or in close vicinity of the GTPase or heptad repeat (HR) domains of MFN2. In 6 of the 7 severe cases with dominant MFN2 mutations (patients 1-5 and 9), the mutation was located immediately upstream of or in the GTPase domain, and all of them involved highly conserved amino acids (Figure 1 and Table 2). Two severe cases harbored the p.R94W mutation, which has been previously reported to be a hot spot for MFN2 mutations. The p.R104W mutation (which had been found in 3 families before this study) was present in 3 unrelated patients in this series. This residue, therefore, seems to be a new hot spot for MFN2 mutations. Both p.R94W and p.R104W mutations result from a C→T transition in a CpG dinucleotide (the CGG codon translated to arginine being replaced by a TGG codon translated to tryptophan). CpG dinucleotides are known to be more prone to point mutations (approximately 10-fold over other di-nucleotides) because they frequently contain a methylcytosine that can be deaminated to thymine.

The only dominant mutation responsible for a severe neuropathy and not localized in close vicinity of the GTPase domain was p.R364P (patients 17 and 18). This mutation replaces the conserved arginine by a proline residue, which may severely affect protein stability. Another mutation replacing arginine 364 by a tryptophan residue (which is also predicted to be damaging for the secondary structure of MFN2) had previously been found in several patients with severe forms of CMT2. In the present series, we also identified another mutation at position 364 that results in substitution of glutamine for arginine beginning in childhood and harbored compound heterozygous mutations in several patients with severe forms of CMT2.

The remaining 2 severe cases (patients 11 and 12) were apparently sporadic and harbored compound heterozygous MFN2 mutations: in each case, one mutation was from http://www.molgen.ua.ac.be/cmtmutations. Red indicates dominant mutations identified in severe cases; blue, recessive mutations. GTPase indicates GTPase domain; HR1, heptad repeat 1 domain; HR2, heptad repeat 2 domain; and TM, transmembrane domain.

Figure 1. Localization of mitofusin 2 (MFN2) mutations. Previously reported mutations are from http://www.molgen.ua.ac.be/cmtmutations. Red indicates dominant mutations identified in severe cases; blue, recessive mutations. GTPase indicates GTPase domain; HR1, heptad repeat 1 domain; HR2, heptad repeat 2 domain; and TM, transmembrane domain.
homozygous p.R707W mutation. This HR2 mutation may, therefore, be associated with less severe forms of axonal CMT. In our family, both heterozygous parents had no signs or symptoms of peripheral neuropathy and normal electroneuromyographic findings. This is the first multiplex family reported to date, to our knowledge, with recessively inherited CMT2 due to MFN2 mutations.

It has been known for a long time that autosomal recessive disorders tend to occur earlier and have greater severity than autosomal dominant disorders. This has been illustrated by William Allan in 1939 for CMT, considered as a single clinical entity at that time. He observed that individuals with dominantly inherited CMT had a milder phenotype and a later onset than did those affected by a recessive form of CMT. In MFN2-related neuropathies, the situation seems less clear-cut: in the present series, 3 of 11 patients with dominantly inherited CMT2 had severe forms and 2 of 11 had onset before 5 years of age, whereas 3 siblings with recessively inherited CMT2 had a moderate phenotype. However, mild forms were encountered in patients with dominant inheritance only, and 3 of 11 patients with dominant forms had an age at onset older than 10 years (vs 0 of 5 with recessive forms, 0 of 4 with de novo forms, and 1 of 5 with sporadic forms).

In conclusion, these observations demonstrate that MFN2 mutations can be responsible for peripheral neuropathies of various severities, with dominant or recessive inheritance. This highlights the importance of screening all patients with axonal forms of CMT for MFN2

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Abbreviations: Ce, Caenorhabditis elegans (Fzo protein); Cf, dog; Dr, Danio rerio fish; Dm, Drosophila melanogaster (Marf protein); Gg, chicken; Mm, mouse; Rn, rat; +, present; −, absent.
mutations, irrespective of the apparent mode of inheritance or disease severity.

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REFERENCES