Sir, we recently showed in Brain that the MFN2 gene, typically responsible for autosomal dominant axonal Charcot–Marie–Tooth disease, is a novel gene associated with ‘mitochondrial DNA breakage’ syndrome (Rouzier et al., 2012). We described a novel MFN2 heterozygous missense mutation in Tunisian patients who presented with optic atrophy beginning in early childhood, axonal neuropathy and mitochondrial myopathy in adult life with accumulation of mitochondrial DNA deletions in muscle. Renaldo et al. (2012) confirm that MFN2 is involved in mitochondrial DNA maintenance disorders. Indeed, they show that this gene is responsible for a severe phenotype in a child who presented a developmental delay with hypotonia and failure to thrive at the age of 6 months. Evolution was marked by the gradual appearance of ataxia, areflexia, abnormal movements and axial sensorimotor neuropathy. At the age of 9 years, weight, height and head circumference were <3 SD. The child presented a bilateral optic atrophy and was deaf. Brain MRI was normal. A mitochondrial myopathy, with cytochrome c-oxidase negative fibres, was diagnosed on muscle biopsy and a multiple respiratory chain deficiency was found both in muscle and fibroblasts. Furthermore, Renaldo et al. (2012) found mitochondrial DNA depletion in the patient’s muscle with a low amount of mitochondrial DNA (28% of controls). The association of optic atrophy and axonal neuropathy led the authors to analyse the MFN2 gene and to identify a novel missense heterozygous mutation (p.D210Y) that affects the same aspartic acid residue modified in the Tunisian family that we described previously (p.D210V).

A defect in the maintenance of mitochondrial genome can lead to a reduction of mitochondrial DNA copy number and/or multiple mitochondrial DNA deletions. In mouse models, conditional inactivation of Mfn2 in muscle leads to a mitochondrial myopathy with a severe mitochondrial DNA depletion above the accumulation of point mutations and deletions (Chen et al., 2010). In patients, mutations in POLG1, encoding the catalytic subunit of the DNA polymerase γ, are found in a large spectrum of clinical presentations (Stumpf and Copeland, 2011). They are involved in Alpers syndrome, a devastating recessive disorder associated with mitochondrial DNA depletion in young children. They are also responsible for dominant and recessive forms of progressive external ophalmoplegia. Progressive external ophalmoplegia manifests during adulthood and is characterized by muscle weakness, exercise intolerance with accumulation of multiple mitochondrial DNA...
deletions in muscle. Other genes involved in mitochondrial DNA maintenance disorders, like PEO1 encoding the Twinkle helicase, TK2 or RRM2B, can lead either to severe mitochondrial depletion syndrome or late-onset progressive external ophthalmoplegia with mitochondrial DNA deletions. The patient described by Renaldo et al. (2012) is highly interesting because it confirms that the aspartic acid residue at position 210 is critical for the function of Mfn2, particularly in terms of mitochondrial DNA stability. Furthermore, it seems that the p.D210Y mutation leads to an early-onset multi-systemic disease with depletion while the p.D210V mutation is responsible for a less severe phenotype with mitochondrial DNA deletions in the Tunisian family that we described. Among these patients, one child who carries the p.D210V mutation presented with an early-onset severe phenotype. Nevertheless, the situation is clearly different from that of the patient reported by Renaldo et al. (2012). Firstly, clinical presentations are completely divergent and secondly, we found no reduction of mitochondrial DNA copy number in the muscle of our patient. This child was born at 36 weeks of gestation after an uncomplicated pregnancy with a birth weight of 2500 g (5th percentile), a length of 43 cm (3rd percentile) and a head circumference of 33 cm (25th percentile). Psychomotor development was initially normal but deteriorated at the age of 18 months with loss of walking and sitting posture. At 2 years of age, clinical examination showed lower limbs and axial hypertonia. After the initial regression, the child progressed slowly despite a global psychomotor delay. At 8 years of age, clinical examination showed lower limbs and axial hypotonia. EEG was normal. Brain MRI showed diffuse hyperintense signal of white matter with a moderate brain atrophy (not shown).

EMG did not exhibit signs of neuropathy and ophthalmological examination was normal, without optic atrophy. Muscle analysis showed a lipidic myopathy with a major decrease of succinate dehydrogenase activity (data not shown) that was confirmed by spectrophotometric measurements showing a complex II deficiency. The amount of mitochondrial DNA in muscle was 91% of age-matched controls. We excluded mutations in nuclear genes known to be involved in complex II deficiency and in mitochondrial DNA instability syndromes but it is possible that a variant in an unknown modifier gene could play a role in the severity of the phenotype presented by this child.

Crystal structure can help to explain phenotypes of disease-causing mutations. For example, the 3D structure of the human polymerase γ confirmed that the holoenzyme is a heterotrimer including two pol γ/C13/C0/C12 (accessory) subunits and one pol γ/C13/C0/C11 (catalytic) subunit encoded by POLG1. The most common POLG1 mutation (p.A467T) is located in the thumb domain that interacts with the pol γ/C13/C0/C12 subunit and substitution to threonine disrupts the hydrophobic area formed by nearby leucine residues thus explaining the decreased binding to pol γ/C13/C0/C12 subunit (for review, see Stumpf and Copeland, 2011). To try to understand the role of the mutations affecting the 210 residue, we modelled the 3D structure of the human mitofusin 2 (residues 121–306) by comparative protein modelling and energy minimization, using the Swiss-Model program in the automated mode (http://swissmodel.expasy.org/). The 3.10Å coordinate set for an EHD ATPase (pdb code: 2QPT) was used as the template (Daumke et al., 2007) and Swiss Pdb Viewer 3.7 (http://www.expasy.org/spdb) was used to analyse the structural insights into MFN2 mutations and to visualize the structure. The 210 aspartic acid residue is located at the beginning of an α-helix (Fig. 1A) and in an area
that corresponds to the EHD2 oligomerization surface. Concerning the corresponding oligomerization surface of Mfn2, one of the lateral chains of the 210 aspartic acid residue probably interacts with a basic residue via an ionic stabilizing bond. The p.D210V mutation will disrupt this electrostatic attraction, destabilizing locally the interface of interaction. The structural impact of the p.D210Y mutation will also alter the electrostatic properties of the interface of interaction, corresponding to a major destabilization. In this case, the disruption of the protein–protein interaction can be explained by the synergy of structural effects including the loss of an electrostatic salt bridge and the formation of a steric clash due to the introduction of an aromatic residue; these cumulated events lead to a more deleterious effect than that of the p.D210V mutation. Another mutation located in the GTPase domain near the 210 residue has been described in a patient with a recessive form of Charcot–Marie–Tooth type 2A (Calvo et al., 2009). At 34 years of age, he presented a distal motor and sensory neuropathy with hearing loss caused by compound heterozygous mutations in MFN2 (p.D214N and p.C390R). The parents, who carried the mutations in a heterozygous state, had normal electrophysiological explorations without objective clinical signs of neuropathy. These results show that the p.D214N variant is much less deleterious than mutations that affect the 210 residue. The 214 aspartic acid residue is located in the same α-helix as the 210 residue but in the middle of the secondary structure (Fig.1B). The destabilization due to the p.D210V or p.D210Y mutations can be likened to the opening of a zipper with a more drastic effect than that observed when the mutation occurs in the middle of the helix.

In conclusion, the observation reported by Renaldo et al. (2012) confirms that mutations in MFN2 are associated with a large clinical spectrum. This gene is responsible for mitochondrial DNA instability disorders with both mitochondrial depletion syndrome and less severe phenotype associated with mitochondrial DNA deletions in muscle. Further experiments will be necessary to explain the variability in disease severity that is observed. Nevertheless, our findings suggest that this discrepancy in terms of severity could at least, in part, be explained by the effects of some mutations on the oligomerization capacity of Mfn2.

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**References**


