LETTER TO THE EDITOR

MFN2, a new gene responsible for mitochondrial DNA depletion

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Sir, we read with great interest the paper by Rouzier et al. (2012) reporting a novel dominant MFN2 missense mutation (c.629A>T, p.D210V) in a large family displaying an optic atrophy ‘plus’ phenotype. Importantly, the authors identified MFN2 as a new gene implicated in mitochondrial DNA instability as they found multiple mitochondrial DNA deletions in skeletal muscle of two adult patients. Moreover, they have shown that mitochondrial fusion is necessary to repair stress-induced damage to mitochondrial DNA.

Here, we report a child with an early-onset progressive multisystemic disorder and carrying a novel MFN2 missense mutation responsible for mitochondrial DNA depletion in skeletal muscle. This girl was the second child of healthy unrelated parents, born after an uneventful pregnancy. Her birth weight was 2700 g [−2 standard deviations (SD)], her length 45.5 cm (−2 SD) and her head circumference 35 cm (mean). At the age of 6 months she was examined because of developmental delay with hypotonia, decreased rate of growth in head circumference (−2 SD), failure to thrive and severe gastro-oesophageal reflux. Brain MRI, EEG, brainstem auditory evoked potentials, metabolic and genetic investigations were all normal. At 3 years of age, the patient could only stand up when aided and had no language acquisition although her level of understanding seemed good as did her social interaction and behaviour. Drooling, abnormal ocular pursuit, ataxia, dysmetria, areflexia, weakness of limbs and near permanent abnormal movements appeared progressively thereafter. EMG disclosed an axonal sensorimotor neuropathy. At 5 years of age, brainstem auditory evoked potentials revealed hearing loss on the right side and the amplitude of visual evoked responses was decreased while the electroretinogram was normal. Complementary aetiological explorations were negative. Orthopaedic surgery on foot deformities was performed at the age of 6 years. She was referred to our neuropaediatric department at the age of 9 years. Failure to thrive persisted despite adequate caloric intake (weight −3.5 SD, length −4 SD) and the head circumference had reached −3.5 SD.
her speech was dysarthric and limited to a few words she smiled frequently and was able to use pictograms and signs to communicate. Severe muscle wasting and orthopaedic deformities were obvious and she had difficulties in walking with a walker and in using her hands. In addition, motor abilities were impaired by the occurrence of near permanent choreic movements (Supplementary Video 1). Ocular pursuit and orofacial movements were impaired and she had permanent facio-bucco-lingual dyskinesia with mild chewing difficulties and drooling. Fundoscopy revealed bilateral optic atrophy. CSF lactate concentration was increased (2.4 mmol/l). MRI was normal. Abnormal movements partially improved with trihexyphenidyl therapy. Orthopaedic surgery of hand deformities became necessary and at the same time muscle and skin biopsies were performed following parental consent.

The progressive multi-systemic disorder of this patient with increased lactate concentration in the CSF led to suspicions of a mitochondrial disorder. At first, as the parents were uncertain about muscle biopsy, we sequenced mitochondrial DNA in peripheral leukocytes, which was normal. Consequently and considering the association of an axonal sensorimotor neuropathy with optic atrophy, we looked for mutations in OPA1 and MFN2. The sequencing of OPA1 was normal while MFN2 analysis identified a novel missense heterozygous mutation in exon 7 (c.628G>T, p.D210Y). This mutation was absent in both parents and in 300 control chromosomes.

In order to further investigate the consequences of the MFN2 mutation in this child with an unusual phenotype, we performed both muscle and skin biopsies during her next surgical procedure. The biopsy from the quadriceps showed denervation and revealed that most fibres were cytochrome c-oxidase negative (Fig. 1). At the ultrastructural level, muscle displayed mild signs of dystrophy with increased collagen and lipid deposits in the extracellular matrix. Ultrastructural findings included an accumulation of mitochondria throughout interfibrillar and subsarcolemmal spaces and the occurrence of abnormal dystrophic cristae (Fig. 2). Global mitochondrial activity was low in fibroblasts and associated with poor cell respiration and complex I and III activities were 20 and 45%, respectively, below the lower range in muscle extracts (Table 1). Analysis of mitochondrial DNA from the muscle biopsy displayed no deletion but a significant reduction in the mitochondrial copy number (72% lower relative to the control mean) (Table 1).

Our patient presenting an early-onset progressive disorder including developmental delay/mental retardation, microcephaly, failure to thrive, followed by abnormal movements, axonal sensorimotor neuropathy, optic atrophy and hearing loss broadens the clinical spectrum of MFN2 mutations. MFN2 mutations are the most common cause of the axonal form of Charcot–Marie–Tooth disease (Züchner et al., 2004). Nevertheless, the related phenotypes can be ‘pure’ or ‘complex’ with tremor, optic atrophy, pyramidal signs, sensorineural hearing loss or white matter changes on brain MRI (Züchner et al., 2004; Zhu et al., 2005; Chung et al., 2006; Brockmann et al., 2008; Del Bo et al., 2008; Klein et al., 2011; Rouzier et al., 2012). While a few authors have reported cognitive impairment and ataxia previously (Zhu et al., 2005; Del Bo et al., 2008; Genaria et al., 2011; Rouzier et al., 2012) near permanent and disabling chronic choreic movements have, to our knowledge, never before been described, although one adult patient with CMT2A linked to MFN2 did suffer an acute fatal brainstem syndrome with chorea (Boaretto et al., 2010). Moreover, in contrast with the progressive macrocephaly described in two patients (Brockmann et al., 2008), microcephaly and failure to thrive are also new features for MFN2 mutations. The patients described by Rouzier et al. (2012) also displayed an unusual phenotype with an early childhood optic atrophy.
associated with axonal neuropathy and mitochondrial myopathy in adult life. While CNS involvement was described in four of their patients (learning difficulties, psychomotor regression and late-onset progressive CNS disorders), the phenotype does differ from that of our patient.

The two novel missense \textit{MFN2} mutations identified in this family and in our patient affected the same Asp residue, but led to a different amino acid substitution (p.D210V versus p.D210Y). Mutations of this residue, highly conserved and located in the GTPase domain of the protein, have never before been reported. Remarkably, both \textit{MFN2} mutations were associated with mitochondrial DNA instability. Rouzier \textit{et al}. (2012) found multiple mitochondrial DNA deletions but no depletion in skeletal muscle of two adult patients but neither deletion nor depletion in two affected children. By contrast, we found significant mitochondrial DNA depletion which could explain the unusual phenotype of our 9-year-old patient. Therefore, \textit{MFN2} mutations can be responsible for both deletion and depletion, as previously described for other nuclear genes and in mouse models of inactivated mitofusins (Chen \textit{et al}.,. 2010; Copeland, 2012). Among these mitochondrial DNA instability disorders, depletion is frequently associated with early-onset and severe phenotypes such as was observed in our patient.

In conclusion, our findings broaden the clinical spectrum of \textit{MFN2} mutations and confirm that the \textit{MFN2} gene can be

### Table 1

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<thead>
<tr>
<th></th>
<th>Muscle P/N</th>
<th>Fibroblasts P/N</th>
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<tbody>
<tr>
<td><strong>Polarographic analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyruvate oxidation</td>
<td>88/20–83</td>
<td>2.2/3.3–6.8</td>
</tr>
<tr>
<td>Succinate oxidation</td>
<td>115/33–141</td>
<td>3.2/6.5–14.3</td>
</tr>
<tr>
<td><strong>Enzyme activities</strong></td>
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<td></td>
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<tr>
<td>Complex IV</td>
<td>1640/1100–2800</td>
<td>37/72–143</td>
</tr>
<tr>
<td>Complex III</td>
<td>540/1000–2465</td>
<td>66/98–180</td>
</tr>
<tr>
<td>Complex II</td>
<td>260/105–270</td>
<td>7/10.8–17</td>
</tr>
<tr>
<td>Complex I</td>
<td>49/60–123</td>
<td>–</td>
</tr>
<tr>
<td>Complex IV (h)</td>
<td>485/175–385</td>
<td></td>
</tr>
<tr>
<td>Citrate synthase (h)</td>
<td>91/70–150</td>
<td>27/32–72</td>
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<tr>
<td><strong>Activity ratios</strong></td>
<td></td>
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<tr>
<td>Succinate/pyruvate</td>
<td>1.3/1.2–2.0</td>
<td>1.5/1.3–2.5</td>
</tr>
<tr>
<td>Complex IV (h)/Citrate</td>
<td>5.3/2.3–3.9</td>
<td>1.4/1.2–2.8</td>
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<tr>
<td>Synthase (h)</td>
<td></td>
<td></td>
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<tr>
<td>Complex IV/Complex III</td>
<td>3.0/0.8–1.6</td>
<td>0.6/0.6–1.4</td>
</tr>
<tr>
<td>Complex II/Complex I</td>
<td>5.4/1.5–2.3</td>
<td>–</td>
</tr>
<tr>
<td>MtDNA copy number</td>
<td>28/100</td>
<td>–</td>
</tr>
</tbody>
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Patient (P)/normal range values (N) were expressed as nmol of O$_2$/min/mg Prot(*) or as nmol of substrate/min/mg Prot(**). Abnormal values are in bold. (h) = homogenate; MtDNA = mitochondrial DNA.

![Figure 2](image.png)

**Figure 2**: Ultrastructural analysis of skeletal muscle (A, C, D: patient; B: control). Mitochondria appear to have accumulated in the subsarcolemmal space (A), in sarcoplasm and throughout myofibrils in which alignment is disrupted (A and C). Mitochondria display abnormal cristae and reduced size (100–200 nm versus 300–400 nm) (D).
responsible for mitochondrial DNA instability leading to multiple deletions but also to depletion. Further investigations are needed to evaluate the existence of a specific link between MFN2 mutations affecting the D210 residue and defects in mitochondrial DNA repair. MFN2 mutations should now be tested in patients with complex and unusual phenotypes involving the CNS and PNS, dominant or sporadic, and in patients carrying mitochondrial DNA depletion or multiple deletions.

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Supplementary material
Supplementary material is available at Brain online.

References


