Letter to the Editor

Functional characterization of the c.462delA mutation in the NDUFS4 subunit gene of mitochondrial complex I

To the Editor:

Isolated deficiency of complex I (CI) is the most commonly identified biochemical defect in childhood-onset mitochondrial diseases (1). Approximately 50% of CI-deficient individuals exhibit Leigh syndrome (LS) or Leigh-like syndrome, a devastating neurodegenerative disorder characterized by specific neuroradiological and neuropathological features. Although CI deficiency has been associated to distinct mutations in all its seven mitochondrial DNA (mtDNA) genes and in 12 of its 38 nuclear genes, the genetic cause of a large proportion of patients is still unknown.

We describe the identification and functional characterization of c.462delA mutation of the CI subunit gene NADH dehydrogenase (ubiquinone) Fe-S protein 4 (NDUFS4) in two siblings with LS.

Patient 1, the second child of a family with a highly possible history of consanguinity, displayed normal psychomotor developmental milestones until the age of 4–5 months when she began to show hyporeactivity, convergent strabismus and failure to thrive. Neurological examination revealed axial hypotonia with increased muscle tone at four limbs, absent head control, poor visual contact, dysphagia and rotatory nystagmus. Serum lactate levels, urinary organic acids, serum amino acids were normal. Brain magnetic resonance imaging (MRI) showed symmetric T2 hyperintensity involving the tegmentum of the brainstem bilaterally, along the course of the medial longitudinal fascicle. There was also bilateral hyperintensity of the substantia nigra and subthalamic nuclei.

The muscle biopsy revealed increased fiber size variability, few type II hypotrophic fibers, no ragged red or cytochrome c oxidase (COX)-deficient fibers and 49% decrease of CI activity when normalized to citrate synthase and to complexes II and III (I+III/CS/II+III) (Table 1). Respiratory support became progressively necessary and the patient died in 9 months.

Patient 2, the younger brother, born at term with normal birth parameters, presented rotatory nystagmus, poor visual contact, no head control, and axial hypotonia at 5 months. Brain MRI showed a similar picture as in Patient 1; magnetic resonance spectroscopy showed mild reduction of N-acetylaspartate and a pathological lactate peak (Fig. 1a). The muscle histology disclosed numerous fibers with peripheral mitochondrial proliferation at the COX, succinate dehydrogenase (SDH) stainings, and no COX deficient fibers. Red oil staining showed a mild neutral lipids overload.

In both patients, the analysis of mtDNA ruled out large-scale deletions and depletions. In contrast, a single nucleotide polymorphism (SNP) array-based homozygosity mapping study (Illumina, Infinium-HumanLinkage-12 BeadChip, Illumina, Inc., San Diego, CA), performed on the basis of suspected relatedness of the parents, identified three loci harboring stretches of consecutive homozygous markers on chromosome 5, 19 and 21 and including 257 genes (Fig. 1b). Prioritization using as baits genes associated to mitochondrial electron transport chain highlighted six genes involved in mitochondrial function (the CI subunits NDUFA7, NDUFS4 and NDUFA11, the pyridine nucleotide transhydrogenase NNT and the proteases LONP1, CLPP). Mutational screening of known disease genes led to the identification of a homozygous c.462delA mutation in NDUFS4 (NM_002495) generating a protein in which the last 22 amino acids of the native protein are replaced with 34 novel amino acids.

Table 1. Biochemical assay of respiratory chain in total muscle lysates from Patient 1a

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Patient 1</th>
<th>Reference values</th>
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<tbody>
<tr>
<td>NADH dehydrogenase (I)</td>
<td>25.1 ↓</td>
<td>33.5 ± 6</td>
</tr>
<tr>
<td>Succinate dehydrogenase (II)</td>
<td>0.83</td>
<td>0.625 ± 0.125</td>
</tr>
<tr>
<td>NADH cytochrome c reductase (I + III)</td>
<td>0.92</td>
<td>1.075 ± 0.425</td>
</tr>
<tr>
<td>Succinate cytochrome c reductase (II + III)</td>
<td>1.05</td>
<td>0.675 ± 0.225</td>
</tr>
<tr>
<td>Cytochrome c oxidase (IV)</td>
<td>3</td>
<td>2.125 ± 0.325</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>12.15</td>
<td>9.35 ± 1.55</td>
</tr>
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</table>

aActivities of mitochondrial enzymes were determined in a 10% muscle homogenate extracted from Patient 1’s muscle biopsy. Activities were measured by spectrophotometric assay and are expressed as micromoles/min/gm proteins. Reference values or activity of controls are expressed as mean of different determinations on 50 samples ± SE.
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Fig. 1. (a) Brain magnetic resonance imaging (MRI) of Patient 2. (A–F) Axial T2-weighted images show symmetric hyperintensity involving the tegmentum of the brainstem bilaterally, along the course of the medial longitudinal fascicle (arrowheads). There is also bilateral hyperintensity of the substantia nigra [thin arrows, (E)] and subthalamic nucleus [thick arrows, (F)]. (G) Axial diffusion-weighted image shows restricted diffusion in the substantia nigra (thick arrows) and midbrain tegmentum (arrowheads) bilaterally. Corresponding hypointensities were detected on the apparent diffusion coefficient (ADC) map (not shown). (H,I) Coronal T2-weighted images confirm involvement of the subthalamic nucleus [thick arrows, (H)] and medial longitudinal fascicle [thin arrows, (I)] bilaterally. (J) MR spectroscopy (single-voxel Point-RESolved-Spectroscopy (PRESS), echo time (TE) 144 ms). Upper right image shows placement of the sampling voxel on the right cerebral peduncle, including the substantia nigra. Corresponding spectrum shows presence of an inverted doublet at 1.3–1.4 ppm, consistent with lactate. (b) Graphical output of homozygosity mapping using the Illumina, Infinium HumanLinkage-12 BeadChip. Homozygous loci on chromosomes 5, 19 and 21 are indicated by red bars. (c) *NDUFS4* c.462delA mutation determines a reduction of the transcript levels by nonsense-mediated decay (NMD). (i) The analysis of the transcript levels of *NDUFS4* was performed by mix Sso Fast EvaGreen on RNA extracted from primary fibroblasts isolated from skin biopsy of both patients. Fibroblasts from two healthy subjects age-matched were used as controls. The relative levels of expression of *NDUFS4* were normalized with two house-keeping genes: beta-2-microglobulin and glyceraldehyde-3-phosphate dehydrogenase. White bars, patients’ RNA (Pt); black bars, controls’ RNA (Cn); *p* < 0.05 patients vs controls. (ii) Analysis of transcript levels of *NDUFS4* after treatment for 4 and 12 h with puromycin. White bars, patients’ untreated cells; grey bars, patients’ cells treated for 4 h; black bars, patients’ cells treated for 12 h; § *p* < 0.05 treated vs untreated cells. (d) Lack of complex I *NDUFS4* protein due to c.462delA mutation in pure mitochondrial fractions. Mitochondria isolated from primary fibroblasts of Pt1, Pt2 and two controls (Cn) were subjected to immunoblot analysis with a primary antibody to NDUFS4 (18 kDa). COX IV was utilized as a loading control (15 kDa).
The analysis of NDUFS4 transcript levels showed a 60% reduction in primary skin fibroblasts isolated from the two patients when compared with two healthy controls. The effect was inhibited by puromycin, a protein synthesis inhibitor blocking nonsense-mediated decay (Fig. 1c (i, ii)).

On a protein level, the c.462delA deletion led to a complete lack of NDUFS4 peptide in isolated mitochondria (Fig. 1d), suggesting an additional defective translation of the mutated transcript and/or proteolytic degradation of this long translated product.

NDUFS4 deficiency caused an inefficient CI assembly in our patients. NDUF4A6, a CI subunit known to be labile and secondarily decreased when the complex is inefficiently assembled, was undetectable in the patient’s muscle sections, while it displayed a uniform distribution in the controls (data not shown).

NDUFS4 is a nuclear encoded CI structural accessory subunit highly expressed in energy-demanding tissues and located in a strategic region of the complex. NDUFS4 has an essential role in the assembly of the functional CI, in its import/maturation in mitochondria and in the activation of the NADH–ubiquinone oxidoreductase activity of the complex (1, 2).

To date, 12 recessive mutations in NDUFS4 cause LS or Leigh-like disease (3); c.462delA was already described in the homozygous state in three affected siblings from an Ashkenazi Jewish family and in a compound heterozygous state (p.D119H/p.Lys154fs) in one child of Ashkenazi–Sephardic ancestry (4, 5). Our study indicates that this mutation has a more widespread occurrence and is not restricted to the Ashkenazi population.

Differently from the previously described NDUFS4 c.462delA cases, we observed a predominant brain stem presentation associated to subthalamic nuclei involvement on MRI. No basal ganglia abnormalities were detected. Bilateral involvement of the subthalamic nucleus and brainstem with mild or even absent basal ganglia abnormalities was considered suggestive of COX deficiency due to SURF1 gene mutations although a variable MRI presentation in LS patients with SURF1 mutation was subsequently reported. Awareness that peculiar MRI patterns could be related to specific gene mutations is important to properly address further diagnostic investigations.

In conclusion, although massive parallel sequencing of candidate genes or all genes (exome) is increasingly and successfully applied to identify the underlying gene defect in patients with mitochondrial disease, our study confirms that, in cases of consanguinity, homozygosity mapping, positional candidate gene analysis and complementary functional studies represent a rapid and effective approach in the diagnostic algorithm of these patients.

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References


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