A novel NDUFV1 gene mutation in complex I deficiency in consanguineous siblings with brainstem lesions and Leigh syndrome


Although deficiency of complex I of the mitochondrial respiratory chain is a frequent cause of encephalopathy in children, only a few mutations have been reported in each of its subunits. In the absence of families large enough for conclusive segregation analysis and of robust functional testing, it is difficult to unequivocally show the causality of the observed mutations and to delineate genotype–phenotype correlations, making additional observations necessary. We observed two consanguineous siblings with an early-onset encephalopathy, medulla, brainstem and mesencephalon lesions on brain magnetic resonance imaging and death before 8 months of age, caused by a complex I deficiency. We used a homozygosity mapping approach and identified a missense mutation in the NDUFV1 gene. The mutation, p.Arg386His, affects a highly conserved residue, contiguous to a cysteine residue known to coordinate an Fe ion. This observation adds to our understanding of complex I deficiency disease. It validates the important role of Arg386 and therefore supports the current molecular model of iron–sulfur clusters in NDUFV1.

Conflict of interest
None of the authors have any financial or personal relationships that might bias this work. There is no potential conflict of interest.

The mitochondrial respiratory chain, involved in oxidative phosphorylation, is located at the inner membrane of the mitochondria and is composed of five complexes. Some components of the complexes are encoded by mitochondrial DNA, but most are encoded by nuclear DNA (1). Respiratory chain deficiencies cause a wide range of clinical problems, often affecting the central nervous system. Among these, Leigh syndrome is an early onset and devastating neurodegenerative disorder characterized by specific neuroradiological and neuropathological features i.e. brainstem lesions as a constant feature and diencephalic involvement as a frequent feature (1, 2). Leigh syndrome most often begins before 12 months of age. Affected children progressively lose motor milestones, develop hypotonia and poor head control, and display pyramidal and extrapyramidal signs. Recurrent vomiting and swallowing difficulties cause failure to thrive. Nystagmus and ophthalmoplegia are often observed. Breathing disorders and acute respiratory failure are frequent. Leigh syndrome is genetically heterogeneous, with mutations identified in both nuclear DNA- and
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mitochondrial DNA-encoded components of the mitochondrial respiratory chain complexes.

Complex I deficiency accounts for 30% of respiratory chain deficiencies in humans, which affects at least 1/10,000 newborns (3–5). Complex I, the largest complex of the respiratory chain, is composed of 7 mitochondrial DNA (mtDNA)-encoded and at least 39 nuclear DNA-encoded subunits. Mutations in a dozen of the nuclear gene-encoded subunits have been reported in patients with encephalopathies, mostly Leigh syndrome or leukodystrophy (1). Most of these patients were sporadic cases however, with only a few cases described for each subunit. Functional assays are difficult for these subunits embedded in the inner mitochondrial membrane. Without large families with high logarithm of the odds (LOD) scores or robust functional assays, additional reports are still needed to document and validate the effects of complex I gene mutations in man.

Nine patients have been reported with complex I deficiency and mutations in NDUFV1, representing 14 mutations (5–8). Five were sporadic cases and only two families were reported each with two affected siblings. None had biallelic null mutations, which in other monogenic recessive diseases is usually considered a strong argument for causality. No functional tests were reported for the missense mutations, and no family was large enough for conclusive linkage.

Here we report two siblings with a very similar course of early-onset, lethal encephalopathy and complex I deficiency, in whom a genome-wide approach identified a novel mutation in NDUFV1.

 Patients and methods

Case report

Patient 1, a boy, was born at term, with normal birth parameters, to healthy first cousin parents of Moroccan origin, after an uneventful pregnancy. Recurrent vomiting, dysphagia and failure to thrive occurred at the age of 3.5 months. He had axial hypotonia, tetraparesis without muscle wasting, irritability, and a rotatory nystagmus. Episodes of hypoventilation of increasing intensity and rapid neurologic degradation occurred. The patient died at the age of 4.5 months. Magnetic resonance imaging (MRI) showed T2 hypersignal and T1 hyposignal in the posterior part of the medulla, the pons (full arrow) and in the mesencephalon (thin arrow) in patient 1. Patient 2 had mild T2 hypersignal in the pons and medulla (d, full arrow).

Fig. 1. T2 hypersignal (a, b) and T1 hyposignal (c) in the posterior part of the medulla, the pons (full arrow) and in the mesencephalon (thin arrow) in patient 1. Patient 2 had mild T2 hypersignal in the pons and medulla (d, full arrow).

was normal (except for increased sebacic acid), serum amino acids were normal except for a mildly elevated Lysine (2158 μmol/g creatinine, N < 1550). Respiratory chain spectrophotometry did not reveal any anomaly in the muscle cellular extract or in fibroblasts. In blood, mtDNA mutations m.3243A>G, m.8344A>G, and m.8993T>C/G were absent, as well as a deletion of mtDNA. Despite the absence of biochemical evidence for respiratory chain anomaly, complex I deficiency was suspected based on the clinical course and brain MRI. The NDUFA12L gene was considered a good candidate (9) and was studied by direct sequencing, but no mutation was found.

Patient 2, the sister of patient 1 was born at term, after an uneventful pregnancy, with normal birth parameters. A rotatory nystagmus and mild peripheral hypotonia were noticed at the age of 3.5 months. Lactate was mildly elevated in serum and CSF (5.2 mmol/l, N < 2 and 3 mmol/l, N < 2.8, respectively). Serum pyruvate was 0.137 mmol/l, and the lactate/pyruvate ratio was elevated: 22.3 (N < 10). Urinary organic acids and plasma amino acids screening were normal. MRI showed discrete symmetric T2 hypersignal and T1 hyposignal lesions in the pons and the medulla (Fig. 1). Feeding difficulties and respiratory insufficiency developed. Respiratory support became necessary at the age of 5.5 months. The neurologic status further deteriorated, and she died at the age of 7.5 months. An open muscle
biopsy performed on vastus lateralis disclosed normal Gomori trichrome staining and no cytochrome oxidase deficiency, but red oil red staining showed a mild neutral lipids overload. Electron microscopy showed moderate myofibrillar disorganization and atypical mitochondrial crests. Respiratory chain spectrophotometry and blue native page analysis revealed a diminished complex I activity in the muscle cellular extract (Fig. 2) but normal results in a liver cellular extract.

A recessive defect in a nuclear encoded gene of complex I was suspected because of the consanguinity of the parents, both asymptomatic, and the recurrence of the same severe clinical course in siblings.

Homozygosity mapping and direct sequencing of candidate genes

Both patients were genotyped using 250 K Gene Chip SNP arrays (Affymetrix™, Inc). Samples were processed, hybridized, and scanned following the instructions of the manufacturer. Homozygous stretches were delineated using the HOMOZYGOSITYMAPPER software (10).

Primers for polymerase chain reaction (PCR) amplification were designed by the web software exon primer (http://ihg2.helmholtz-muenchen.de/ihg/ExonPrimer.html). After PCR amplification, coding regions and exon–intron junctions of the candidate genes were sequenced by the Sanger’s method, using the Big Dye Terminator cycle sequencing kit v2 (Applied Biosystems, Foster City, CA), and analyzed by a 3130 Genetic Analyzer sequencing machine (Applied Biosystems). Sequences were inspected in silico for mutations by the SeqScape software V.2.0 (Applied Biosystems).

Results

Homozygosity mapping

Genotyping 250K single nucleotide polymorphisms (SNPs) identified four genomic segments of significant length (>2 Mb), which were homozygous and concordant in both siblings, encompassing 74.3 Mb and 1186 protein-encoding genes. Of those 1186, 4 genes encoded complex I subunits: NDUFB2, NDUFS3, NDUFS8 and NDUFV1.

Mutation identification

No mutations were found by Sanger sequencing in NDUFB2, NDUFS3, and NDUFS8. A missense...
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Fig. 3. NDUFV1 gene mutation analysis. (a) The c.G1156A mutation, homozygous in both affected siblings and heterozygous in both parents, replaces an arginine codon by a histidine codon. (b) Sequence alignment shows conservation of the mutated Arginine residue in different species (boxed) and position of the four cysteine residues, which are thought to coordinate the Fe ion (arrows).

Discussion

We investigated a family where two siblings presented a very similar, early-onset encephalopathy, which followed a lethal course before 8 months of age. Despite normal values of lactic acid in blood and CSF and a normal spectrophotometry of the respiratory chain in patient 1, Leigh syndrome was suspected based on the clinical course and brain lesions, which were suggestive of complex I deficiency (11). This suspicion was confirmed by biochemical investigations in patient 2.

The affected siblings presented a very similar clinical course with very similar MRI lesions, suggesting a genotype/phenotype correlation from a mutation that was highly penetrant for all features of the disease. The presentation in patients reported here is similar to the clinical course in patients described in the literature (Table 1). All patients with NDUFV1 mutations described so far had clinical signs by the age of 1 year, including hypotonia, progressive loss of motor milestones, and epilepsy in some. Brain MRI lesions consisted in leukodystrophy or, like in these two patients, Leigh syndrome (Table 1). Diencephalic involvement, particularly in the putamen, has been previously considered as the hallmark of Leigh syndrome (12, 13). More recently, neuropathologic and MRI studies have shown that brainstem abnormalities are constant and characteristic features of Leigh syndrome and a potential predictor of complex I deficiency, although not specific for the underlying genetic defect (11). The MRI lesions observed in patients 1 and 2 were of very limited size, restricted to the medulla, brainstem and mesencephalon, and could have been missed if not carefully looked for.

Because of its genetic heterogeneity, identifying mutations in Leigh syndrome is a difficult task. As complex I subunits are encoded by more than 40 different genes, mostly nuclear, we used a homozygosity mapping strategy and identified only one potentially disease-causing mutation, p.Arg386His, in the NDUFV1 gene. The mutation affects a highly conserved residue. Another mutation of the same codon, p.Arg386Cys, had already been reported in another family with complex I deficiency (7), giving a strong independent argument for causality. Arg386 is contiguous to Cys385, one of the four cysteine residues (with Cys 379, Cys 382 and Cys 425), which coordinate Fe atoms in the iron–sulfur cluster of NDUFV1, an essential structure in the electron transfer chain (14, 15). We hence speculate that the mutation hampers the stability of the iron–sulfur cluster and/or the electron transfer flow.

There is no evidence that this homozygous mutation would be a null mutation, abolishing gene function. Interestingly, as noted by Laugel et al. (16), no patient has been reported with a convincingly null mutation (e.g. a stop codon) of both alleles. This suggests that complete deficiency of NDUFV1 is not compatible with life, in spite of the fact that complex I and complex II of the respiratory chain provide parallel supplies of electrons to complex III and might hence bypass one another.

The first clue to complex I deficiency were the MRI brainstem lesions in patient 1, which, in spite of normal metabolic studies, prompted us to study the known complex I NDUFVA12L gene and mtDNA nucleotides (m.3243, m.8344,
<table>
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<tr>
<th>Patients in sibship</th>
<th>Consanguinity</th>
<th>Geographic/ethnic background</th>
<th>sex</th>
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<th>MRI</th>
<th>Metabolic findings</th>
<th>References</th>
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<tr>
<td>2</td>
<td>No</td>
<td>The Netherlands</td>
<td>M</td>
<td>mat: c.1268 C&gt;T, p.T423M</td>
<td>Cys425 (Fe/S)</td>
<td>Similar course from the age of 5 months. Progressive muscular hypotonia, myoclonic epilepsy, psychomotor regression. Died at 14 and 17 months, respectively.</td>
<td>Brain atrophy (CT scan).</td>
<td>Elevated lactate in blood and CSF. Complex I deficiency in muscle tissue and fibroblast culture.</td>
<td>Schuelke et al. (9)</td>
</tr>
<tr>
<td>1</td>
<td>No</td>
<td>The Netherlands</td>
<td>M</td>
<td>pat: c.175 C&gt;T, p.R59X</td>
<td>–</td>
<td>Progressive muscular hypotonia, myoclonic epilepsy from the age of 6 months.</td>
<td>Brain atrophy.</td>
<td>Macrocystic leukodystrophy.</td>
<td>Lactate normal in blood, elevated in CSF. Complex I deficiency in muscle tissue and fibroblast culture.</td>
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<tr>
<td>1</td>
<td>No</td>
<td>France</td>
<td>M</td>
<td>mat: c.1192 +4 A&gt;C (skipping of exon 8)</td>
<td>Loss of the last Fe/S</td>
<td>Seizures at 1 year, progressive psychomotor regression at 28 months. Died at 3 years.</td>
<td>Brain atrophy, multiple symmetric areas of hypersignal in the brainstem.</td>
<td>Elevated lactate in blood. Complex I deficiency in muscle tissue and liver extract.</td>
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<tr>
<td>1</td>
<td>No</td>
<td>France</td>
<td>F</td>
<td>mat: c.990delTG</td>
<td>Loss of the four Fe/S sites</td>
<td>Vomiting and progressive hypotonia from the age of 6 months. Died at the age of 18 months.</td>
<td>Hypersignal in the basal ganglia.</td>
<td>Elevated lactate in blood. Complex I deficiency in muscle tissue.</td>
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<tr>
<td>Patients in sibship</td>
<td>Consanguinity</td>
<td>Geographic/ethnic background</td>
<td>sex</td>
<td>Mutation</td>
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<td>Clinical data</td>
<td>MRI</td>
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<td>Yes</td>
<td>Unknown</td>
<td>F</td>
<td>mat: c.1155 C&gt;T, p.R386C</td>
<td>Cys385 (Fe/S)</td>
<td>Hypertonicity and irritability from the age of 7 months.</td>
<td>Diffuse hyperintense periventricular and subcortical white matter.</td>
<td>–</td>
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<td>pat: c.1155 C&gt;T, p.R386C</td>
<td>Cys385 (Fe/S)</td>
<td>Developmental regression from the age of 11 months.</td>
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<td>pat: c.632 T&gt;C, p.A211V</td>
<td>FMN</td>
<td>–</td>
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<td>2</td>
<td>Yes</td>
<td>Morrocco</td>
<td>M</td>
<td>mat: c.1156 G&gt;A, p.R386H</td>
<td>Cys385: (Fe/S)</td>
<td>Hypotonia, nystagmus, at 3 months, rapid neurologic degradation. Death before 8 months.</td>
<td>Discrete symmetric T2 hypersignal and T1 hyposignal lesions in the pons and the medulla.</td>
<td>Mildly elevated lactate, and low complex I activity in muscle extract in one sib only.</td>
<td>Present report</td>
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<td>F pat: c.1156 G&gt;A, p.R386H</td>
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CT, computed tomography; Fe/S, describe a cysteine residue known to coordinate a Fe ion; FMN, residues 199-247 FMN binding site; mat, maternal allele; pat, paternal allele.
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and m.8993) where no mutations were found. We started homozygosity mapping when patient 2 (patient 1’s sister) developed the same clinical signs and a biochemical deficiency of complex I was documented in her muscle extract, along with mild lactic acidemia. It appears from the literature that MRI brainstem lesions are not systematically observed in complex I deficiency patients, but some were not investigated by MRI, and in some, no details were mentioned about the brainstem. We conclude that the subtle brainstem lesions observed in our patients, consistent with previous observations, are a clue to complex I deficiency.

Acknowledgements

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References