Novel mutations in COX15 in a long surviving Leigh syndrome patient with cytochrome c oxidase deficiency

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Background: Isolated cytochrome c oxidase (COX) deficiency is usually associated with mutations in several factors involved in the biogenesis of COX.

Methods: We describe a patient with atypical, long surviving Leigh syndrome carrying two novel mutations in the COX15 gene, which encodes an enzyme involved in the biosynthesis of heme A.

Results: Only two COX15 mutated patients, one with severe neonatal cardiomyopathy, the other with rapidly fatal Leigh syndrome, have been described to date. In contrast, our patient had a slowly progressive course with no heart involvement. COX deficiency was mild in muscle and a normal amount of fully assembled COX was present in cultured fibroblasts.

Conclusions: The clinical and biochemical phenotypes in COX15 defects are more heterogeneous than in other conditions associated with COX deficiency, such as mutations in SURF1.

CASE REPORT

The proband is the third born of unrelated Italian parents; two older sisters are healthy. From the first days of life he displayed poor sucking, feeding difficulties, and failure to thrive. A severe psychomotor delay was noted at 4 months of age, with diffuse hypotonia and muscle wasting and weakness. Subsequently he developed brisk deep tendon reflexes, cerebellar tremor, and eye movement incoordination. A first MRI at 18 months of age showed symmetric signal changes in the posterior part of the putamina and bilateral cerebellar white matter abnormalities. Lactate and pyruvate were markedly elevated in plasma (lactate 3.6 mM, normal values (nv) 0.5–1.8; pyruvate 0.19 mM, nv 0.06–0.13) and in CSF (lactate 3.8 mM, nv 0.8–2.0; pyruvate 0.17 mM, nv 0.045–0.125). Clinical and MRI features were consistent with the diagnosis of Leigh syndrome. Symptoms slowly worsened thereafter with virtual arrest of body growth, progressive loss of postural control, and onset of dystonic postures in the upper limbs, while the cognitive functions remained relatively better preserved. A follow up MRI study at 4 years of age disclosed a mild progression of the cerebellar white matter and putaminal lesions, and the appearance of bilateral signal changes in the head of the caudate nuclei (fig 1). His clinical features remained grossly unchanged thereafter, although plasma lactate and pyruvate progressively decreased to normal levels. Apart from the central nervous system and skeletal muscle, the patient showed no abnormality in other tissues or organs, including the heart, gastrointestinal tract, liver, kidneys, and haemopoietic system. The boy is now 16 years old.

METHODS

Morphological and biochemical analyses

Morphological analysis of skeletal muscle and biochemical assays of the individual respiratory chain complexes on muscle homogenate and cultured skin fibroblasts were carried out as described. Enzymatic activity of each complex was normalised to that of citrate synthase.

Genetic analysis

Molecular analysis was performed on genomic DNA extracted from cultured fibroblasts. Overlapping fragments of the COX15 coding region were PCR amplified and products were assessed on agarose gel, purified, and sequenced according to Antonicka et al with slight modifications.

Western blot analysis of protein fraction resolved by 2D-BNE

For two dimensional Blue Native electrophoresis (2D-BNE), crude mitochondrial pellets, obtained as described, were resuspended in 100 μl of 1.5 M 6-aminohexanoic acid, 50 mM Bis-Tris, pH 7.0. Then 20 μl of 10% β-lauryl maltoside was added.

Abbreviations: 2D-BNE, two dimensional Blue Native electrophoresis; COX, cytochrome c oxidase; mtDNA, mitochondrial DNA.
RESULTS AND DISCUSSION

A muscle biopsy, performed at the age of 18 months, was morphologically normal. Biochemical assays (table 1) showed an isolated COX deficiency both in skeletal muscle and cultured fibroblasts (42% and 22% residual activity compared to the mean of controls, respectively). Accordingly, the cytochemical reaction to COX was moderately reduced in cultured fibroblasts as compared to normal control cells, but much stronger than that observed in fibroblasts from SURF1 mutant Leigh syndrome patients (fig 2).

Mutation analysis revealed a compound heterozygous state for two novel mutations in the COX15 gene. These mutations were absent in 40 alleles from ethnically matched controls, and are not listed in any SNP database. The first mutation, a 700C→T, R217W) in exon 5 on one allele and a (B) Axial T2 weighted image shows extensive cerebellar white matter involvement (arrows).

![Image](https://via.placeholder.com/150)

were added and the samples were incubated for 15 min on ice. Clearing of the samples was performed by centrifugation at 12 000 x g for 20 min at 4°C. The supernatant was removed and 10 μl of 5% Serva Blue G in 1 M 6-aminohexanoic acid was added prior to loading. 2D-BNE and electroblotting procedures were performed as described. The blots were immunostained using mouse monoclonal antibodies raised against bovine COX subunit I (Molecular Probes, Eugene, OR), as previously described.

![Table 1](https://www.jmg.bmj.com/)

![Figure 1](https://www.jmg.bmj.com/)

**Figure 1** Brain MRI. (A) Coronal proton density weighted image shows bilateral lesions in the putamina and in the head of caudate nuclei (arrows). (B) Axial T2 weighted image shows extensive cerebellar white matter involvement (arrows).
extreme reduction of COX activity in cardiac mitochondria. Similar to yeast COX15 mutants, the cytochrome spectrum showed low levels of heme A and accumulation of heme O.

Figure 2  Analysis of COX. Left panel: Immunoblot staining of 2D-BNE using an anti-COX I specific monoclonal antibody. The analysis was performed on cultured skin fibroblasts from a control, from our COX15 patient, and from a SURF1 patient. S1 and S2 are assembly intermediates of the COX holoenzyme, corresponding to S4. The arrows indicate the direction of the electrophoretic run in the first and second dimensions, respectively. Right panel: COX cytochemical staining of the cultured fibroblast lines used for the 2D-BNE immunoblot analysis.

However, wide tissue variability of the biochemical defect was observed also in the previous two COX15 mutant cases; therefore, a mild reduction of COX activity in muscle and fibroblasts does not exclude a more severe biochemical defect in other tissues, including the brain. An interesting possibility is that the S344P change has a hypomorphic, rather than null, effect, determining a partial reduction, rather than abolition, of the COX15 catalytic activity on heme A biosynthesis. This hypothesis could also explain the presence of a normal amount of fully assembled COX found in our patient, in contrast with the lower than normal amount found in the first COX15 mutant case, and also in other defects affecting the heme A biosynthesis, notably mutations in the COX10 gene.

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