



CASE STUDY

# Neonatal seizures and limb malformations associated with liver-specific complex IV respiratory chain deficiency

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## KEYWORDS

Mitochondrial; Complex IV; Limb anomaly; Malformation syndromes

**Summary** An eight-week-old infant, the fourth child of consanguineous parents presented with intractable neonatal seizures. The mother had two previous miscarriages. The infant initially presented on day one with multifocal myoclonus, complex partial and generalised tonic-clonic seizures. On examination, there were dysmorphic hands and feet, with absent nails and terminal phalanges of the fingers and toes, hepatomegaly, marked axial and peripheral hypotonia and severe global developmental delay. Ophthalmological assessment showed 'salt and pepper' pigmentary retinopathy. The urinary organic acid profile revealed a marked increase in tricarboxylic acid metabolites. Urinary phosphate reabsorption was reduced at 84%. Type I fibre atrophy was seen on muscle histology, and a cytochrome c oxidase deficiency was found only on enzymology of liver tissue.

Limb malformations associated with respiratory chain defects have rarely been reported. To our knowledge, this child has the most severe limb anomaly associated with a tissue-specific complex IV respiratory chain defect.

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## Introduction

Oxidative phosphorylation is the main energy supplier in almost all tissues and organs. Defects in

oxidative phosphorylation may result in multisystemic dysfunction, which may affect an individual from conception to fetal and postnatal development. Such mitochondriocytopathies display a wide range of clinical phenotypes. Chemical aetiology may be due to defects in any of five multienzymatic complexes: NADH-coenzyme Q (CoQ) reductase (complex I), succinate CoQ reductase (complex II),

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CoQ-H2 cytochrome c reductase (complex III), cytochrome c oxidase (cox, complex IV) and ATP-synthase (complex V). Genetically, the respiratory chain is uniquely encoded by both nuclear and mitochondrial DNA (with the exception of complex II).

Our patient represents an unusual case of complex IV deficiency associated with severe prenatal limb malformation syndrome. It further confirms the need to consider the role of genetic mitochondrial defects in prenatal developmental disorders.

### Case study

An eight-week-old infant presented, the fourth child of first cousin parents. The father had an older female sibling who had died on day 2 of life from an unexplained cause. The three older children, all female, were healthy. The mother had two previous miscarriages, both in the first trimester.

The pregnancy was uneventful. The birth, at 41 weeks gestation was a spontaneous vaginal delivery with birth weight at 3.4 kg (50th percentile) and head circumference at 36.5 cm (90th percentile). The Apgar scores were 8 at 1 min and 9 at 5 min.

The child initially presented on day one with neonatal seizures. There were multiple seizure types, including multifocal myoclonus, complex partial events and generalised tonic-clonic episodes. Treatment of seizures was of limited success. Despite multiple anti-epileptic agents, seizure control was never achieved, with almost daily episodes of status epilepticus.

Clinical examination revealed a well-grown male infant. Subtle facial dysmorphia was evident (Fig. 1). Dysmorphic hands and feet were noted, with absent nails and absent terminal phalanges of the fingers (Fig. 2) and toes. A skeletal survey showed complete absence of the distal phalanges of the hands and feet (Fig. 3). Abdominal examination revealed hepatomegaly 2 cm below the costal margin. Systemic examination, including cardiac assessment was otherwise normal. There was marked axial and peripheral hypotonia with global developmental delay. Deep tendon reflexes were normal. He was also noted to have global developmental delay. Ophthalmological assessment demonstrated fine 'salt and pepper' retinal pigmentation.

Urinary organic acid profile revealed a marked increase in tricarboxylic acid cycle metabolites with raised citrate,  $\alpha$ -ketoglutarate, fumarate and succinate. Additionally, urinary phosphate reabsorption was 84% (normal value  $>96\%$ ) suggestive of a proximal tubulopathy. The serum and



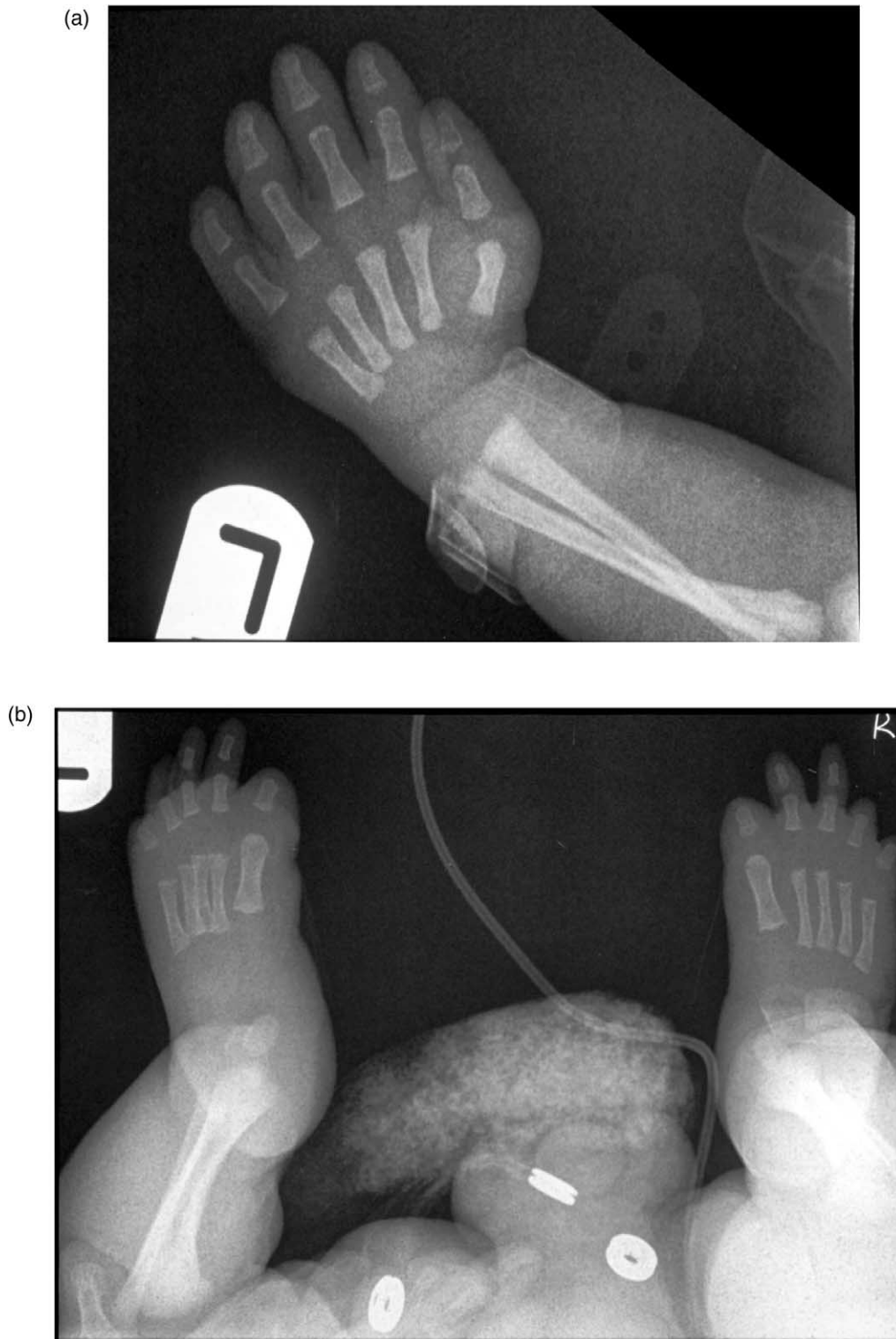
**Figure 1** Mild facial dysmorphia with a flattened nasal bridge, a flattened philtrum, low set ears and a short neck.

cerebrospinal fluid (CSF) lactate were normal, however, CSF analysis (performed when the infant was 8-weeks-old) showed a mildly raised protein level of 564 and 658 mg/l (normal range 0-450 mg/l) on two separate occasions.

Marked slowing of the background but no specific epileptiform changes were seen on the interictal electroencephalogram.



**Figure 2** Right hand showing absent nails and terminal phalanges.



**Figure 3** Images from the skeletal survey indicating absent terminal phalanges of limbs. (a) Anteroposterior (AP) view of left hand, (b) AP view of both feet.

Liver histology and electron microscopy was normal with no evidence of excess fat deposition. Respiratory chain enzyme analysis of liver tissue demonstrated complex IV deficiency (Table 1). All

measured enzyme activities were found to be somewhat low including the activity of the non-mitochondrial enzyme, lactic dehydrogenase. However, the complex IV activity was much more

**Table 1** Quantitative respiratory chain enzymology in liver tissue.

Enzyme assay	Activity (nmol/min/mg protein)	Control ( <i>n</i> = 20) (nmol/min/mg protein)
Complex I	12	15-28
Complex II	107	111-167
Complex III	190	289-453
Complex IV	55	125-231
Complex V	74	61-105
Citrate synthase (CS)	44	47-78
Fumarase	185	256-386
Lactate dehydrogenase	758	889-1473
	Ratio	Control ( <i>n</i> = 20)
Complex IV/I	4.8	8.6 ± 1.5
Complex IV/II	0.5	1.5 ± 0.2
Complex IV/III	0.3	0.6 ± 0.1
Complex IV/V	0.7	2.2 ± 0.4
LDH/complex IV	13.7	6.7 ± 1.6
Complex IV/CS	1.2	2.9 ± 0.4

reduced as compared to control values. This specific reduction was more easily detected by analysing enzyme activity ratios which all indicated a pronounced and specific complex IV deficiency of liver tissue.

Histological analysis of muscle tissue showed non-specific type I fibre atrophy. There were no diagnostic features on electron microscopy. Normal oxidative phosphorylation was seen on muscle enzymology studies. Cytochrome oxidase activity in cultured fibroblasts was also normal.

As both the clinical presentation of our patient and the enzyme deficiency were analogous to those reported in association with *SCO1* gene mutation, a molecular analysis of this latter gene was performed without revealing any disease-causing mutation.

An extensive neurometabolic screen was otherwise unremarkable. Serum lactate, a 24 h glucose-lactate profile, pyruvate, lactate/pyruvate ratio, liver function tests, urea, electrolytes, alkaline phosphatase, creatine kinase, ammonia, cholesterol, triglycerides, thyroid function tests, iso-electric focusing of transferrin, a peroxisomal screen, carnitine and acylcarnitine pattern were all normal. Urinary sulphite, amino acids and mucopolysaccharides screen were also normal. Cytogenetic analysis revealed a normal male karyotype. Magnetic resonance imaging of the brain was also normal.

Over the next few months, the child continued to have intractable epilepsy, with limited response to multiple anti-epileptic agents. His clinical course showed marked global developmental delay.

## Discussion

We report a rare case of a severe limb malformation syndrome (consisting of absent terminal phalanges and nails in the hand and feet) and neonatal seizures associated with a defect in complex IV of oxidative phosphorylation in the respiratory chain. Such malformations may represent antenatal disease expression in the skeletal and connective tissue system.

Developmental anomalies may be present in a wide range of metabolic diseases.<sup>1</sup> Developmental malformations such as a high forehead, flat philtrum, low set ears, short neck, hypoplastic nails, cryptorchidism and duodenal atresia have rarely been described in mitochondrial cytopathies.<sup>2</sup> Severe prenatal intrauterine growth retardation has also been described in a series of four patients.<sup>2</sup> Antenatal anomalies are also described in mitochondrial disorders. A series of 300 patients with mitochondriocytopathies were retrospectively reviewed and the incidence of fetal anomalies detected on antenatal scanning was found to be 6.7% (20 cases).<sup>3</sup> Defects included cardiac abnormalities, tracheoesophageal fistula, gastrointestinal malformations, anal atresia, renal agenesis/dysplasia and vertebral anomalies. Limb defects such as arthrogyrosis and VACTERL association were also reported.

The mechanism of mitochondrial disease-related malformation is unclear. Two pathogenetic processes have been proposed. Anomalies may be secondary to dysplasia, where there is abnormal morphological organisation of tissues. They may also be due to disruption, where the morphological defect of an organ results from the breakdown of an originally normal developmental process. It may be due to decreased ATP formation and an alteration of apoptotic events controlled by the mitochondria.

This association is rare but may be underdiagnosed as the combination of neonatal seizures and dysmorphism may be attributed to a primary malformation sequence with subtle brain dysgenesis, rather than a primary metabolic disorder. Muscle and liver biopsies are rarely contemplated in patients with refractory seizures without strong biochemical indicators of a metabolic disease process (for example, elevated blood or CSF lactate, abnormal intermediary metabolites or organic acid abnormalities). This patient had tricarboxylic aciduria in the urinary organic acids, impaired tubular phosphate reabsorption, a mildly raised CSF protein, but normal blood and CSF lactate. Unusually, there were no characteristic pathological changes in skeletal muscle.

The fibroblast cytochrome oxidase was also normal, as seen in other cases of COX deficiency.<sup>4,5</sup>

The specific defect seen in our patient was a complex IV (cytochrome c oxidase, COX) deficiency, found in liver but not in muscle or fibroblasts. Of note, muscle and liver samples used for analysis were snap frozen immediately after biopsy to ensure accurate respiratory chain enzyme analysis. It may be postulated that there is brain COX deficiency in the light of the severe neurological signs.

Cytochrome c oxidase is a multisubunit complex in the inner mitochondrial membrane and responsible for the terminal event in electron transport, in which molecular oxygen is reduced. COX catalyses both electron transfer from cytochrome c to molecular oxygen and the concomitant vectorial proton pumping across the inner mitochondrial membrane. COX consists of 13 polypeptide subunits (of which three are encoded by mitochondrial DNA). There is thus considerable heterogeneity in the clinical and biochemical presentation of complex IV deficiency.

For complex IV deficiency, mutations of mitochondrial encoded genes for COX subunits I-III or nuclear encoded genes for complex assembly proteins have been implicated to date. Among the assembly protein defects, mutations involving the *surf1* gene usually manifest as clinical Leigh's syndrome;<sup>6</sup> *SCO2* mutations present as fatal infantile cardioencephalomyopathy;<sup>7</sup> *SCO1* mutations as early onset hepatic failure and neurological dysfunction;<sup>8</sup> and mutations of *COX10* as tubulopathy and leukodystrophy.

Although no known mutations were identified in this case, the clinical picture is similar to that associated with the *SCO1* mutation. Of note, patients with *SCO1* mutations also show a consistently early onset of disease with no symptom free period, which may indicate antenatal expression of disease.

In conclusion, we have described a severe neonatal encephalopathy and skeletal malformation syndrome in association with complex IV

deficiency in liver tissue. To our knowledge, such severe limb malformations associated with a mitochondrial disorder have not been reported. Genetic defects of oxidative phosphorylation may be considered in the differential diagnosis of malformation syndromes, including minor facial, developmental and limb anomalies.

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