

# G8363A mitochondrial DNA mutation is not a rare cause of Leigh syndrome – clinical, biochemical and pathological study of an affected child

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## Abstract

*Leigh syndrome (LS), or subacute necrotizing encephalomyelopathy, having relatively homogeneous clinical symptomatology and pattern of neuropathological changes, shows remarkable heterogeneity in biochemical and molecular background. G8363A mitochondrial DNA mutation typical for MERRF syndrome and progressive cardiomyopathy may also be associated with LS. Clinical, biochemical and pathological findings in a boy aged 28 months who died with classical COX-deficient LS associated with mtG8363A is described in detail. Hyperlactataemia, LCHAD-like organic acids profile and respiratory alkalosis (pH 7.47, pCO<sub>2</sub> 4.9 mmHg, HCO<sub>3</sub> 3.0 mmol/l) were observed. Spectrophotometric assay showed deficit of respiratory chain complexes IV and I. Skeletal muscle biopsy revealed mosaic cytochrome oxidase deficit, lipid accumulation and ultrastructural abnormalities of mitochondria. Post mortem examination confirmed the presence of typical LS central nervous system lesions as well as hypertrophy of the left ventricle of the heart.*

**Conclusion:** *mtG8363A “MERRF-like” mutation should be included in the differential diagnosis of classical LS in infants. This case is in agreement with our hypothesis that hyperventilation plays a substantial role in progression of central nervous system damage.*

**Key words:** *LS, COX deficiency, mtDNA, G8363 tRNA lys mutation, MERRF-like mutation*

## Introduction

A morphological description of brain lesions in a 6-month-old infant who died in the course of unknown progressive disease published in 1951 by Le-

igh led to the recognition of a new syndrome – subacute necrotizing encephalomyelopathy [5]. For a long time the disease could be diagnosed only post mortem. Nowadays the development of techniques of brain imaging allows for diagnosis of Leigh syn-

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drome (LS) in a living patient. LS may result from various mitochondrial disorders, including respiratory chain complex defects (mitochondrial or nuclear origin), pyruvate dehydrogenase deficiency, and others. It was not found which of them caused the death of Leigh's case.

In spite of evidence of aetiological heterogeneity of LS its diagnosis is still in practical use.

The aim of the paper is to describe in detail the next aetiological factor leading to LS presentation – G8363A mutation in a mitochondrial DNA tRNA (Lys) gene (OMIM 590060).

This mutation found in a few patients with mitochondrial cardiomyopathy and/or MERRF phenotype [1,3,12] was recently reported in a number of patients with LS or Leigh-like phenotype. The aim of the paper is to describe an additional patient of ours with classical LS who died in 1997 in whom a G8363A mutation was identified in 2002 [11].

## Material

### Case report

First child of healthy non-consanguineous parents. Family history was negative. The boy was born in the 37th week of gestation with birth weight 2350 g, and Apgar score 10. Hyperbilirubinaemia was observed in the neonatal period (18.2 mg/dl on the fourth day). Infantile period was uneventful although some developmental delay was noted. At the age of 20 months his level of development was that of an 11-month old child (Burnet Lezine test LR=72). His weight was 11.1 kg (10 percentile).

At the age of 20 months he was admitted to the regional hospital due to loss of appetite, tachypnoea and worsening of general condition which developed during the treatment of rhinitis. Somnolence, loss of muscle tone and dehydration were observed. First acid base balance test revealed marked metabolic acidosis (pH 7.27, pCO<sub>2</sub> 9.8 mmol/l HCO<sub>3</sub> 4.4, pO<sub>2</sub> 107.1, O<sub>2</sub> saturation 96.7, with increased anion gap 23.4 mmol/l, normal value 9-16 mmol/l). Ammonia level was slightly increased (107 µmol/l), ketones were found in urine (+++) with pH 5-6, no abnormal sediment. No changes were found in the levels of creatinine, urea, electrolytes, aminotransferases, coagulation factors or creatine phosphokinase (CPK). Leukocyte count was 20 600 (P 4%, S 48%, Mo 3%, L 45%), mild normoplastic anaemia, anisocytosis, poikilocytosis.

Infusion of bicarbonates resulted in normalisation of blood pH (7.47) on the second day of hospitalisation. During the following month three sequential tests of acid base balance showed compensated respiratory alkalosis: pH 7.45-7.47, pCO<sub>2</sub> 17-21.9 mmol/l, BE 7.3-49 mmol/l, HCO<sub>3</sub> 12.1-15.4 mmol/l. Oxygen partial tension and saturation of capillary blood were increased (pO<sub>2</sub> 62.5-98.4, SatO<sub>2</sub> 92.7-97.9%).

With the suspicion of organic acidemia two urine samples were sent to the metabolic laboratory (CMHI). Due to persisting LCHAD-like organic acid profile with increased lactic acid and 2-ketoglutaric acid excretion the child was referred to our metabolic clinic with the suspicion of FAO defect.

On admission general condition was good. Examination revealed insufficient body mass, lowered muscle tone (the boy was not walking), thinning of the skin and increased perspiration. Emotional contact and comprehension were good but the boy was not talking. Internal organs were normal on examination. Periodic respiratory cycle disturbances were noted.

Laboratory tests showed lactic acidemia (40-82 mg/dl) and hyperalaninaemia (516 µmol/l; normal value 186-318 µmol/l). FAO defects were excluded on the basis of labelled palmitate oxidation test and disappearance of LCHAD profile, also in the test of prolonged fast. During the next hospitalisation the muscle biopsy was done and COX-deficient Leigh syndrome was eventually diagnosed.

During the following months the boy was twice admitted to the regional hospital due to worsening of his condition in the course of upper respiratory tract infections, with accompanying disturbances of respiratory rhythm. Acid base balance tests performed at that time showed pCO<sub>2</sub> decrease (the lowest value was 4.9 mmHg; pH 7.41; HCO<sub>3</sub> 3.0 mmol/l), and tendency for ketosis. Lactic acid levels were in the range 19-82 mg/dl. Increased level of copper was also noted (222 mg/dl), and discrete features of renal tubular damage (fractional phosphate resorption 80%), as well as tendency for hyponatraemia. Test with synacthen was normal (increase from 13.1 to 45.5 µg/dl after one hour).

Between attacks the general condition reversed to normal. Only fits of refractory cough were observed. An attempt at introduction of propranolol resulted in bronchial obturation, which was successfully treated with naloxone preparations. CT scan of the brain performed at the age of two years revealed the

presence of hypodense areas not enhanced by contrast and dilatation of ponto-cerebellar cisternae.

At the age of 28 months, during admission to the hospital for planned MRI examination the boy again developed infection with accompanying severe worsening of general condition, apnoea, cyanosis and flaccidity. He died within 24 hours. Resuscitation was not attempted as agreed with his parents due to diagnosis of Leigh syndrome and unfavourable prognosis.

## Methods

Surgical biopsy of the vastus lateralis muscle under local anaesthesia was performed and samples for the following procedures were obtained:

1. Spectrophotometric measurement of enzymatic activities of respiratory chain complexes was performed as described earlier [4].
2. Pathological examination. Histological and histochemical assessment of muscle included light microscopic examination of frozen sections in the following stains and histochemical reactions: haematoxylin and eosin (HE), modified Gomori trichrome, oil red O, succinate dehydrogenase, NADH dehydrogenase, cytochrome c oxidase (COX), acid phosphatase, myosin ATP-ase preincubated at pH 4.3, 4.6 and 9.4. Ultrastructural study of muscle samples was performed. Autopsy was done 24 hours after death.
3. Molecular analysis. Screening for common mtDNA and *SURF1* gene mutations was applied at the beginning. Sequencing of part (nucleotides 1777-4218, 4850-9847, 14753-15399, 16515-1173) of the patient's mitochondrial genome was performed by Sanger method and analysed on an AB1 Prism 377 DNA Sequencer.

## Results

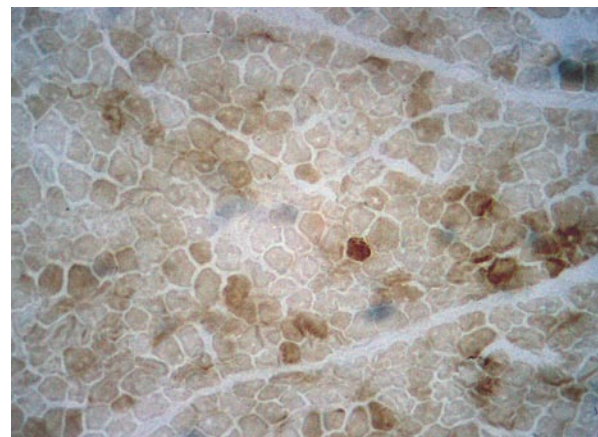
### Enzymatic data

Spectrophotometric examination of muscle revealed defect of respiratory chain function in the form of IV complex deficit (6.8% CS activity, normal value  $22.3 \pm 6.5\%$ ), and I complex deficit (at the border of sensitivity of the method) as well as increase of citrate synthase activity (212.1 normal value  $103.4 \pm 37.1$  nmol/min/mg protein).

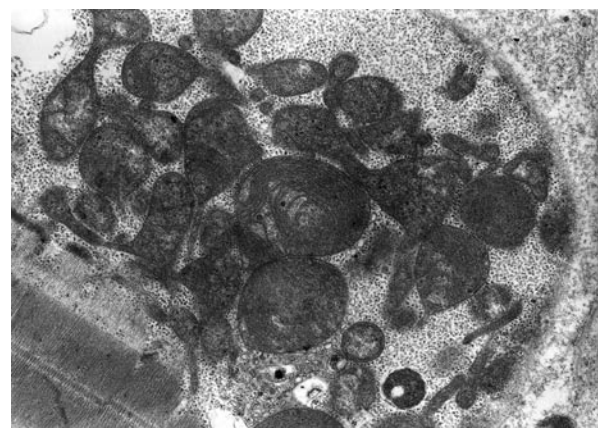
### Skeletal muscle pathology

Muscle histology and histochemistry revealed mosaic pattern of cytochrome oxidase deficiency (Fig. 1), with no evidence of ragged red fibres. Some fibres showed distinct COX positivity, other were totally negative. Other findings included moderate lipid accumulation in muscle fibres, and slight type I fibre predominance. Routine HE stain showed no detectable pathology.

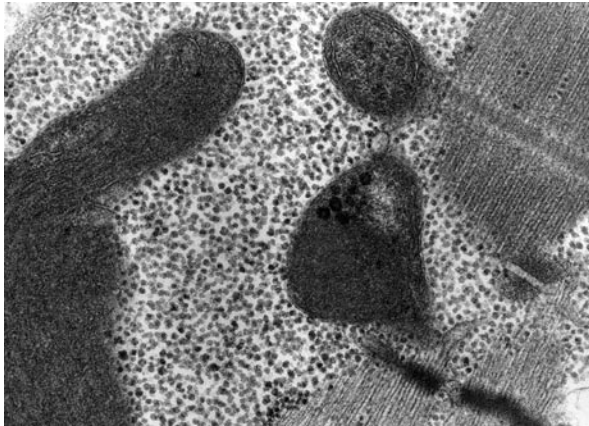
Ultrastructural examination of muscle disclosed collections of abnormally shaped mitochondria sometimes with concentric or irregular cristae and few electron dense inclusions (Figs. 2 and 3).



**Fig. 1.** Skeletal muscle. Cytochrome oxidase histochemical reaction shows mosaic deficit of an enzyme. Original magnification  $\times 200$



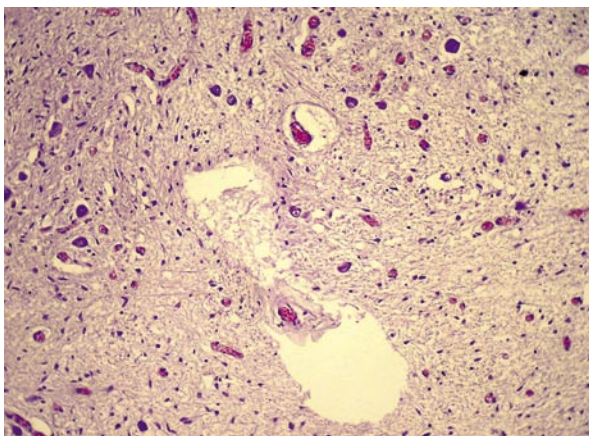
**Fig. 2.** Ultrastructure of skeletal muscle mitochondria showing altered shape and concentric or irregular cristae. Transmission electron microscopy  $\times 15\,000$



**Fig. 3.** Ultrastructure of skeletal muscle mitochondria showing concentric cristae and few electron dense inclusions. Transmission electron microscopy  $\times 20\ 000$

### Post mortem examination

Macroscopically the central nervous system showed symmetrical focal grey discolorations localized in the basal ganglia and pons, similar more distinctly outlined ones in the dorsal part of the medulla approximately 0.4 cm in diameter, and bilateral delicate discolorations in the vicinity of the dentate nuclei. Microscopically the above foci showed spongy degeneration, astroglial and capillary proliferation as well as neuronal damage. In several foci central microcystic areas had developed (Fig. 4).



**Fig. 4.** Microscopic view of post mortem brain stem lesions typical for LS. Vascular proliferation, spongy degeneration, neuronal damage and small pseudocyst formation. Original magnification  $\times 100$

Gross assessment of internal organs revealed hypertrophy of left ventricle of the heart.

### Molecular data

Search for *SURF* mutation and molecular screening towards common mitochondrial DNA mutation were negative. Due to histochemical mosaic pattern of COX deficit sequencing of part of the patient's mitochondrial genome was performed. This revealed a G3010A polymorphism and one mutation – G8363A. Sequencing chromatogram suggested very high level of heteroplasmy. The mother's DNA is not available for study.

### Discussion

Since 1996 clinical, biochemical and molecular characteristics of 10 probands carrying A8363G mtDNA mutation and 31 affected relatives have been described [3,5,7,8,12]. The majority of the patients are adults including four asymptomatic persons.

Cardiomyopathy was the cause of death of the first reported patient [12], was found (or suspected) in other patients [3,4] and is considered specific for this type of mtDNA mutation. Deafness, myoclonic epilepsy, muscle RRFs associated with mitochondrial tRNA-Lys mutation(s), and especially "horse collar" lipomas were relatively frequent [7,8,12]. All symptomatic patients showed signs of mitochondrial encephalopathy or neuropathy. A correlation between clinical severity and level of A8363G heteroplasmy was shown.[13] In adult patients studied muscle biopsy revealed common presence of RRFs and mosaic COX deficiency.

For several old cases the data of brain MRI, CT and/or autopsy were not reported.

A8363G mutation with LS was described for the first time in a 13-month-old girl who died at the age of 27 months [14]. LS was proven at autopsy of her maternal half-sister who died at the age of 2.5 years. There are at least three more LS or LS-like cases reported during the last few years. Increased MRI T2-weighted signal in the putamen and posterior medulla was found in an older sister of the proband with A8363G mutation presenting autistic behaviour in the second year of age [9]. Zeman's group included in their study [2] well documented LS in a 10-year old Czech boy with  $>95\%$  of A8363G heteroplasmy in all tissues studied. Bilateral basal ganglia hyperintensities of T2 signal and increased lactate were found

in MRS of the mother of a 9-year-old patient with A8363G mutation and diffuse leukodystrophy [8].

In our affected child with A8363G mtDNA mutation the onset and course of the disease and pattern of spectrophotometric muscle examination do not differ from those of classical autosomal recessive COX-deficient Leigh syndrome associated with *SURF1* gene mutation. In our practice we have observed over 30 such children [5,10,11]. The signs that prompted us to search for mtDNA mutation in this case were the absence of *SURF1* gene mutation and mosaic pattern of histochemical COX deficit. Autopsy examination confirmed the presence of neuropathological lesions typical for LS accompanied by hypertrophy of left cardiac ventricle. Skeletal muscle showed no ragged red fibres, which is unusual for typical MERRF mutation (A8344G tRNA Lys), although ultrastructural features of mitochondrial pathology were present as well as COX deficit and lipid accumulation.

The natural history of Leigh syndrome in our patient and association of outbreaks of symptoms with infection and respiratory alkalosis (hypocapnia) is evident. This is in agreement with our "hypocapnic" hypothesis of LS [10]. In a representative group of *SURF1*-deficient LS patients we have demonstrated evidence of compulsory hyperventilation evoked by infection or other stress [9,10]. Mitochondrial defect may explain inefficient ventilation effort in response to increased energy demand. We speculate that such an event contributes to LS brain damage [10] of specially vulnerable areas through decrease in cerebral pCO<sub>2</sub> pressure, hypervascularity, intracellular alkalosis and neuron apoptosis. We suppose that this cause-effect chain of events comprises a common mechanism of LS damage of CNS, especially in young children with various respiratory chain dysfunctions.

In this context we sustain our suggestion that all stresses increasing hyperventilation (i.e. fever, fear, anaesthesia) should be avoided in all patients suffering from any type of mitochondrial disease, including those with mitochondrial A8363G mutation.

While describing this patient we would like to remark that bicarbonates should not be administered in hyperlactataemic patients with low HCO<sub>3</sub> if low blood pH is not evidently confirmed (excluding respiratory alkalosis).

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