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## CYTOCHROME *c* OXIDASE DEFICIENCY PRESENTING AS RECURRENT NEONATAL MYOGLOBINURIA

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**Abstract**—Markedly reduced cytochrome *c* oxidase (COX) activity was found in cultured skin fibroblasts of an infant with recurrent episodes of acute myoglobinuria, hypertonia, muscle stiffness and elevated plasma levels of sarcoplasmic enzymes (creatine kinase 96950 U/l, normal below 150) since the age of 3 weeks (COX activity: 36 nmol/min/mg protein; normal 65–440; COX/succinate cytochrome *c* reductase ratio: 1.4, normal  $3.0 \pm 0.4$ ). The expression of the disease in cultured fibroblasts allowed us to carry out a prenatal diagnosis during the next pregnancy.

Hitherto, mitochondrial respiratory chain deficiency has not been established as a cause of recurrent myoglobinuria in childhood. Since most cases of myoglobinurias remain poorly understood, we suggest giving consideration to respiratory chain deficiency in elucidating the origin of unexplained recurrent myoglobinuria in childhood, especially when seemingly unrelated symptoms are present.

**Key words:** Mitochondria, cytochrome, *c* oxidase, myoglobinuria.

### INTRODUCTION

Myoglobinuria is defined by the urinary excretion of myoglobin as a result of acute destruction of skeletal muscle fibres. This condition, also called acute rhabdomyolysis, results in myalgia, muscle weakness, elevated sarcoplasmic enzyme levels in plasma and occasionally acute renal failure. Several genetic diseases have been recognized as possible causes of recurrent myoglobinuria, including inborn errors of glycolysis, glycogenolysis, fatty acid oxidation and muscular dystrophy [1, 2]. Hitherto, however, genetic defects of oxidative phosphorylation have not been established as a cause of recurrent myoglobinuria in childhood. This metabolic pathway leads to the oxidation of fuel molecules by oxygen and the concomitant energy transduction into ATP, via four multienzymatic complexes: NADH-coenzyme

Q reductase (complex I), succinate coenzyme Q reductase (complex II), coenzyme Q cytochrome *c* reductase (complex III) and cytochrome *c* oxidase (complex IV).

Here, we report on a severe cytochrome *c* oxidase deficiency in an infant presenting with recurrent episodes of myoglobinuria and on the pre- and postnatal findings in the subsequent pregnancy. Considering that most cases of recurrent myoglobinuria remain poorly understood, we suggest giving consideration to a genetic defect of mitochondrial energy supply in elucidating the origin of unexplained recurrent myoglobinuria in childhood.

### PATIENT

A girl (case 1) was born to second-cousin Tunisian parents after a full-term pregnancy and normal delivery (birth weight: 2700 g; length: 49 cm; head circumference: 35 cm). There were no miscarriages, neonatal deaths or other symptoms that might represent myoglo-

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binuria on the maternal or paternal part of the family. At three weeks of age, she refused milk and this was ascribed to a mild upper respiratory tract infection. Shortly thereafter, she became lethargic, had recurrent attacks of upper limb hypertonias and was admitted to an intensive care unit (Hôpital Saint-Vincent-de-Paul, Paris) after an episode of milk aspiration. She was comatose with generalized hyporeflexia and trunk hypotonia, but she experienced no anoxia or hypoglycemia and had appropriate reactions to stimulation. Marked stiffness of limb muscles with major pigmenturia was noted and this was ascribed to myoglobinuria both by immunological methods and electrophoresis. Serum creatine kinase (CK) and other sarcoplasmic enzyme levels were markedly elevated (CK: 96950 U/l, normal below 150; AST: 254 U/l, ALT: 1956 U/l, normal below 20; LDH: 9250 U/l, normal below 500). The liver was mildly enlarged with no metabolic acidosis or major evidence of hepatocellular dysfunction (pH 7.46; PCO<sub>2</sub>: 33 Torr; plasma bicarbonates: 21 mM; acetate: +; fibrinogen: 5.7 g/l; coagulation factor II: 50% V: 100%; VII + X: 45%; prothrombin time: 60%). The hypothesis of a genetic defect of mitochondrial energy supply was considered because of urinary elimination of the following Krebs cycle intermediates: citrate, fumarate, succinate (355 µmol/mmol creatinine, normal below 120), α-ketoglutarate (29 µmol/mmol creatinine, normal below 15). However, plasma lactate (1.8 mM, normal below 2), pyruvate (0.17 mM, normal below 0.19) and their molar ratios were normal (below 18, normal below 20). Free and total plasma carnitine were also normal (total carnitine 45 mM, normal above 28; free carnitine 32 mM, normal above 20). She gradually recovered normal consciousness with spontaneous ventilation and normal deep tendon reflexes. However, muscle stiffness persisted and plasma CK did not return to normal values.

At 3 months, she had a second attack of muscle stiffness with trunk hypotonia and hyporeflexia but consciousness remained normal and she could smile and follow adequately with the eyes. At this age, a painful enlargement of the right calf was observed and this was ascribed to hemorrhagic muscular necrosis, especially as platelets and leukocytes were consumed (blood platelet count; 27,000/mm<sup>3</sup>; blood leukocytes: 3000/mm<sup>3</sup>; poly-

morphonuclears: 300/mm<sup>3</sup>; hemoglobin: 12.79%; reticulocyte count: 140,000/mm<sup>3</sup>; direct Coombs test and circulating antinuclear antibodies: negative). She recovered thereafter and did relatively well during the next few weeks.

At 6 months, she suddenly developed acute fever with hematuria (105 erythrocytes/mm<sup>3</sup>), proteinuria (1.2 g/l) and hemorrhagic diarrhoea with severe anemia and thrombopenia (hemoglobin: 8.7 g/l; platelet count: 39,000/mm<sup>3</sup>; leukocyte count: 6600/mm<sup>3</sup>; presence of schizocytes). The liver was moderately enlarged but no evidence of hepatocellular dysfunction was noted. The association of severe anemia with thrombopenia, schizocytosis and renal failure was suggestive of hemolytic uremic syndrome. The patient died after sudden gastrointestinal bleeding and the parents refused autopsy.

## METHODS

Plasma lactate and lactate/pyruvate and ketone body molar ratios were determined in the proband, and postnatally in the subsequent sibling and in controls to establish oxidation-reduction status in cytoplasm and mitochondria, respectively. Urinary organic acids were studied by gas chromatography-mass spectrometry.

Lymphocytes were isolated from 10 ml venous blood on Ficoll cushion as described [3] and fibroblasts were grown in RPMI 1640 (Gibco) supplemented with 10% fetal calf serum, 2 mM glutamine, 2.5 mM pyruvate, 200 µM uridine, 100 µg streptomycin and 100 U/ml penicillin at 37°C under standard conditions [4]. Oxygen consumption was measured with a Clark-type oxygen electrode (Hansatech, Norfolk, UK) in a 250-µl cell thermostated at 37°C, containing 0.3 M mannitol, 5 mM MgCl<sub>2</sub>, 10 mM KCl, 10 mM NaH<sub>2</sub>PO<sub>4</sub> (pH 7.4), 1 mg BSA ml<sup>-1</sup>. Succinate oxidation was triggered by the addition of 0.05% (w/v) digitonin, 10 mM succinate, 0.1 mM ATP and 3 µM rotenone. Subsequently, duroquinol (0.6 mM) oxidation was measured in the presence of 5 mM malonate and 2 mM EDTA. Cytochrome *c* oxidase and succinate cytochrome *c* reductase were measured spectrophotometrically by following either the oxidation or the reduction of cytochrome *c* (at 550 nm minus 570 nm) as previously described [3].

For prenatal diagnosis of the next pregnancy,

enzyme activities were measured in a confluent culture of amniocytes ( $1.5 \times 10^6$  cells). Results were expressed not only as absolute values but also as ratios, since a constant ratio of respiratory enzyme activities is a consistent feature, regardless of the tissue tested [5].

For Southern blotting, total DNA derived either from lymphocytes or from cultured fibroblasts was digested, separated by agarose gel (0.7%) and transferred on to nylon filters (Hybond N<sup>+</sup>, Amersham). The filters were hybridized with [<sup>32</sup>P] dCTP-labelled mtDNA probes [3].

## RESULTS

Table 1 shows that a markedly reduced cytochrome *c* oxidase activity and a decreased cytochrome *c* oxidase/succinate cytochrome *c* reductase ratio were found in cultured fibroblasts of case 1. Accordingly, low levels of oxygen consumption by both intact fibroblasts and permeabilized fibroblasts were observed (Table 1). Similar investigations in circulating lymphocytes showed normal results (Table 1). The  $\beta$ -oxidation of <sup>14</sup>C-long chain, medium chain and short chain fatty acids in circulating

lymphocytes of the patient was normal and Southern blot analysis of fibroblast and lymphocyte DNA did not reveal major rearrangements of the mtDNA (not shown). The screening for mtDNA point mutations is currently in process. Based both on the expression of the enzyme deficiency and the controlled stability of respiratory chain activities in cultured skin fibroblasts [6, 7], a prenatal diagnosis was proposed for the next pregnancy. Enzyme activities were determined on chorion villi at 9 weeks and on cultured amniocytes at 19 weeks. Normal results were found in both cell types (Table 1, Individual 2). This result was subsequently confirmed by determination of respiratory enzyme activities in cultured skin fibroblasts of the neonate (Table 1). The child, a girl, is presently 9 months old and healthy.

## DISCUSSION

The present study reports on recurrent episodes of acute myoglobinuria and terminal hemolytic uremic syndrome in an infant with repeated attacks of hypertonia, muscle stiffness and elevated plasma levels of sarcoplasmic enzymes from the age of 3 weeks. Only at

Table 1. Investigation of oxidative phosphorylation in the proband (1) and during the next pregnancy (2)

	1	2	Controls
<b>Fibroblasts</b>			(n = 20)
Enzyme activities (nmol cyt <i>c</i> min <sup>-1</sup> mg <sup>-1</sup> protein)			
Cytochrome <i>c</i> oxidase (COX)	36	140	65-439
Succinate cytochrome <i>c</i> reductase (SCCR)	25	42	22-166
COX/SCCR ratio	1.4	3.3	3.0 ± 0.4
O <sub>2</sub> uptake by intact fibroblasts (nmol O <sub>2</sub> min <sup>-1</sup> mg <sup>-1</sup> protein)	7	7.4	5-19
Succinate oxidation by digitonin-permeabilized cells (nmol O <sub>2</sub> min <sup>-1</sup> mg <sup>-1</sup> protein)	8.5	12.5	9-49
<b>Lymphocytes</b>			(n=16)
Enzyme activities (nmol cyt <i>c</i> min <sup>-1</sup> mg <sup>-1</sup> protein)			
Cytochrome <i>c</i> oxidase	147	—	51-240
Succinate cytochrome <i>c</i> reductase	42	—	16-98
COX/SCCR ratio	3.5	—	3.1 ± 0.4
O <sub>2</sub> uptake by intact lymphocytes (nmol O <sub>2</sub> min <sup>-1</sup> mg <sup>-1</sup> protein)	5	—	3-6
Succinate oxidation by digitonin-permeabilized cells (nmol O <sub>2</sub> min <sup>-1</sup> mg <sup>-1</sup> protein)	13	—	5-15
<b>Chorionic villi</b>			(n = 4)
Enzyme activities (nmol cyt <i>c</i> min <sup>-1</sup> mg <sup>-1</sup> protein)			
Cytochrome <i>c</i> oxidase	—	79	36-193
Succinate cytochrome <i>c</i> reductase	—	22	14-68
COX/SCCR ratio	—	3.6	2.9 ± 0.3
<b>Cultured amniocytes</b>			(n = 6)
Enzyme activities (nmol cyt <i>c</i> min <sup>-1</sup> mg <sup>-1</sup> protein)			
Cytochrome <i>c</i> oxidase	—	79	20-80
Succinate cytochrome <i>c</i> reductase	—	21.5	12-33
COX/SCCR ratio	—	3.6	3.1 ± 0.3

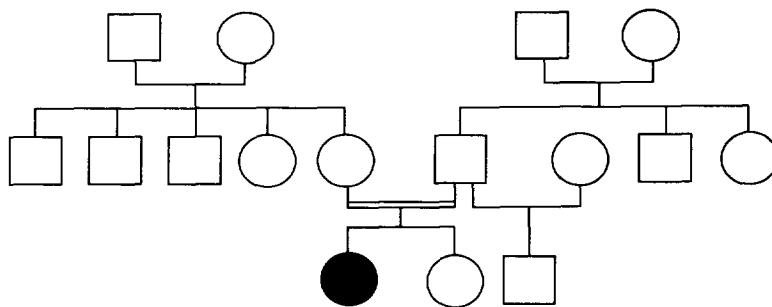


Fig. 1. Family pedigree. The blackened symbol indicates the clinically affected individual.

the point when urinary elimination of Krebs cycle intermediates was observed, could the hypothesis of a genetic defect of energy metabolism be considered and eventually confirmed by the discovery of a markedly reduced complex IV activity in cultured skin fibroblasts of the proband. Indeed, no elevation of plasma lactate and lactate/pyruvate molar ratios was noted during attacks of myoglobinuria while these are frequent and reliable clues for the diagnosis of respiratory enzyme deficiency [8, 9]. Why no elevation of plasma lactate and/or lactate/pyruvate molar ratios was observed is questionable and could be accounted for by either partial COX deficiency or the uneven distribution of the enzyme deficiency in the different organs. The prevailing skeletal muscle involvement and the apparently absent involvement of the central nervous system are suggestive of a muscle-specific respiratory enzyme deficiency. Unfortunately, a needle biopsy of the muscle or the liver was not practical prior to the death of the child. However, expression of the trait in cultured cells allowed us to complete the characterization of the enzyme deficiency and provide what we believe to be one of the first evidences of respiratory enzyme deficiency in childhood myoglobinuria. It is worth noting, however, that the reduced cytochrome *c* oxidase (COX) activity in cultured fibroblasts did not alter the initial cell respiration by intact fibroblasts as COX activity was not rate-limiting under our experimental conditions.

Recurrent myoglobinuria has been reported in two sisters with coenzyme Q10 deficiency [10] and multiple mitochondrial DNA deletions have been reported in two adult siblings with recurrent exertional myoglobinuria and alcohol intolerance associated with distinct morphological anomalies of muscle mitochondria [11]. Apart from these two observations, nothing is known regarding the possible involvement of

oxidative phosphorylation in recurrent myoglobinuria. The other genetic diseases reported to cause recurrent myoglobinuria are muscular dystrophy [12] and inborn errors of muscle glycolysis (phosphofructokinase, phosphoglycerate kinase, phosphoglycerate mutase, lactate dehydrogenase) [1, 2], muscle glycogenolysis (glycogen phosphorylase) [1, 2] and fatty acid oxidation (carnitine palmityl transferase and long chain acyl CoA dehydrogenase) [13–15]. Why myoglobinuria is never observed in children with generalized cytochrome oxidase deficiency is questionable, especially as it is often relatively severe in muscle. One possible answer may be that the severe brain involvement and the subsequent hypotonia in affected children may 'protect' them from exercise-related myoglobinuria, in the same way that the severe weakness of children with Duchenne muscular dystrophy may 'protect' them from exercise-related myoglobinuria which is much more common in Becker dystrophy.

Since most cases of recurrent myoglobinuria remain poorly understood, the present study prompts us to suggest that consideration should be given to the possibility of genetic defects of mitochondrial energy supply in cases of unexplained recurrent myoglobinuria in childhood, especially when seemingly unrelated symptoms are present and even when the plasma oxidation–reduction status is normal.

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*Note added in proof*—A young woman with recurrent myoglobinuria and a small deletion in subunit III of cytochrome *c* oxidase was recently reported at the meeting of the American Academy of Neurology by Dr Nancy Kennaway's group (Neurology 1994; 44: A335).

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