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ORIGINAL INVESTIGATION

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A deletion in the human QP-C gene causes a complex III deficiency resulting in hypoglycaemia and lactic acidosis

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Abstract Mitochondrial respiratory chain complex III (ubiquinol-cytochrome *c* reductase) consists of 11 subunits, only one (cytochrome *b*) being encoded by the mitochondrial DNA. Disorders of complex III are comparatively rare but are nevertheless present as a clinically heterogeneous group of diseases. To date, no mutation in any of the nuclear-encoded subunits has been described. We report here a deletion in the nuclear gene UQCRB encoding the human ubiquinone-binding protein of complex III (QP-C subunit or subunit VII) in a consanguineous family with an isolated complex III defect. In the proband, a homozygous 4-bp deletion was identified at nucleotides 338–341 of the cDNA predicting both a change in the last seven amino acids and an addition of a stretch of 14 amino acids at the C-terminal end of the protein. Both parents were found to be heterozygous for the deletion, which was absent from 55 controls. Low temperature (–196°C) spectral studies performed on isolated mitochondria from cultured skin fibroblast of the proband showed a decreased cytochrome *b* content suggestive of a role for the QP-C subunit in the assembly or maintenance of complex III structure.

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Introduction

Mitochondrial oxidative phosphorylation defects are a genetically heterogeneous group of diseases with a wide spectrum of clinical manifestations. Organ and tissue involvement is remarkably variable, the defect often being expressed in the neuromuscular system (DiMauro and Schon 2001; Wallace 2000; Munnich and Rustin 2001; Leonard and Schapira 2000). Respiratory chain complex III (CIII; ubiquinol-cytochrome *c* reductase; EC 1.10.2.2) deficiency is a comparatively rare condition in humans (Mourmans et al. 1997; Von Kleist-Retzow et al. 1998). CIII channels electrons from the ubiquinone pool to cytochrome *c*, simultaneously extruding protons from the mitochondrial matrix space to the inter-membrane space. CIII contains four redox centers, i.e. two haem groups (b_h and b_l of the *b*-type cytochromes), cytochrome c_1 and the iron-sulphur cluster of the Rieske protein (Schägger et al. 1995).

In mammals, CIII is made up of 11 subunits, all of which are encoded in the nucleus with the exception of the mitochondrially encoded cytochrome *b*. Because of its dual genetic origin, CIII deficiency can be subject to either an autosomal or a maternal mode of inheritance. To date, about 20 different mutations have been identified in cytochrome *b* (Marin Garcia et al. 1995, 1996; Dumoulin et al. 1996; Bouzidi et al. 1996; Andreu et al. 1999a, 1999b, 2000; De Coo et al. 1999; Valnot et al. 1999; Mitomap 2002), mostly in patients with skeletal muscle weakness and exercise intolerance. Myoglobinuria has been less frequently observed (Andreu et al. 1999a). Recently, mutations have been reported in the nuclear BCS1 gene encoding an essential factor for CIII assembly in a series of patients with early liver failure associated with tubulopathy and/or encephalopathy (de Lonlay et al. 2001) or in patients with GRACILE syndrome (Visapaa et al. 2002).

In an attempt to identify additional nuclear genes responsible for CIII deficiency in humans, we have performed direct sequencing of the genes encoding the eleven structural subunits of CIII and the BCS1 assembling gene in a patient with isolated CIII deficiency.

Materials and methods

Nomenclature

Gene mutation nomenclature used in this article follows the recommendations of den Dunnen and Antonarakis (2001). Gene symbols used in this article follow the recommendations of the HUGO Gene Nomenclature Committee (Povey et al. 2001).

Case report

The patient, a girl, was the first child of healthy Turkish consanguineous parents and was born at term after a normal pregnancy and delivery. She developed normally until 8 months of age, when she presented with an episode of acute gastroenteritis. Clinical examination revealed slight dehydration, moderate tachypnea and liver enlargement (2 cm). Her weight, length and head circumference were normal. Laboratory investigations revealed hypoglycaemia (1.8 mmol/l; normal: 3.8–5.8 mmol/l), metabolic acidosis (bicarbonates: 6 mmol/l; normal: 23–29 mmol/l; hyperlactataemia (11 mmol/l; normal: <1.7 mmol/l) during metabolic crisis only. Intravenous infusion of glucose and bicarbonate corrected both clinical and biological alterations in a few hours, except for the persistent hepatomegaly and a mild elevation of serum transaminase levels (ASAT: 125 IU/l; normal: <50 IU/l; ALAT: 65 IU/l; normal: <60 IU/l). The amino acid profile was normal in serum but a moderate elevation of alanine (160 µmol/µmol creatinine; normal: <115 µmol/µmol creatinine) was noticed in urine. Ammonia, serum levels of muscular enzymes, serum cortisol and growth hormone levels, and total and free serum carnitine levels were normal. Normal overall fatty acid oxidation and normal activities of very long- and medium-chain fatty acylCoA dehydrogenases were demonstrated in cultured fibroblasts. Fructose 1–6 bisphosphatase and pyruvate carboxylase activities were found to be normal in a liver biopsy. During the course of a fasting test, hypoglycaemia occured 19 h after the last meal and became symptomatic after 21 h (1.1 mmol/l) associated with hyperlactataemia (4.16 mmol/L) and low ketogenesis suggesting a functional defect of fatty acid oxidation in these conditions. An abnormality of the respiratory chain was considered as an explanation of the impairement of fatty acid oxidation.

Lactate and pyruvate levels monitored in blood before and after meals revealed no abnormality. Nevertheless, enzymatic studies in lymphocytes, liver and fibroblasts demonstrated the respiratory chain defect.

The child is now 4 years of age with normal growth and no signs of psychomotor retardation or neurological impairment. The initial liver enlargement progressively disappeared and liver size was normal at 19 months of age. Cardiac and abdominal ultrasound and ophthalmological work-up was consistently normal. Biological parameters remained unaltered, except for two further episodes of hypoglycaemia (1.3 mmol/l) with metabolic acidosis at 2.0 years and 2.5 years of age.

Methods

Informed consent was obtained from all tested individuals. Fibroblasts were grown in Ham's F10 medium supplemented with 10% fetal calf serum, 2.5 mM pyruvate and 200 µM uridine. Oxygen consumption was measured with a Clark oxygen electrode (Hansatech, UK) in a 250-µl cell, thermostated at 37°C according to Rustin et al. (1994). Results are presented as rates of O_2 consumption in digitonin-permeabilized cells. Respiratory chain complex activities were measured spectrophotometrically in lymphocytes, fibroblasts and liver biopsy by standard procedures (Rustin et al. 1994). Low temperature difference spectra was performed at liquid nitrogen temperature (–196°C) according to published procedures (Chance and Williams 1955; Bourgeron et al. 1992).

For mutation analysis, direct sequencing of BCS1 and the eleven structural CIII subunits genes (cyt c1, cyt b, core 1, core 2, FeS protein, Rieske 1, QP-C, Hinge, 9.5-kDa protein, 7.2-kDa protein and 6.4-kDa protein) was performed in our patient and five controls.

Total RNA and DNA were extracted from liver biopsy, lymphocytes and skin fibroblasts. Reverse transcription/polymerase chain reaction (RT-PCR) for cDNA preparation and PCR amplification of genomic fragments were conducted as described by Valnot et al. (1999) and de Lonlay et al. (2001) with specific oligonucleotides generously given by I. Valnot. PCR amplification was performed in a 50-µl volume containing 100 ng DNA or cDNA, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 20 pmol each primer and 0.6 U *Taq* polymerase. Amplification conditions included an initial denaturation at 94°C for 5 min, 30 cycles of 30 s at 95°C, 30 s at 45–55°C (depending on the primers) and 1 min at 72°C and a final extension at 72°C for 10 min. After purification, sequencing was performed on an automated Abi Prism 310 sequencer by using a Big Dye Terminator reaction kit (Applied biosystems).

Table 1 Biochemical values of respiratorry chain function and enzymes activities in lymphocytes, fibroblasts and liver biopsy were measured according to Rustin et al. (1994). Results are expressed as extreme absolute values or absolute values for controls or patient, respectively (*bold* abnormal values). In controls, activity ratios only were calculated and are given as means ± SD

	Lymphocytes		Fibroblasts		Liver biopsy	
	Controls (*n*=15)	Patient	Controls (*n*=25)	Patient	Controls (*n*=10)	Patient
Substrate oxidations[a]						
10 mM succinate	9–15.2	**6.0**	6.5–14.3	**4.6**		
10 mM Pyruvate + 1 mM malate	5–8.4	4.5	3.3–6.8	3.8		
Decyl Ubiquinol	11–15.7	**7.5**	8.5–23.2	**2.7**		
Respirtory chain complexes[b]						
Complex I					19–26	12
Complex II	14–33	32	10.8–17	10.6	168–277	179
Complex III	75–237	**55**	98–180	**17**	143–192	**10**
Complex IV	85–269	170	72–143	**55**	202–319	210
Complex V					74–167	281
Citrate synthase (matrix)	36–85	**145**	32–72	32	63–131	131
Activity ratio						
Complex IV/III	1.5±0.2	**3.1**	1.0±0.2	**3.2**	1.4±0.2	**21**
Complex IV/II	6.8±0.5	5.3	6.0±0.9	5.2	1.5±0.3	1.2
Complex IV/CS	2.0±0.4	1.2	2.0±0.4	1.7	2.9±0.2	1.6
Succinate/pyruvate	2.1±0.4	1.3	1.9±0.3	1.2		

[a] Values in nanomol O_2/min per milligram protein
[b] Values as nanomol substrate/min per milligram protein

Screening for the absence of the 4-bp deletion in controls was performed by denaturing high-presure liquid chromatography (DHPLC) as previously reported (de Lonlay et al. 2001). Exon 4 was amplified by PCR from 50 Turkish individuals. The primers used (forward/reverse, 5'–3') for amplification products from genomic DNA were as follows: CAGATAGTTCTTAGGTTGAGA, CAGCTGCATCCACAGACTTCA.

PCR product (10 µl) from the patient was mixed with PCR product (10 µl) from a normal reference DNA. Amplification products were then denaturated at 95°C for 5 min and allowed to cool to 25°C for the formation of heteroduplexes. DHPLC was carried out on a Transgenomic WAVE HPLC and DNASep column. Separation was carried out at a flow rate of 0.9 ml/min over a 3.5-min period though a linear acetonitrile gradient (54–63%) at 55.9°C.

Results

Biochemical results

A severe decrease of CIII activity was shown in lymphocytes with the impairment of both absolute and relative CIII activities (Table 1). Accordingly, low rates of substrate oxidation in detergent-permeabilized cells were demonstrated, particularly when decylubiquinol, which feeds electrons directly to CIII, was used as a substrate. CIII activity was also strongly decreased in liver associated with a mild decrease of complex I activity. A seemingly low activity of all measured red cell (RC) enzymes was observed in cultured skin fibroblasts; however, all activities except CIII activity became normal when expressed as a ratio to citrate synthase activity.

Because of the low CIII activity measured in all investigated tissues, a low temperature (–196°C) differential spectrum analysis was further performed on mitochondria isolated from cultured skin fibroblasts by using dithionite as a potent reducing agent. Whereas a normal cytochrome aa_3 (absorption peaks: 601 and 440 nm) and cytochrome *c* (absorption peak: 550 nm) content was detected in mitochondria from the fibroblasts of the patient when compared with controls, a strongly decreased absorption of cytochrome *b* at 560 nm was demonstrated, together with severe reduction of the absorption peak at 427 nm, representing the mixed absorption of both cytochromes *c* and *b*. (Fig. 1).

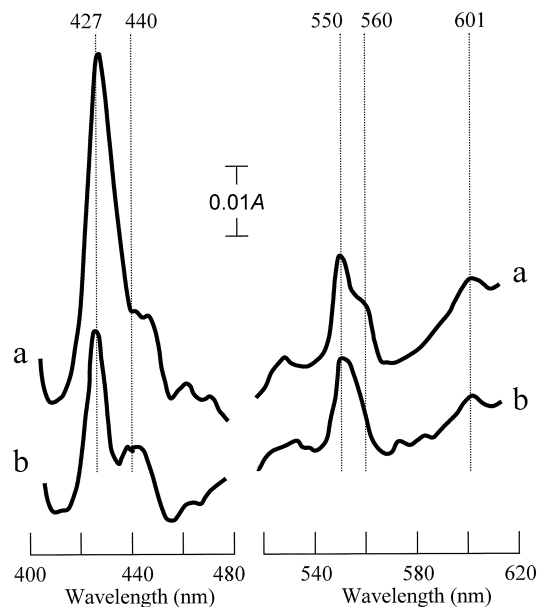

Fig. 1a, b Low temperature (–196°C) difference spectra in isolated mitochondria from control (**a**) and patient (**b**) fibroblasts, under the experimental conditions described

Mutation analysis of CIII subunit genes

Since the cytochrome *b* content was found to be very low in these cells, we first looked for mutations in the mitochondrial cytochrome *b* gene, despite consanguinity in this family. However, no divergence from the Cambridge reference sequence (Anderson et al. 1981) was shown except for four common polymorphisms (nt 14983:TTA→CTA, nt 15043:GGC→GGA, nt 15301:TTG→TTA and nt 15326: ACA→GCA). We further sequenced the ten nuclear-encoded CIII subunits and the BCS1 gene. A 4-bp deletion (AAAA) was uncovered in the QP-C cDNA at the nt 338–341 and three non-conservative amino acid changes were observed in the patient and all controls: G901A and G1078C (reported by Valnot et al. 1999) and A206T for the core1, core2 and HINGE proteins, respectively. Genomic DNA analysis indicated that the patient was homozygous for the deletion located in exon 4 of the gene. Both parents were found to be heterozygous for the deletion. The deletion was not seen in five French controls by direct sequencing and in 50 controls of similar ethnic origin (Turkey) by DHPLC analysis. The resulting protein is predicted to have seven changed amino acid residues plus an additional stretch of 14 amino acids at the C-terminal end (Fig. 2a).

Multiple sequence alignment of CIII subunit VII shows a considerable homology across species (Fig. 2b). This holds particularly true for the C-terminus with a large number of both acidic and basic residues, making this part of the protein highly hydrophilic and predicting a conserved helical structure. The 4-bp deletion identified in exon 4 of the QP-C and resulting in an abnormal elongation with a less hydrophilic amino acid chain may be expected to disrupt this helical structure. This might in turn affect the interactions of this subunit within the complex, thus lowering CIII stability and possibly accounting for the observed decrease in cytochrome *b* content accompanying the loss of CIII activity in the fibroblasts of the patient.

Discussion

We report here for the first time a respiratory chain complex deficiency in which a mutation in a nuclear-encoded complex III subunit has been demonstrated. A 4-bp deletion (nt 338–341; AAAA del) has been found in the exon 4 of the QP-C gene and is homozygous in the proband and heterozygous in both parents. The identification of the molecular defect accounting for CIII deficiency in this family offers the possibility of a confident prenatal diagnosis.

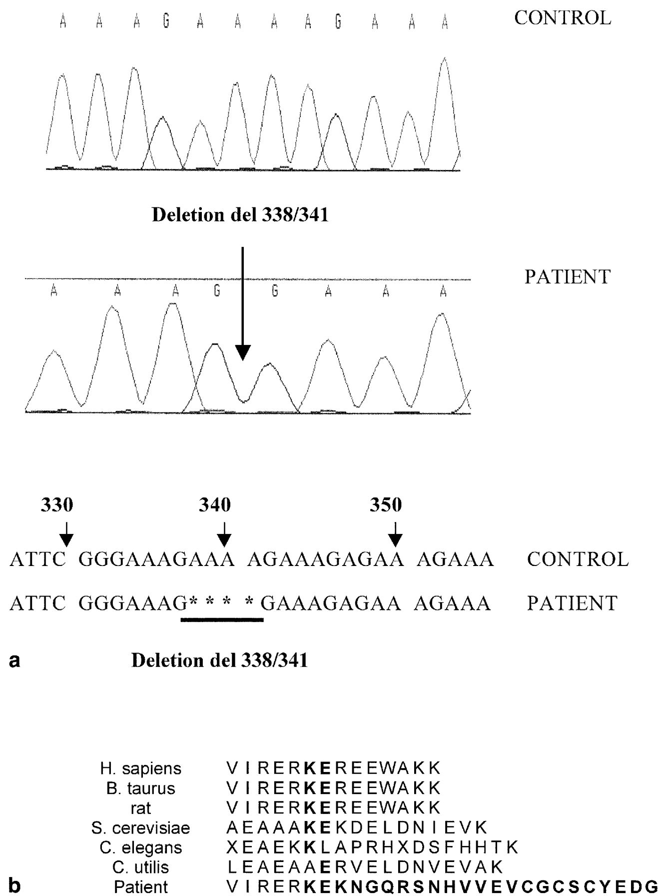

Fig. 2a, b Sequence analysis of the QP-C gene. *Arrows* Location of the AAAA deletion at nt 338–341 (**a**) and sequence alignment of QP-C (subunit VII) from various species (**b**)

The molecular change in the QP-C gene predictably results in a protein with seven modified amino acids plus an additional 14-amino-acid-long stretch at the C-terminal end. The pathogenicity of this abnormal elongation, which was absent in 55 controls and which occurs in a highly conserved region of the protein (Suzuki et al. 1988, 1989), is highly likely in view of the important function confered by this helical domain on CIII subunit VII in the maintenance (or assembly) of the complex. Indeed, previous experiments on the yeast *Saccharomyces cerevisiae* have shown that various deletions in the helical domain of the C-terminal part of the yeast counterpart of QP-C result in reduced residual CIII activities associated with decreased cytochrome *b* and other CIII subunit content (Hemrika et al. 1994). Both the experimental data obtained on the yeast and the reduced cytochrome *b* content measured in the cultured skin fibroblasts from the patient described in this study suggest that this particular domain of CIII subunit VII plays a prominent role in the maintenance (or assembly) of complex III.

Multisystem disorders and tissue-specific diseases, such as myopathy or cardiomyopathy, have been shown to result from complex III deficiency (Marin Garcia et al. 1995, 1996; Dumoulin et al. 1996; Bouzidi et al. 1996; Andreu et al. 1999a, 1999b, 2000; De Coo et al. 1999; Valnot et al. 1999). Here, we show that such a deficiency can also result in hypoglycaemia and lactic acidaemia expending the spectrum of symptoms possibly associated with this defect. Mutations in the gene encoding the BCS1 protein involved in CIII assembly trigger severe liver CIII deficiency and have been shown to cause early hepatic failure in humans (de Lonlay et al. 2001). We report here a case of severe CIII deficiency expressed in liver but with no evidence of permanent liver dysfunction, except for metabolic crises episodes occuring after fasting, with hepatomegaly, hypoglycaemia and mild elevation of liver enzymes. As observed in the cases of deficiencies affecting the other RC complexes (Zhu et al. 1998; Papadopoulou et al. 1999; Valnot et al. 2000; Benit et al. 2001), it is difficult to provide an explanation for these highly variable clinical presentations. So far, too few patients with proven molecular defects have been reported for a correlatation to be made between the nature of the gene involved and a given clinical phenotype.

Whereas the BCS1 gene mutation appears to be a frequent cause of CIII deficiency, we have found the first molecular abnormality in the QP-C gene. A systematic study attempting to identify mutations in nuclear genes encoding CIII structural subunits in another cohort of five patients has failed to identify any mutations (Valnot et al. 2000). This supports the view that, in addition to mutations in mitochondrial cytochrome *b*, mutations resulting in CIII deficiency most often lie in genes involved either in the assembly or, more generally, in the maintenance of the complex. At present, only one such gene, BCS1, is known. However, this is likely to change as the search for the molecular bases of human mitochondrial diseases takes advantage of the ever-increasing functional data available for yeast and other organisms.

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