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ORIGINAL INVESTIGATION

**Isabelle Valnot · Johanna Kassis · Dominique Chretien
Pascale de Lonlay · Béatrice Parfait · Arnold Munnich
Jean Kachaner · Pierre Rustin · Agnès Rötig**

A mitochondrial cytochrome *b* mutation but no mutations of nuclearly encoded subunits in ubiquinol cytochrome *c* reductase (complex III) deficiency

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Abstract Ubiquinol cytochrome *c* reductase (complex III) deficiency represents a clinically heterogeneous group of mitochondrial respiratory chain disorders that can theoretically be subject to either a nuclear or a mitochondrial mode of inheritance. In an attempt to elucidate the molecular bases of the disease, we first determined the nucleotide sequence of three unknown subunits (9.5 kDa, 7.2 kDa, 6.4 kDa) by cyberscreening of human expressed sequence tag data bases and sequenced the 11 cDNA subunits encoding complex III in five patients with isolated complex III deficiency. No mutation in the nuclearly encoded complex III subunits was observed, but a mutation in the cd2 helix of the mitochondrial (mt) cytochrome *b* gene was found to alter the conformation of the bc_1 complex in one patient with severe hypertrophic cardiomyopathy. The present study is highly relevant to genetic counseling as the absence of mtDNA mutations in all but one patient in our series strongly supports autosomal rather than maternal inheritance in the majority of patients with complex III deficiency.

Introduction

Mitochondrial respiratory chain defects are a genetically heterogeneous group of diseases accounting for a large spectrum of clinical manifestations in humans. Among them, complex III (CIII) deficiency represents a relatively rare cause of respiratory enzyme dysfunction (Mourmans et al. 1997). Indeed, among 160 respiratory-chain-enzyme-deficient patients, only 7% had a CIII deficiency, whereas complex I (CI) and CIV deficiencies accounted for 33% and 28% of the patients, respectively (Von Kleist-Retzow et al. 1998).

CIII (ubiquinol cytochrome *c* reductase, E.C. 1.10.2.2) forms the central segment of the respiratory chain and catalyzes the transfer of electrons from ubiquinol to cytochrome *c*. In mammals, CIII is made up of eleven subunits, one of them, cytochrome *b* (cyt *b*), being mitochondrially encoded. Cytochrome c_1, iron-sulfur protein and cyt *b* carry the prosthetic groups (Shägger et al. 1995). Because of its two-fold genetic origin, CIII deficiency can theoretically be subject to either an autosomal or a maternal mode of inheritance. Maternal transmission of cyt *b* mutations has been reported in Leber's hereditary optic neuropathy (Brown et al. 1995). However, since most cases are isolated, it is difficult to decide which mode of inheritance is involved.

In an attempt both to identify the nature of the underlying mutations and to improve genetic counseling, we have sequenced the cDNA subunits encoding CIII in five isolated cases of CIII deficiency. For the purpose of this study, the hitherto unknown nucleotide sequence of three subunits (9.5 kDa, 7.2 kDa, 6.4 kDa) has been determined by cyberscreening of the human expressed sequence tag (EST) data base. Interestingly, no mutations in the nuclearly encoded subunits have been found, but one mitochondrial (mt) DNA mutation has been identified in the cyt *b* gene of a patient presenting with hypertrophic cardiomyopathy.

I. Valnot · J. Kassis · D. Chretien · P. de Lonlay · B. Parfait
A. Munnich · P. Rustin · A. Rötig (✉)
Unité de Recherches sur les Handicaps Génétiques de l'Enfant,
INSERM U393, Hôpital des Enfants-Malades,
149 Rue de Sèvres; F-75015 Paris, France
e-mail : roetig@necker.fr,
Tel.: +33-1-44-495161, Fax: +33-1-47-348514

J. Kachaner
Service de Cardiologie Pédiatrique, Hôpital des Enfants-Malades,
149 Rue de Sèvres, F-75015 Paris, France

Materials and methods

Patients

Patient 1, a girl, was born to non-consanguineous parents after a term pregnancy and normal delivery. Her development was normal until the age of 2 years. She then developed deafness, psychomotor retardation, and epilepsy at the age of 6 years. At 11.5 years, she presented with myopathy, facial diplegia, and lordosis. Plasma lactate, lactate/pyruvate, and ketone bodies molar ratios were normal.

Patient 2, a boy born to non-consanguineous parents, was small for gestational age (weight 2000 g at 37 weeks). Neonatal hypotonia and metabolic acidosis were detected immediately after birth (pH 6.99; plasma lactate: 15 mmol/l, N < 2 mmol/l), and he died at 7 days of age. His elder sister had neonatal lactic acidosis and presented psychomotor retardation from the age of 1 year. She is now 12 years old.

Patient 3, a boy born to non-consanguineous parents, was small for gestational age (weight 1925 g, height 43 cm at 37 weeks). His development was unremarkable until 3 years of age, when he developed strabismus and retinopathy, and his neurological condition gradually worsened. At 5 years of age, he presented pyramidal signs, mild dystonia, strabismus, and poor visual acuity because of retinal microangiopathy. Cerebral magnetic resonance imaging showed diffuse white matter hypodensity and multiple calcifications. His electroretinogram (ERG) was altered, but heart ultrasound, plasma amino acids, lactate, lactate/pyruvate, and ketone body molar ratios were normal.

Patient 4, a girl, was born to non-consanguineous parents after a term pregnancy and normal delivery. Her development was unremarkable until 3 years of age. Elevated sarcoplasmic enzymes (AST and ALT: 6xN) were noted during an infection at the age of 3 years, and cardiomegaly was first detected at 5 years. Heart ultrasound revealed a severe hypertrophic cardiomyopathy at 8 years of age. At this time, metabolic acidosis with elevated plasma lactate was noted (5 mmol/l). Gas chromatography/mass spectrometry of urine and a cerebral computerized tomography (CT) scan were normal. The ERG was altered, and the endomyocardial biopsy showed macrovesicular lipid storage. The patient died of cardiac failure at 9 years of age.

Patient 5, a girl born to first cousin Iranian parents exhibited normal development until the age of 7 months (Bodemer et al. 1999; Rötig et al. 1995). At this time, she developed failure to thrive, abdominal pain, and hair loss. Her condition gradually worsened. At 4 years of age, she showed severe growth retardation with hypotonia, asthenia, mental retardation, and proximal tubulopathy. She also presented alopecia of the scalp related to trichothiodystrophy and a transient cerebral stroke with normal CT scan. Plasma lactate was elevated (5 mmol/l).

Methods

Cytochrome *c* oxidase (CIV; EC 1.9.3.1), antimycin-sensitive decylubiquinol cytochrome *c* reductase (CIII; EC 1.10.2.2), malonate-sensitive succinate quinone dichlorophenolindophenol (DCPIP) reductase (CII; EC 1.3.99.1), succinate cytochrome *c* reductase (CII+CIII), and NADH cytochrome *c* reductase (CI+CIII) activities were measured spectrophotometrically by standard procedures (Rustin et al. 1994). Results were expressed both as absolute values and as activity ratios (Chretien et al. 1997). Kinetic parameters of CIII reactions were determined by fitting the rates obtained at various concentrations of decylubiquinol to a Michaelis-Menten equation modified to provide estimates of the Hill number, in addition to Vmax and Km (Meyer et al. 1990).

Total DNA extracted either from muscle biopsies or from cultured skin fibroblasts were submitted to polymerase chain reaction (PCR) amplification by using specific primers designed from the genomic sequence of the mitochondrial cyt *b* gene, the nuclear iron-sulfur protein (ISP) gene, and the nuclear ISP targeting protein gene (Table 1). Total RNA extracted from either muscle biopsies or cultured skin fibroblasts were reverse-transcribed (RT) by using the GeneAmp RNA PCR kit (Applied Biosystems), and each cDNA was amplified with specific oligonucleotides (Table 1). PCR amplification was performed in a 25-µl volume containing 100 ng DNA, 10 mM TRIS-HCl pH 8, 1.5 mM $MgCl_2$, 50 mM KCl, 80 mM dNTP, 20 pmol of each primer, and 0.6 U *Taq* polymerase (Life Technology). Amplification conditions included

Table 1 Oligonucleotides used for RT-PCR and sequence analyses of CIII cDNA subunits. Nucleotide positions refer to Genbank entries of the CIII subunit genes. Accession numbers are between *brackets*

	Forward primers	Reverse primers
cyt *c1* (J04444)	nt 1347–nt 1366	nt 2261–nt 2241
	nt 2197–nt 2215	nt 3650–nt 3631
	nt 2660–nt 2679	
	nt 3019–nt 3038	
cyt *b* (J01415)	nt 14248–nt 14268	nt 15021–nt 15001
	nt 15000–nt 15020	nt 15255–nt 15225
		nt 15480–nt 15460
		nt 15740–nt 15720
		nt 16062–nt 16042
		nt 15264–nt 15244 (ACRS)
Core 1 protein (L16842)	nt 385–nt 405	nt 672–nt 652
	nt 619–nt 639	nt 909–nt 889
	nt 856–nt 876	nt 1164–nt 1144
	nt 1111–nt 1131	nt 1404–nt 1384
	nt 1351–nt 1371	nt 1644–nt 1624
	nt 1585–nt 1605	nt 1893–nt 1873
Core 2 protein (J04973)	nt 22–nt 44	nt 366–nt 348
	nt 313–nt 335	nt 638–nt 618
	nt 585–nt 605	nt 902–nt 881
	nt 849–nt 869	nt 1196–nt 1176
	nt 1143–nt 1163	nt 1448–nt 1428
Iron-sulfur protein (L32977)	nt 309–nt 329	nt 674–nt 654
	nt 618–nt 638	nt 983–nt 963
Rieske 1 (L32917)	nt 121–nt 141	nt 382–nt 364
HUMQPC (M22348)	nt 3–nt 23	nt 398–nt 378
Hinge protein (Y00764)	nt 10-nt 30	nt 387–nt 367

30 cycles of 30 s at 95 °C, 30 s at 45–55 °C (depending on the primer used), and 1 min at 72 °C. The bovine amino acid sequences of the 9.5-kDa (SwissProt P13271), 7.2-kDa (SwissProt P00130), and 6.5-kDa (SwissProt P07552) subunits were used as templates to screen the human dbEST database by using the BLAST similarity searching program (TBLASTN option), and primers for RT-PCR amplification of total RNA were designed from the derived sequences. For sequence analyses, amplification products were purified on a 2% low-melting-point agarose gel and recovered by heating for 5 min at 65 °C. Direct sequencing was performed by using 3.2 pmol amplification primer (Table 1), 100 ng DNA, and 8 μl sequencing reaction mixture (Dye terminator Cycle Sequencing kit, Perkin-Elmer Cetus) on an automated 377 DNA sequencer (Applied Biosystems).

Results

Enzyme activities

The absolute activities of ubiquinol cytochrome *c* reductase (CIII), NADH cytochrome *c* reductase (CI+III), and succinate cytochrome *c* reductase (CII+III) in our patients were either decreased or at the lowest values of the control ranges (Table 2 a). However, activity ratios involving CIII, which best reflect the balanced respiratory enzyme activity (Chretien et al. 1997), were consistently and severely abnormal in all five patients (Table 2b). CII (succinate quinone-dichlorophenol indophenol reductase) and CIV activities were in the normal ranges. These results indicated that an isolated CIII deficiency was involved in the five patients.

Table 2 a, b Enzymological investigation of patients 1–5 (*SQDR* succinate quinone-dichlorophenol indophenol reductase, *COX* cytochrome *c* oxidase, *NCCR* NADH cytochrome *c* reductase, *SCCR* succinate cytochrome *c* reductase, *QCCR* decylubiquinol cytochrome *c* reductase). Abnormal values are in **bold**

a Activities are given in nanomoles/min per milligram protein

Patients	Activities				
	SQDR (CII)	COX (CIV)	NCCR (CI+III)	SCCR (CII+III)	QCCR (CIII)
Muscle homogenate					
2	30	130	15	**9.5**	**14**
3	39	214	14	24	50
Controls	18–42	65–138	11–45	13–39	41–113
Muscle mitochondria					
5	201	1561	225	270	405
Controls	61–254	214–2303	47–483	68–648	354–1104
Heart homogenate					
4	133	822		**55**	**110**
Controls	34–218	236–1315		74–456	191–789
Fibroblasts					
1	21	107		**15**	50
3	23	149		35	55
5	18	93		25	**29**
Controls	18–34	43–182		23–45	58–136

b The ratio values are presented as mean ± 1 SD

Patients	Ratios			
	COX/QCCR	COX/SCCR	COX/SQDR	COX/NCCR
Muscle homogenate				
2	**9.3**	13.7	**4.3**	**8.7**
3	**4.3**	8.9		**15.3**
Control	1.5 ± 0.4	4.1 ± 0.4	5.5 ± 0.9	4.1 ± 0.4
Muscle mitochondria				
5	**3.9**	**5.8**	7.8	**6.9**
Control	1.4 ± 0.2	3.1 ± 0.5	8.84 ± 1.48	3.4 ± 0.8
Heart homogenate				
4	**7.5**	**14.9**	6.2	
Control	1.4 ± 0.3	3.36 ± 0.54	5.4 ± 0.9	
Fibroblasts				
1	2.1	**7.1**	5.1	
3	2.7	4.3	6.5	
5	**3.2**	3.7	5.2	
Control	1.2 ± 0.2	3 ± 0.4	5.2 ± 1	

Identification of the 9.5 kDa, 7.2 kDa, and 6.4 kDa human coding sequences

Screening the dbEST database with the bovine amino acid sequences for the 9.5 kDa, 7.2 kDa, and 6.5 kDa subunits of CIII by using the TBLASTN option led to the identification of various human ESTs showing significant smallest sum probability values ($P < 10^{-5}$). EST_AA026288, EST_AA703740, and EST_T58471 corresponded to the human core-associated protein (9.5 kDa), the cyt *c1*-associated protein (7.2 kDa), and the ISP-associated protein (6.4 kDa) cDNAs, respectively. The three EST sequences contained the translation initiation codons and stop codons (TAA or TAG). All three subunits contained a canonical polyadenylation sequence (AATAAA) located 21–22 nucleotides upstream from the poly(A) tail. RT-PCR amplification of total RNA from control cultured fibroblasts with primers designed from these ESTs and sequence analysis allowed us to confirm the nucleotide (nt) sequence of these three human cDNA subunits. The 9.5 kDa subunit coding sequence spans 249 nt, and the corresponding polypeptide is 82 amino acids in length (Fig. 1), with the human and bovine proteins being 75.6% homologous. The 7.2 kDa subunit coding sequence spans 192 nt, and the deduced protein sequence contains 63 amino acids, with the human and bovine proteins being 97.8% homologous. Finally, sequence analysis of the 6.4 kDa subunit cDNA revealed an open reading frame of 171 nt encoding a 56 amino acid protein, giving an 83.9% homology with the bovine sequence.

Sequence analysis of CIII subunits in the patients

No mutation in any of the ten nuclearly encoded CIII cDNA subunits tested was found in our series. Several conservative and non-conservative amino acid changes were observed in both patients and unrelated controls (Table 3).

The mitochondrial cyt *b* gene was sequenced in all patients. Alignment of the nucleotide sequence of the cyt *b* gene of patient 4 with the Cambridge reference sequence (Anderson et al. 1981) led us to identify a G to A substitution at nt 15,243, changing a glycine into a glutamic acid in a highly conserved region of the protein (G166E). PCR amplification with the ACRS (allele created restriction site) technique revealed that 90% of mtDNA molecules were mutated in the heart of patient 4 (Fig. 2). In skin fibroblasts cultured from the patient and grown in the presence of uridine (100 µM) and pyruvate (2 mM), 100% of mtDNA molecules harbored the G to A transition at nt 15,243. Conversely, no G to A transition mutation was found in leuko-

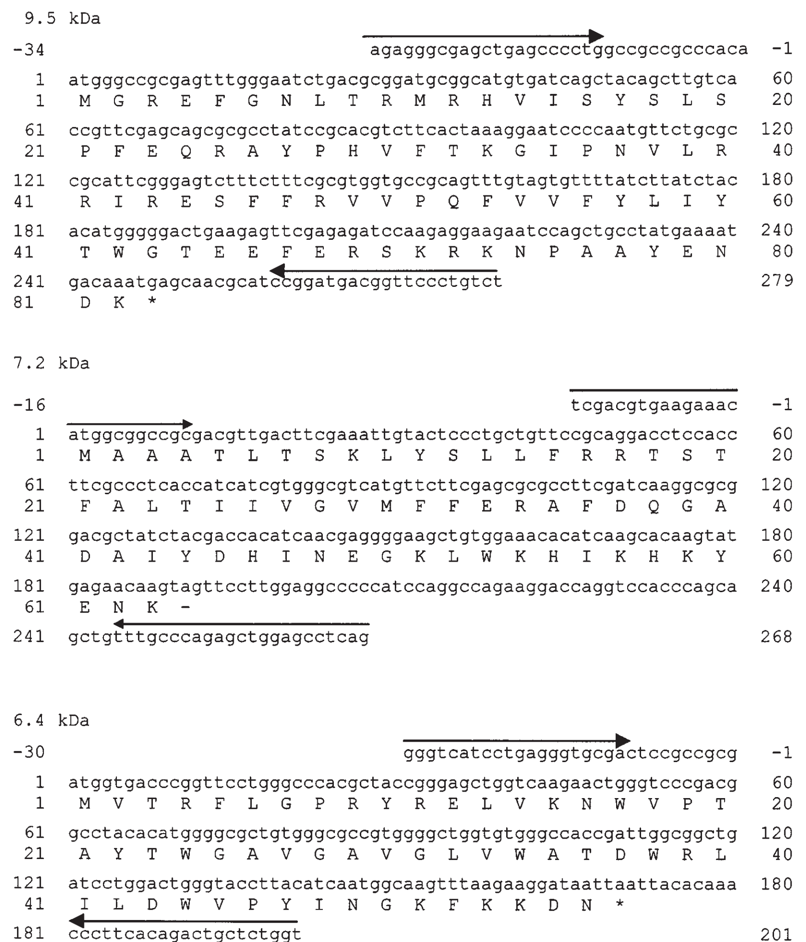

Fig. 1 Nucleotide and deduced amino acid sequences of the human 9.4 kDa, 7.2 kDa, and 6.5 kDa subunits (*arrows* oligonucleotide primer sequences)

Table 3 Polymorphic substitutions in cDNAs of CIII subunits in the patients

Nucleotide substitutions	Amino acid changes	Patients
Core 1 protein		
G473A	Asp158Asp	1, 2, 3, 4, 5, control (*n* = 8)
G901A	Ser301Asn	1, 2, 3, 4, 5, control (*n* = 8)
Core 2 protein		
T547C	Gly183Arg	2, 3, 5, control (*n* = 8)
G1078C	Arg359Thr	1, 2, 3, 4, 5, control (*n* = 8)
Cyt c_1		
G265C	Val89Leu	3, 5, control (*n* = 8)
Cyt *b*		
A14766G[a]	Ile7Thr	2
A14769C	Asn8Ser	2, control (*n* = 8)
T14798C[a]	Phe18Leu	5
T15115C	Thr123Thr	5
A15326G[a]	Thr194Ala	2
C15452A[a]	Leu236Ile	1, 2, 3, 4, 5
T15670C	His308His	5
T15693C[a]	Met316Thr	5
		3

[a] Previously reported (MITOMAP 1999)

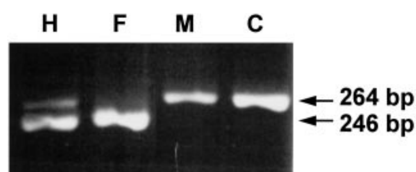

Fig. 2 Quantitation of the G15243A cyt *b* gene mutation in heart *(H)* and cultured skin fibroblasts *(F)* of patient 4. Normal and mutant restriction fragments after *Hin*dIII digestion are 264 bp and 246 bp in length, respectively. *M* Mother, *C* control

cytes of the patient's mother, suggesting that a de novo mutation was involved. This substitution was absent in 50 unrelated controls. An A to C substitution at nt 14,769 changed an asparagine into a serine both in patient 2 and in a control. Finally, two unreported conservative polymorphisms were identified in patients 2 and 5 (T15,115C and T15,670C, respectively; Table 4), and several non-conservative substitutions previously reported as polymorphisms (MITOMAP 1999) were observed (Table 3). No known mtDNA mutations (MELAS, MERRF, NARP) or deletions could be found in any of the other patients. Sequence analysis of tRNA genes (tRNA-Pro, tRNA-Glu, tRNA-Thr) surrounding cyt *b* did not reveal any mutations.

Pathogenicity of the G166E cyt *b* mutation

The G166E amino acid change (patient 4) occurred in the cd2 surface helix of cyt *b* (Fig. 3 a). The cd1 and cd2 he-

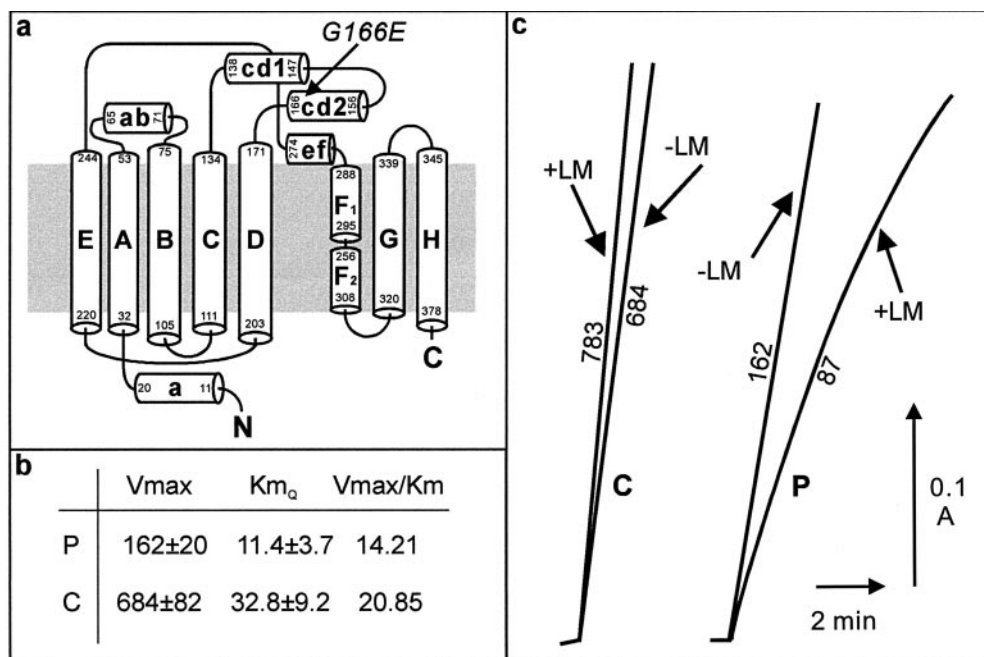

Fig. 3 a–c Kinetic studies of CIII activity in patient 4 and control heart homogenates. **a** Secondary structure of human cytochrome *b* (from Zhang et al. 1998). The G166 E mutation observed in patient 4 is indicated *(arrow)*. **b** Kinetic properties of patient *(P)* and control CIII activity *(C)*. **c** Effect of lauryl maltoside (*LM*, 0.25 mM) on CIII activity measured as decylubiquinol cytochrome *c* reductase. Activities are expressed as nmol cyt *c*/min per milligram protein. Values are means ± 1 SD

lices are responsible for the stability of the cavities created by the transmembrane helices of cyt *b*, thus controlling access of the quinone to its binding site (Xia et al. 1997). Therefore, we investigated the kinetic parameters of CIII in post-mortem heart homogenates of the patient. Interestingly, a four-fold decrease of Vmax (Fig. 3 b) with a marked increase of the apparent Km for decylubiquinone was observed in heart homogenate of patient 4. However, the Vmax/Km ratio, which best reflects changes in the binding activity of the protein (Meyer et al. 1990), did not significantly differ between patient 4 and controls, suggesting that the affinity of the enzyme for its decylubiquinone substrate was normal. Lauryl maltoside has been suggested to act on the conformation of the enzyme (Nalecz et al. 1985). Interestingly, whereas control enzyme activity could be stimulated by lauryl maltoside (Birch-Machin et al. 1994), we observed a significant and gradual inhibition of CIII activity upon the addition of lauryl maltoside to patient heart homogenate, supporting the view that an abnormal conformation of the bc_1 complex was involved (Fig. 3 c).

Discussion

The genes encoding the catalytic subunits of ubiquinol cytochrome *c* reductase were originally regarded as obvious candidate genes in CIII deficiency. Surprisingly, however, no mutation in the nuclearly encoded genes has been found in our series. Conversely, a point mutation in the mitochondrial cyt *b* gene has been observed in one patient. This mutation converts a highly conserved glycine into a glutamic acid in the cd2 helix, which plays an important role in maintaining the protein in its catalytically active conformation (Xia et al. 1997). Indeed, kinetic studies are highly suggestive of an abnormal conformation of the complex in heart homogenate of the patient. Although germline mosaicism cannot be formally excluded, the absence of the G166E mutation in the leukocytes of the proband's mother is highly suggestive of a de novo mtDNA mutation. Interestingly, a point mutation at the same codon of the cyt *b* gene (G to A at nt 15,242), changing a glycine into an arginine, has been reported in a patient with exercise intolerance (Kennaway et al. 1998). The reason for mutations in the same amino acid in the same gene giving rise to different clinical presentations is still unknown. A cyt *b* mutation (G to A at nt 15,615) has been also related to CIII instability in another patient with exercise intolerance (Bouzidi et al. 1996).

Sequence analysis of the nuclearly encoded CIII subunits has failed to detect any deleterious mutation or microdeletion in our patients, a result that is also surprising. It is unlikely that disease-causing mutations lie in mitochondrial tRNAs or rRNAs, as these mutations are expected to produce multiple or generalized respiratory chain deficiency. Indeed, sequencing of the flanking tRNA genes has not revealed deleterious mutations. Similarly, mtDNA mutations in the CI, CIV, and CV genes are unlikely, as they usually result in deficiencies of the corresponding complex. For these reasons, the present study supports the view that the mutations causing CIII deficiency lie in nuclear genes encoding proteins involved in the assembly or regulation of the complex as suggested for CIV deficiencies (Parfait et al. 1997) and recently shown for the SURF-1 gene mutation (Zhu et al. 1998, Tiranti et al. 1998). In yeast, at least four genes have been shown to be involved in CIII assembly, namely CBP3 (Wu and Tzagoloff 1989), CBP4 (Crivellone 1994), BCS1 (Nobrega et al. 1992), and ABC1 (Bousquet et al. 1991). The human counterparts of these genes are presently unknown. When cloned, they will obviously represent major candidate genes in human CIII deficiency.

The present study is highly relevant to genetic counseling as the absence of mtDNA mutations in all but one patient in our series strongly supports an autosomal rather than a maternal mode of inheritance in CIII deficiency.

References

Anderson S, Bankier AT, Barrell BH, Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG (1981) Sequence and organization of the human mitochondrial genome. Nature 290: 457–465

Birch-Machin MA, Briggs HL, Saborido AA, Bindoff LA, Turnbull DM (1994) An evaluation of the measurement of the activities of complexes I–IV in the respiratory chain of human skeletal muscle mitochondria. Biochem Med Metab Biol 51: 35–42

Bodemer C, Rötig A, Rustin P, Cormier V, Niaudet P, Saudubray JM, Rabier D, Munnich A, Prost Y de (1999) Hair and skin disorders as signs of mitochondrial disease. Pediatrics 103: 428–433

Bousquet I, Dujardin G, Slonimski PP (1991) ABC1, a novel yeast nuclear gene has a dual function in mitochondria: it suppresses a cytochrome *b* mRNA translation defect and is essential for the electron transfer in the *bc1* complex. EMBO J 10: 2023–2031

Bouzidi MF, Carrier H, Godinot C (1996) Antimycin resistance and ubiquinol cytochrome *c* reductase instability associated with a human cytochrome *b* mutation. Biochim Biophys Acta 1317: 199–209

Brown MD, Torroni A, Reckord CL, Wallace DC (1995) Phylogenetic analysis of Leber's hereditary optic neuropathy mitochondrial DNAs indicates multiple independent occurrences of the common mutations. Hum Mutat 6: 311–325

Chretien D, Gallego J, Barrientos A, Casademont J, Cardellach F, Munnich A, Rötig A, Rustin P (1997) The biochemical parameters for the diagnosis of respiratory chain deficiency in man and their lack of age-related changes. Biochem J 329: 249–254

Crivellone MD (1994) Characterization of CBP4, a new gene essential for the expression of ubiquinol-cytochrome *c* reductase in *Saccharomyces cerevisiae*. J Biol Chem 269: 21284–21292

Kennaway NG, Keightley JA, Burton MD, Quan F, Libby BD, Buist NRM (1998) Mitochondrial encephalomyopathy asociated with a nonsense mutation in cytochrome *b*. Mol Genet Metab 63: 49

Meyer CR, Rustin P, Wedding RT (1990) The influence of pH on substrate form specificity of phosphoenolpyruvate carboxylase purified from *Crassula argentea*. Arch Biochem Biophys 278: 365–372

MITOMAP (1999) Human mitochondrial genome database. Center for molecular medicine, Emory University, Atlanta, Ga. USA (http://www.gen.emory.edu/mitomap.html)

Mourmans J, Wendel U, Bentlage HACM, Trijbels JMF, Smeitink JAM, Coo IFM de, Gabreëls FJM, Sengers RCA, Ruitenbeek W (1997) Clinical heterogeneity in respiratory chain complex III deficiency in childhood. J Neurol Sci 149 : 111–117

Nalecz MJ, Bolli R, Azzi A (1985) Molecular conversion between monomeric and dimeric states of the mitochondrial cytochrome *bc1* complex: isolation of active monomers. Arch Biochem Biophys 236 : 619–628

Nobrega FG, Nobrega MP, Tzagoloff A (1992) BCS1, a novel gene required for the expression of functional Rieske iron-sulfur protein in *Saccharomyces cerevisiae*. EMBO J 11 : 3821–3829

Parfait B, Percheron A, Chretien D, Rustin P, Munnich A, Rötig A (1997) No mitochondrial cytochrome oxidase (COX) gene mutations in 18 cases of COX deficiency. Hum Genet 101 : 247–250

Rötig A, Lehnert A, Rustin P, Chretien D, Bourgeron T, Niaudet P, Munnich A (1995) Kidney involvement in mitochondrial disorders. Adv Nephrol 24 : 367–378

Rustin P, Chretien D, Bourgeron T, Gérard B, Rötig A, Saudubray JM, Munnich A (1994) Biochemical and molecular investigation s in respiratory chain deficiencies. Clin Chim Acta 228 : 35–51

Schägger H, Brandt U, Gencic S, Jagow G von (1995) Ubiquinol-cytochrome *c* reductase from human and bovine mitochondria. Methods Enzymol 260 : 82–96

Tiranti V, Hoertnagel K, Carrozzo R, Galimberti C, Munaro M, Granatiero M, Zelante Gasparini P, Marzella R, Rocchi M, Bayona-Bafaluy MP, Enriquez JA, Uziel G, Bertini E, Dionisi-Vici C, Franco B, Meitinger T, Zeviani M (1998) Mutations of SURF-1 in Leigh disease associated with cytochrome *c* oxidase deficiency. Am J Hum Genet 63 : 1609–1621

Von Kleist-Retzow JC, Cormier-Daire V, Lonlay P de, Parfait B, Chretien D, Rustin P, Feingold J, et al (1998) A high rate (20%–30%) of parental consanguinity in cytochrome oxidase deficiency. Am J Hum Genet 63 : 428–435

Wu M, Tzagoloff A (1989) Identification and characterization of a new gene (CBP3) required for the expression of yeast coenzyme QH_2-cytochrome *c* reductase. J Biol Chem 264 : 11122–11130

Xia D, Y C-A, Kim H, Xia J-Z, Kachurin AM, Zhang L, Yu L, Deisenhofer J (1997) Crystal structure of the cytochrome *bc1* complex from bovine heart mitochondria. Science 277 : 60–66

Zhang Z, Huang L, Shulmeister VM, Chi Y-I, Kim KK, Hung L-W, Crofts AR, Berry EA, Kim S-H (1998) Electron transfer by domain movement in cytochrome *bc1*. Nature 392 : 677–684

Zhu Z, Yao J, Johns T, Fu K, De Bie I, Macmillan C, Cuthbert AP, Newbold RF, Wang J, Chevrette M, Brown GK, Brown RM, Shoubridge EA (1998) SURF1, encoding a factor involved in the biogenesis of cytochrome *c* oxidase, is mutated in Leigh syndrome. Nat Genet 20 : 337–343