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SHORT COMMUNICATION

UQCRC2 mutation in a patient with mitochondrial complex III deficiency causing recurrent liver failure, lactic acidosis and hypoglycemia

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An isolated mitochondrial complex III (CIII) defect constitutes a rare cause of mitochondrial disorder. Here we present the second case involving *UQCRC2* gene, which encodes core protein 2, one of the 11 structural subunits of CIII. The patient has the same mutation (c.547C > T; p.Arg183Trp) as the first case and presented with neonatal lactic acidosis, hypoglycemia and severe episodes of liver failure. Our study expands the few reported cases of CIII deficiency of nuclear origin.
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INTRODUCTION

Complex III (CIII) of the mitochondrial respiratory chain (RC) conveys electrons from ubiquinol to cytochrome *c* and forms the respirasome when associated with complexes I and IV (CI and CIV). CIII is composed of 11 subunits; one is encoded by mitochondrial DNA (*CYB)* and the others by nuclear DNA, as well as their assembly factors. CIII deficiency represents between 2 to 15% of primary mitochondrial diseases and its molecular origins have been elucidated in only approximately 50% of cases. Mutations in *CYB* and in *BCS1L*, *TTC19* and *LYRM7* that encode assembly factors are the most frequently identified to date.[1,2] Recently, defects in accessory proteins UQCC2 and UQCC3 have been associated with CIII deficiency.[3,4] Only five familial cases with mutations in nuclear genes encoding structural subunits (*UQCRB*, *UQCRQ*, *UQCRC2* and *CYC1*) were reported.[5–8] We report here a second family case with an *UQCRC2* mutation leading to CIII deficiency and enlarge the clinical phenotype from lactic acidosis to hepatocellular insufficiency.

CASE HISTORY

Clinical presentation

The patient was born at term following an uneventful pregnancy of French Caucasian parents presumably non-consanguinous. At few days of life, he presented poor feeding and lactic acidosis with hypoglycemia and hepatocellular deficiency (prothrombin time for the patient/control 52.6/13.1 s, factor V 5 UI dl^{-1}). During the first year of life, he exhibited five episodes of hypoglycemia (about 1 mM, normal range $N = 2.8–4.4$ mM) with ketonuria, hyperlactatemia (8–10 mM, $N = 0.6–2.4$ mM), high lactate/pyruvate ratio (30–40, $N = 12–18$) and elevated serum transaminases (AST 131 UI l^{-1}, $N = 15–40$ UI l^{-1}, ALT 100 UI l^{-1}, $N = 10–40$ UI l^{-1}) triggered by minor infections. Total recovery occurred under glucose perfusion after each recurrence. A fractioned − enriched diet and carnitine, biotin and thiamine supplementations were initiated.

Metabolic investigations during a decompensation episode showed elevated plasma alanine (1858 μM, $N = 234–390$ μM) and proline (526 μM, $N = 133–227$ μM), reflecting hyperlactatemia. Concomitant urine organic acid analysis revealed elevated 3-OH-butyrate (134 mmol per mmol creatinine, $N < 11$ mmol per mmol creatinine) and dicarboxylic acid (adipate 85 mmol per mmol creatinine, $N < 35$ mmol per mmol creatinine; suberate 86 mmol per mmol creatinine, $N < 10$ mmol per mmol creatinine) levels, indicating activation of fatty acid oxidation and ketone production. Moreover, increased succinate (1772 mmol per mmol creatinine, $N = 17–80$ mmol per mmol creatinine), a tricarboxylic acid (TCA) cycle intermediate and ethylmalonate (30 mmol per mmol creatinine, $N < 2$ mmol per mmol creatinine) levels might suggest a defect in mitochondrial RC. The ammonemia was slightly elevated (60 μM, $N < 40$ μM).

At 2.5 years of age, a metabolic work-up independent of decompensation episodes revealed a constant and moderate metabolic acidosis with elevated lactatemia (about 2.5 mM). Neurological, cardiac, muscular and psychomotor functions were normal. The patient presented a slight liver enlargement without splenomegaly and a discrete facial dysmorphia with epicanthus.

The patient is now 9 years old, with normal growth and neurological functions, with the exception of slight scholastic retardation. He is frequently hospitalized for episodes of vomiting and sleepiness with acute hepatocellular deficiency, cytolysis and hyperlactatemia without bleeding. He always recovers without sequelae with intravenous hydration. Standard laboratory tests are normal between crises.

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Mitochondrial function analyses and molecular analyses

Specific investigations excluded tyrosinemia, fructosemia, galactosemia, fructose-1,6-diphosphatase deficiency and a beta-oxidation defect. Mitochondrial function was then explored in the patient's fibroblasts.[9,10] The enzymatic activity of CIII and the rate of oxygen consumption in the presence of decyl-ubiquinol, which feeds electrons directly to CIII, were very low. The activity of CI was decreased slightly. Activities of CIV and TCA cycle enzymes were not affected. Immunoblot detection of native complexes showed a marked decrease of the quantities of CIII and CI (Table 1).

Mitochondrial DNA Sanger sequencing of patient's fibroblasts first excluded mitochondrial DNA origin (especially *CYB* mutation). Targeted resequencing of 150 nuclear genes involved in mitochondrial defects (including all nuclear genes encoding CIII subunits) revealed a homozygous mutation c.547C>T, p.Arg183Trp in the *UQCRC2* gene (the list of the sequenced genes and the targeted sequencing methods are in Supplementary Table S1). The c.547T>C homozygous mutation and parental alleles segregation were then confirmed by Sanger sequencing of exon 7 (Ref Seq NM_003366.2, electrophoregrams in Supplementary Figure S1). The minor allele frequency of this variant is 1/121054 in ExAC (http://exac.broadinstitute.org) database and 0/8598 in ESP (http://evs.gs.washington.edu/EVS) database and the Polyphen score[11] predicted a probably damaging amino-acid substitution (score = 1.0). This mutation is the same as that found in a Mexican consanguineous family (patients P1, P2 and P3) described by Miyake *et al.* in the first and only description of *UQCRC2* deficiency.[7] This previous study proved the pathogenicity of this mutation by its absence in control alleles, the high conservation of the Arg183 residue across species, and the deleterious structural consequences of the p.Arg183Trp mutation.

Table 1 Analyses of mitochondrial function in the patient's fibroblasts

	Patient	*Controls (n = 50) ± s.d.*
Oxygen consumption in permeabilized cells (nmol O_2 per min per mg protein)		
In the presence of pyruvate	5.0	3.3–6.8
In the presence of succinate	12.9	6.5–14.3
In the presence of decyl-ubiquinol	**6.7**	8.5–23.2
Mitochondrial RC enzymatic activities (nmol per min per mg protein)		
Complex I (mitochondrial fraction)	**8.6**	10.0–15.0
Complex II	15	11–17
Complex III	**30**	98–180
Complex II+III	**20**	21–42
Complex IV	87	72–143
TCA cycle enzymatic activities (nmol per min per mg protein)		
Citrate synthase	69	32–72
Aconitase	6.4	3.7–8.5
Isocitrate dehydrogenase	33	21–37
α-Ketoglutarate dehydrogenase	4.7	3.6–7.2
Fumarase	50	40–60
Malate dehydrogenase	19	9–35
Immunoblot quantifications of native complexes		
CI	**24%**	81–119%
CII	110%	84–116%
CIII	**26%**	94–106%
CIV	93%	75–125%
CV	83%	81–119%

Abbreviations: CI, Complex I; CII, Complex II; CIII, Complex III; CIV, Complex IV; CV, Complex V.
Abnormal results are indicated in bold.
Fibroblasts were cultured in HAM F10 medium with 10% fetal calf serum. Oxygen consumption, enzymatic activities of RC complexes CII, CIII, CIV and CII+III, and enzymatic activities of TCA cycle enzymes were performed in permeabilized cells. Activity of CI was measured in mitochondria-enriched fraction. Immunoblot with a non-denaturing electrophoretic separation (Blue-Native PAGE) was performed on a part of mitochondria-enriched fraction as already described. Briefly, 10 μg of solubilized proteins were loaded on a 4–16% gradient acrylamide non-denaturing gel and immunoblotting was performed with monoclonal antibody (Abcam) raised against CI (subunit GRIM19, 1:1200), CII (subunit SDHA, 1:2400), CIII (subunit UQCRC2, 1:3000), CIV (subunit MT-CO1, 1:2000) or complex V (subunit ATPB, 1:3000). Quantification was performed using NIH ImageJ software (Bethesda, MD, USA) and the results were normalized to control cells set at 100%.

DISCUSSION

We present here the second reported case of mutation in the *UQCRC2* gene, causing mitochondrial defect. Our study confirmed that the p.Arg183Trp mutation in core protein 2 leads to a marked decrease in CIII activity and quantity in fibroblasts, as observed for P1 in the study of Miyake *et al.*[7] Immunoblot analysis revealed severe reduction in the quantity of free CI in our own and in the previous study (respectively −76 and −50% of control). These results confirm that the mutation in core protein 2 alters the stability of free CI.

Based on the increase in urinary dicarboxylic acid levels and TCA cycle intermediates, additional roles of CIII core protein 2 in beta-oxidation or TCA cycle function were suggested.[7] A comparable metabolic pattern was observed in our patient. However, beta-oxidation function was normal in our patient's fibroblasts and we showed that TCA cycle enzyme activities were normal, along with oxygen consumption in the presence of pyruvate or succinate (Table 1). Our investigations suggested therefore that the urinary metabolite profile observed in *UQCRC2* deficiencies reflected probably the RC blockade.

The clinical presentations of our patient and the three related patients previously reported were all characterized by recurrent access of lactic acidosis with glycemic disturbance. However, transient but severe hepatocellular deficiency observed in our patient was not reported in the other cases. Interestingly, the patients had a normal neurological function at 9, 5 and 4 years (patient P3 is too young to determine long-term neurological outcome). The other three cases with CIII deficiency that we previously described, due to mutations in structural subunits cytochrome c_1 (*CYC1*) and protein Hum-QPC (*UQCRB*), all presented neonatal lactic acidosis also and a normal cognition at 7, 22 and 19 years, respectively.[5,8] Intriguingly, the 20 familial cases with mutations in assembly genes *LYRM7* and *TTC19* were almost all marked by long-term neurological impairment.[2,12] The number of patients is too small to determine if this results from random difference in prevention of toxic encephalopathy during decompensation or if the defect in *CYC1*, *UQCRC2* and *UQCRB*-encoded proteins is less deleterious for brain development. However, *LYRM7* and *TTC19* mutation-induced encephalopathy was not systematically preceded by metabolic crisis, leading some authors to suggest that the LYRM7 and TTC19 proteins play additional roles in brain function. CIII deficiency observed in these cases may not constitute the primary cause of encephalopathy.[12]

In conclusion, our study reports a new case of primary deficiency of CIII due to a mutation in a structural subunit and characterized by a neonatal lactic acidosis without long-term neurological impairment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)