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SHORT COMMUNICATION

Mutation analysis of *COX18* in 29 patients with isolated cytochrome *c* oxidase deficiency

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Isolated cytochrome *c* oxidase (COX) deficiency (MIM#220110) is a relatively common biochemical finding in pediatric patients with mitochondrial disorder. It has been associated with different clinical phenotypes ranging from isolated myopathy to severe multisystem disorder. It is a genetically heterogeneous trait, and the most frequent genetic defects affect *SURF1* and *SCO2*, two genes required for COX assembly. However, a significant proportion of patients lacks mutation in these genes and in other known genes that require COX biogenesis. *COX18* is a novel COX assembly gene required for membrane insertion of the C-terminal portion of COX subunit II. We have studied 29 pediatric patients with isolated COX deficiency in the skeletal muscle associated with different clinical phenotypes. Mutations in *SURF1*, *SCO2*, *SCO1*, *COX10*, *COX15* and in mitochondrial DNA, had been ruled out earlier. The *COX18* gene was analyzed using a PCR-single-stranded conformation polymorphism (PCR-SSCP) protocol, and in 15 patients, the analysis was repeated by direct sequencing. No pathogenic mutations were detected in our cohort of patients indicating that *COX18* mutations may be very rare or associated with other phenotypes than isolated COX deficiency in infancy.

INTRODUCTION

Cytochrome *c* oxidase (COX), the terminal component of the mitochondrial electron transport chain, comprises 13 protein subunits, 3 encoded by mitochondrial DNA (mtDNA) and 10 by nuclear DNA (nDNA).[1] COX requires other ancillary proteins for its biogenesis, encoded by COX assembly genes, which synthesize the prosthetic groups, transport and insert the metal cofactors, as well as assemble the different subunits to form a holoenzyme.[2] In humans, isolated COX deficiency (MIM#220110) is a common cause of mitochondrial dysfunction in infancy and is clinically and genetically heterogeneous.[3] Mutations in the mtDNA-encoded subunits are rare; most defects are inherited as autosomal recessive traits, but mutations in nDNA-encoded subunits have been found only in one patient.[4] However, mutations have been detected in six COX assembly genes, namely *SURF1*, *SCO1*, *SCO2*, *COX10*, *COX15* and *LRPPRC* (see Barrientos *et al.*[2] for individual references). *SURF1* and *SCO2* account for the majority of reported defects;[5] mutations in *COX10*, *COX15* and *SCO1* are less frequent, whereas those in *LRPPRC* are specific to Leigh Syndrome French Canadian type, a distinct clinical entity found (almost) exclusively in patients from the Saguenay-Lac Saint-Jean region of Canada.[6] Nevertheless, it is not possible to find any defect in a large proportion of patients.[7,8]

We have identified a novel human COX assembly gene, *COX18*,[9] which encodes a mitochondrial protein of 333 amino acids. It belongs

to the Alb3–Oxa1–YidC protein family,[10] which is required for the processing of transmembrane proteins in mitochondria, chloroplasts and bacteria. In yeast, Cox18p is required for the insertion of the C-terminal tail of COX subunit II into the mitochondrial inner membrane. *COX18^null Saccharomyces cerevisiae* show severe isolated COX deficiency, and the human gene can complement the defect.[11,12] Therefore, we tested whether mutations of human *COX18* could play a role in patients with isolated COX deficiency.

MATERIALS AND METHODS

Patients

We have studied 29 patients, of different ethnical background, who had biochemically documented isolated COX deficiency in muscle and different clinical phenotypes. A total of 21 patients had been screened earlier for mutations in the 3 mitochondrial COX subunits, the 22 mtDNA transfer RNA (tRNA) genes and in 7 COX assembly genes, namely *SCO1*, *SCO2*, *SURF1*, *COX10*, *COX11*, *COX15* and *COX17* (patients 3–7, 9–11, 13–16, 19–22, 24–27 and 29–30 as reported by Sacconi *et al.*[7]). Eight previously unpublished patients were included in the study. Five presented with encephalomyopathy and liver failure but lacked radiological features of the LS. Two patients had LS, and two had encephalomyopathy without LS. Muscle biopsies had been obtained with the informed consent of the patients' legal tutors, and were analyzed as reported.[7] All had isolated COX deficiency in muscle,

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and histochemistry showed a diffuse reduction in COX staining. They tested negative for mutations in *COX10*, *SCO1*, *SCO2*, *SURF1*, *COX15*, mitochondrial tRNA genes, mitochondrial COX subunits and in *COX6B1*. Eight of the nine patients with hepatic involvement had also been screened for mutations in *LPPRC*. Phenotypes have been summarized in Table 1.

The six coding exons of *COX18* and intron–exon boundaries amplified using primers and conditions are summarized in Table 2. The amplicons were analyzed by single-stranded conformation polymorphism (SSCP) using a reported protocol.[13] Owing to its size, exon 1 was analyzed by direct sequencing in all patients. Fragments showing abnormal patterns were sequenced as described.[13]

In a subset of patients, the whole coding region of *COX18* was also sequenced directly using the same primer set.

RESULTS AND DISCUSSION

Single-stranded conformation polymorphism analysis did not detect pathogenic mutations in our cohort of patients. As *COX18* is highly expressed in the liver, we focused on the group of patients with predominant hepatic involvement. To exclude that, our protocol had overlooked some mutations (we estimate its sensitivity to be ~80–90%); we sequenced the whole coding region of *COX18* in these nine patients, and also in four patients with LS, in one patient with encephalomyopathy and in the patient with isolated myopathy. Again, no pathogenic mutations were detected. It should be noted that there were no discrepancies between the results of the SSCP analysis and of direct sequencing in these patients.

We considered that *COX18* could be an interesting candidate gene in patients with isolated COX deficiency, because *COX18^{null}*

S. cerevisiae strains retain some residual COX activity,[11] and this fact may be important for avoiding the embryonic lethality-associated complete mitochondrial respiration defects.[14]

Our analysis did not detect pathogenic mutations in this gene, even in the 15 patients who were analyzed by direct sequencing. *COX18* mutations might be very rare or associated with other phenotypes than those that we studied.

Mutation screening in these patients is problematic because of the large (and still growing) number of candidate genes. The clinical picture is often not helpful in directing the genetic analysis, because there is a significant overlap between phenotypes associated with specific gene defects (for example, hypertrophic cardiomyopathy is a feature of *SCO2*, *COX10* and *COX15* mutations[2]), and because of the phenotypic variability of mutations in individual genes, such as *COX10*[15] and *COX15*.[16] Moreover, *SURF1* defects, which are usually associated with LS, may also present with different clinical phenotypes.[17,18]

Most patients with COX deficiency are isolated cases and therefore, it is impossible to use traditional genetic techniques, such as linkage or homozygosity mapping, to direct molecular analyses. Microcell-mediated chromosome transfer has been used successfully for identifying specific gene defects,[19] but it is a complex and manpower-intensive procedure and is not suitable for analyzing large cohorts of patients. Simpler methods have been developed on the basis of functional complementation of cultured fibroblasts using specific lentiviral vectors;[15] however, for most of our patients, the muscle biopsy fragment was the only biological material available for our study. Therefore, we stress the importance of collecting skin fibroblasts in all patients in whom a respiratory chain defect is suspected.

Novel high-throughput techniques will soon become available, and it will be possible to rapidly analyze a large panel of genes in individual patients at reasonable costs. Therefore, it is important to identify other human COX assembly genes as only a portion of the at least 30 different yeast genes involved in COX biogenesis[20] have been characterized in humans.

Table 1 Clinical phenotypes of patients involved in the study

Clinical features	Number of patients
Leigh Syndrome	6
Encephalomyopathy	10
Hepato-encephalomyopathy	9
Hypertrophic cardiomyopathy	4
Isolated myopathy	1
Lactic acidosis, white matter disease	1

All patients had cytochrome *c* oxidase (COX) enzymatic activity in muscle for <30% of controls and diffuse reduction of COX staining at histochemistry.

Table 2 PCR primers used to amplify *COX18*

EXON		
1	For	tgactgggaaggtcaatcgc
	Rev	aatgtcggccttcctccgc
2	For	gagggaataatgtgaaagattatg
	Rev	tatgtgtgcgtatggtcttac
3	For	ctatggaaataaagcacgdattc
	Rev	caaagacaatgttagagaacttc
4	For	ctgctattttaggacttctattg
	Rev	gatttgaagtcaaaacagcagc
5	For	aagcttgtctgatttaatatgcg
	Rev	gctaaagtcagagaggatttag
6	For	tggactgtgatgtccttgtag
	Rev	catttagatgatagtgactcctg

All reactions were carried out in the presence of 5% dimethyl sulfoxide. PCR conditions were 94 °C 3 min; 35 cycles of 94 °C 1 min, 55 °C 1 min, 72 °C 1 min; and a final extension step of 72 °C for 7 min.

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