Studies of COX16, COX19, and PET191 in Human Cytochrome-c Oxidase Deficiency

Stacey K. H. Tay, MD; Claudia Nesti, PhD; Michelangelo Mancuso, MD; Eric A. Schon, PhD; Sara Shanske, PhD; Eduardo Bonilla, MD; Mercy M. Davidson, PhD; Salvatore DiMauro, MD

Background: Cytochrome-c oxidase (COX) is the terminal enzyme of the mitochondrial electron transport chain, and COX deficiency is a common cause of mitochondrial diseases. Cytochrome-c oxidase is composed of 13 subunits, of which 3 are encoded by mitochondrial DNA and 10 by nuclear DNA. Mutations have been identified in each of the 3 mitochondrial DNA genes but in none of the nuclear DNA genes. However, COX deficiency has been attributed to mutations in several nuclear DNA–encoded ancillary proteins needed for COX assembly and function. Despite this progress, the molecular basis of COX deficiency remains elusive in many patients, justifying the identification and screening of additional COX assembly genes, such as COX16, COX19, and PET191.

Objective: To determine if COX16, COX19, and PET191 are implicated in human COX deficiency.

Methods: Mutation screening was performed on 53 patients with isolated COX deficiency by direct sequencing of COX19 and by single-strand conformational polymorphism analysis for COX16 and PET191.

Results: No mutations were found in COX16, COX19, or PET191 in these patients.

Conclusions: The COX16, COX19, and PET191 genes are either not involved or very rarely involved in human COX deficiency. Mutations in additional COX assembly genes remain to be identified.


COX oxidase (COX), the terminal enzymatic complex of the mitochondrial electron transport chain, transfers electrons from cytochrome c to molecular oxygen and pumps protons across the inner mitochondrial membrane. It is a multiheteromeric enzyme that comprises 13 subunits and 2 copper-containing prosthetic heme groups (a and a3). Isolated COX deficiency has been described in numerous mitochondrial disorders, and mutations have been identified in each of the mitochondrial DNA (mtDNA)–encoded COX subunits. Occasionally, selective COX deficiency has been associated with mutations in transfer ribonucleic acid (tRNA) genes. Interestingly, no mutation has been identified as yet in any of the nuclear DNA–encoded COX subunits. However, mutations have been found in several nuclear genes encoding ancillary proteins, needed for the correct assembly of COX. In the yeast Saccharomyces cerevisiae, there are more than 30 different genetic complementation groups for COX assembly.

Methods

PATIENTS

A total of 53 children with COX deficiency were included in this study. We defined COX deficiency as COX activity in muscle (relative to citrate synthase) lower than 33% of the normal mean, with other respiratory chain enzyme activities in the normal ranges. Seventeen of the 53 patients had already tested negative for mutations in the 3 mtDNA COX genes, the 22 mtDNA tRNA genes, and the COX assembly genes SURF1, SCO1, SCO2, COX10, COX11.
COX15, and COX17. The remaining 36 patients were divided into 4 main phenotypic groups and had been screened for the appropriate mutations. Group 1 included patients with Leigh syndrome who tested negative for SURF1 mutations; group 2 included patients with cardiomyopathy who tested negative for mutations in SURF1, SCO2, and COX15; group 3 included patients with hepatoencephalomyopathy who tested negative for mutations in SCO1, SURF1, the mtDNA COX genes, and the tRNA genes; and group 4 included patients with isolated myopathy who tested negative for mutations in SURF1, the mtDNA COX genes, and the tRNA genes.

Biopsies were performed with the informed consent of parents or guardians, and all studies were approved by the institutional review board of Columbia University College of Physicians and Surgeons, New York, NY.

**BIOCHEMICAL AND HISTOCHEMICAL ANALYSIS**

Measurements of respiratory chain enzyme activities were performed in skeletal muscle homogenates as previously described. Muscle biopsy slices were stained for COX and succinate dehydrogenase as previously described.

**DNA ANALYSIS**

DNA was extracted from muscle biopsy specimens, blood, and fibroblasts according to standard protocols. The entire coding regions of COX16, COX19, and PET191 were amplified as summarized in the Table. Mutation screening of COX19 was performed by direct sequencing, using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit and 310 Automatic Sequencer (Applied Biosystems, Foster City, Calif). COX16 and PET191 were studied by single-strand conformational polymorphism analysis.

For single-strand conformational polymorphism analysis, a total of 50 to 100 ng of genomic DNA was amplified as described in the Table. Reactions were performed in 25 µL of 10mM Tris hydrochloride (pH 8.9); 1.5mM magnesium chloride; 0.4µM forward and reverse oligonucleotides each; 0.2mM deoxynucleosine triphosphate, deoxyguanosine triphosphate, and deoxythymidine triphosphate each; 0.02mM deoxyctydine triphosphate; 1µCi (37037 Bq) of α-32P deoxyctydine triphosphate; and 1.25 U of Taq DNA polymerase (Roche Diagnostics, F. Hoffman-La Roche Ltd, Basel, Switzerland). Samples were denatured and separated on a 6% MDE gel solution (Cambrex BioScience Rockland, Rockland, Me), with 5% glycerol, according to the manufacturer’s protocol. Single-stranded conformers were visualized by autoradiography using BIOMAX film (Kodak, Rochester, NY). Samples with abnormal patterns were sequenced as described earlier.

**EXON PRIMERS PCR CONDITIONS**

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primers</th>
<th>PCR Conditions</th>
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<tbody>
<tr>
<td>1</td>
<td>GCCTG6AATTTCTTCTCAC</td>
<td>94°C 3 min (94°C 30 s, 55°C 30 s, 72°C 60 s); 30 cycles, 72°C 7 min</td>
</tr>
<tr>
<td>2</td>
<td>TAGC6ACAAGAGGGGAGGAT</td>
<td>94°C 3 min (94°C 30 s, 53°C 30 s, 72°C 60 s); 30 cycles, 72°C 7 min</td>
</tr>
<tr>
<td>3</td>
<td>CCTAAACGAGAAACATACA</td>
<td>94°C 3 min (94°C 30 s, 54°C 30 s, 72°C 60 s); 30 cycles, 72°C 7 min</td>
</tr>
<tr>
<td>4</td>
<td>TGGTACCACGGAAAAATA</td>
<td>94°C 3 min (94°C 30 s, 55°C 30 s, 72°C 60 s); 30 cycles, 72°C 7 min</td>
</tr>
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</table>

**RESULTS**

We studied 53 unrelated patients with isolated COX deficiency. Cytochrome-c oxidase activity in skeletal muscle ranged from 3% to 30% of the mean control value, while other respiratory chain enzyme activities were normal. The biochemical finding of COX deficiency was confirmed by histochemical analysis. Ragged red fibers were identified in only 1 patient.

All patients had onset of disease in infancy or early childhood, with clinical symptoms suggestive of mitochondrial disorders, such as encephalomyopathy (with or without neuroradiological evidence of Leigh syndrome) (43 of 53), hepatoencephalopathy (2 of 53), hypertrophic cardiomyopathy (6 of 53), or isolated myopathy (2 of 53). No pathogenic mutations were detected in any of the 3 COX assembly genes (COX16, COX19, PET191) that we screened.

**COMMENT**

Cytochrome-c oxidase is the terminal enzyme of the mitochondrial electron transport chain, and COX deficiency...
is a common cause of mitochondrial disorders. To date, pathogenic mutations have been found in the 3 mtDNA-encoded COX subunits and in several nuclear genes encoding COX assembly proteins. These proteins are required for the correct folding and maturation of COX subunits and for the synthesis, delivery, and insertion of prosthetic groups into the holoenzyme.

Despite these advances, the molecular basis of COX deficiency remains elusive in many patients, thus justifying the search for other candidate genes. COX16, COX19, and PET191 are nuclear genes needed for the assembly of COX in yeast, and it is conceivable that their human homologues might harbor pathogenic mutations. Therefore, we screened 53 patients with COX deficiency of unknown cause and heterogeneous clinical phenotypes for mutations in the COX16, COX19, and PET191 genes.

COX16 encodes a novel protein, Cox16p, required for the assembly of COX in S cerevisiae. Cox16p consists of 118 amino acid residues and has a predicted mass of 14.1 kDa. The sequence includes a potential membrane domain, suggesting that Cox16p may be an integral membrane protein. Human COX16 is located on the long arm of chromosome 14, in the interval 14q22.1-14q24.3 (LOC51241). There is high sequence conservation in the region of the transmembrane domain and in the C-terminal half.

COX19 encodes an 11.1-kDa protein, Cox19p, which is present both in the cytoplasm and in the intermembrane space of the mitochondria, suggesting a post-translational role in the assembly of the COX holoenzyme. Similarities in subcellular localization and in the shared presence of 4 cysteines between Cox19p and Cox17p, a copper protein required for the maturation of the CuA center of COXII, suggest that Cox19p may also function in metal transport to the mitochondria. Human COX19, located on the short arm of chromosome 7, is an attractive candidate gene for at least 1 reported patient with COX deficiency but no molecular defect. PET191 is located on the long arm of chromosome 2 (2q11.2). Although the encoded protein has a role in the assembly of active COX in S cerevisiae, its precise function is still unclear.

While COX16, COX19, and PET191 were attractive candidate genes for human COX deficiency, sequence analysis and single-strand conformational polymorphism analysis in a large cohort of patients with isolated COX deficiency did not support an etiological role for any of these genes. One other attractive candidate that we were unable to study owing to insufficient DNA is the LRPPRC gene, described by Mootha et al in patients with Leigh syndrome and COX deficiency. This and other candidate genes will have to be studied to clarify the molecular basis of the many unexplained cases of isolated COX deficiency.

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Correspondence: Salvatore DiMauro, MD, 4-420 College of Physicians and Surgeons, 630 W 168th St, New York, NY 10032 (sd12@columbia.edu).

Author Contributions: Study concept and design: Tay, Nesti, Mancuso, Schon, Shanske, Bonilla, Davidson, and DiMauro. Acquisition of data: Tay and Nesti. Analysis and interpretation of data: Tay, Nesti, and Mancuso. Drafting of the manuscript: Tay and Nesti. Critical revision of the manuscript for important intellectual content: Mancuso, Schon, Shanske, Bonilla, Davidson, and DiMauro. Statistical analysis: Nesti. Obtained funding: Shanske and DiMauro. Administrative, technical, and material support: Tay, Nesti, Schon, Shanske, Davidson, and DiMauro. Study supervision: Mancuso, Bonilla, Davidson, and DiMauro.

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Additional Information: Drs Tay and Nesti contributed equally to this work.