Hum Genet (1997) 101 : 247–250

© Springer-Verlag 1997

RAPID COMMUNICATION

**Béatrice Parfait · Agnès Percheron**
**Dominique Chretien · Pierre Rustin · Arnold Munnich**
**Agnès Rötig**

# No mitochondrial cytochrome oxidase (COX) gene mutations in 18 cases of COX deficiency

Received: 21 May 1997 / Accepted: 5 August 1997

**Abstract** Cytochrome *c* oxidase (COX) deficiency causes a variety of neuromuscular and non-neuromuscular disorders in childhood and adulthood and can theoretically undergo either a nuclear or a mitochondrial (mt) mode of inheritance, making genetic counseling in COX deficiency particularly hazardous. In an attempt to determine the respective roles of mtDNA and nuclear DNA mutations in COX deficiency, we sequenced the three mitochondrially encoded COX subunits (COXI–III) in a series of 18 patients with isolated COX deficiency, especially as COXI-III code for the catalytic site of the enzyme. We failed to detect any deleterious mutations in this series. Moreover, no mtDNA deletion was observed and sequencing of the flanking tRNA genes involved in the maturation of the COX transcripts failed to detect deleterious mutations as well. The present study supports the view that the disease-causing mutations do not lie in the mt genome but, rather, in the nuclear genes encoding either the COX subunits or the proteins involved in assembly of the complex and suggests a recurrent risk of 25% rather than other modes of inheritance in COX deficiencies.

## Introduction

Cytochrome *c* oxidase (COX) deficiency is a group of clinically heterogeneous defects of oxidative phosphorylation accounting for a large variety of neuromuscular and non-neuromuscular symptoms in childhood and adulthood (DiMauro et al. 1990; Cormier et al. 1991; Edery et al. 1994). Owing to the twofold genetic origin of the mitochondrial (mt) respiratory chain, any mode of inheritance can be theoretically observed in COX deficiency, including autosomal and maternal inheritances. This genetic heterogeneity makes genetic counseling in COX deficiency particularly hazardous. In an attempt to determine the respective roles of mtDNA and nuclear DNA mutations in COX deficiency, we sequenced the three mitochondrially encoded COX subunits (COXI–III) in a series of 18 patients with isolated COX deficiency, especially as COXI–III code for the catalytic site of the enzyme. None of our 18 patients harbored mtDNA mutations or deletions, suggesting that nuclear gene mutations largely account for COX deficiency, an observation which is highly relevant to genetic counseling of these devastating conditions.

B. Parfait · A. Percheron · D. Chretien · P. Rustin · A. Munnich
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
Fax: + 33 01 47 34 85 14; e-mail: roetig@necker.fr

## Patients and methods

A total of 18 patients with isolated COX deficiency was included in this study. Their clinical presentation is reported in Table 1 and enzyme activities were measured on various tissues (Rustin et al. 1994). Total DNA extracted from various COX-deficient tissues (lymphocytes, muscle, liver, heart, skin fibroblasts) was submitted to PCR amplification using primers specific for mitochondrial COX genes (COXI: A5′ nucleotides 5881–5901, A3′ 6195-6175, B5′ 6136–6156, B3′ 6450–6430, C5′ 6385–6405, C3′ 6710–6690, D5′ 6450–6470, D3′ 6970–6951, E5′ 6910–6930, E3′ 7230–7210, F5′ 7170–7190, F3′ 7471–7451; COXII: A5′ 7561–7581, A3′ 7983–7963, B5′ 7923–7943, B3′ 8308–8288; COXIII: A5′ 9185–9205, A3′ 9505–9485, B5′ 9441–9461, B3′ 9765–9745, C5′ 9705–9725, C3′ 10014–9994) and tRNA genes ($tRNA^{Ser}$ and $tRNA^{Asp}$: 5′ nucleotides 7170–7190, 3′ 7630–7610; $tRNA^{Lys}$: 5′ 8200–8220, 3′ 8613–8593; $tRNA^{Gly}$: 5′ 9898–9918, 3′ 10700–10680; $tRNA^{Leu}$: 5′ 3130–3149, 3′ 3423–3404). Amplification conditions included 30 cycles of 30 s, 95°C; 30 s, 50°C; 30 s, 72°C.

For sequence analysis, amplification products were purified in 2% low-melting point agarose gels and recovered by heating for 5 min at 65°C. Direct sequencing was performed using 3.2 pmol of the amplification primer, 100 ng DNA, and 8.5 μl sequencing reaction mixture (Prism Ready Reaction Sequencing kit; Perkin-Elmer Cetus) on an automatic fluorimetric DNA sequencer (Applied Biosystems).

## Results

Sequence analysis of the three mitochondrial COX genes in tissues which actually expressed the disease failed to

**Table 1** Clinical presentations of patients with isolated COX deficiencies. Cytochrome c oxidase/succinate cytochrome c reductase ratio points out the COX deficiencies. Control values: 3.2 ± 0.3. +++: 0 to 0.8, ++: 0.8 to 1.5, +: 1.5 to 2.3. *M* Multiplex family, *C* consanguineous family, *IUGR* intrauterine growth retardation

| Patient | | Symptoms/syndromes | Affected tissues | COX deficiency | Reference |
|---|---|---|---|---|---|
| 1 | M | Growth failure, psychomotor delay, deafness, cataract, hepatomegaly | Muscle,<br>lymphocytes,<br>fibroblasts | ++<br>++<br>+++ | Cormier-Daire et al. (1996) |
| 2 | C | Myoglobinuria | Fibroblasts | ++ | Saunier et al. (1995) |
| 3 | M | Myoclonic epilepsy, encephalopathy, liver involvement, myocardiopathy | Fibroblasts | + | |
| 4 | C | Hypotonia, tubulopathy, retinitis pigmentosa, growth failure, pyramidal syndrome | Muscle | +++ | Cormier et al. (1991) |
| 5 | C | de Toni-Debré-Fanconi syndrome and Leigh syndrome | Lymphocytes | ++ | Ogier et al. (1988) |
| 6 | | Myocardiopathy | Muscle | ++ | |
| 7 | | Neonatal hypotonia | Lymphocytes | ++ | |
| 8 | | Alpers disease | Liver,<br>fibroblasts,<br>lymphocytes | +<br>++<br>++ | Chabrol et al. (1994) |
| 9 | | Hypotonia, growth failure, hepatocellular dysfunction | Liver,<br>fibroblasts,<br>lymphocytes | ++<br>++<br>++ | |
| 10 | | IUGR, premature ageing, dwarfism, osteoporosis, diarrhea, mental delay | Fibroblastes | + | |
| 11 | | Liver failure | Liver | +++ | Edery et al. (1994) |
| 12 | M | Hyperlactacidemia | Muscle | +++ | |
| 13 | C | Leukodystrophy, hyperlactacidemia, peripheral neuropathy | Muscle,<br>fibroblasts | +<br>+++ | |
| 14 | | Leigh syndrome | Muscle,<br>lymphocytes | + | |
| 15 | | Ophthalmoplegia, progressive encephalopathy, myopathy, epileptic seizures | Muscle | ++ | |
| 16 | | Myocardiopathy | Liver | + | |
| 17 | | Hypotonia, pyramidal syndrome, cerebellar syndrome | Lymphocytes | +++ | |
| 18 | | Growth failure, tubulopathy, | Muscle,<br>lymphocytes,<br>fibroblasts | +<br>++<br>+++ | |

detect any deleterious mutations in 18 patients with COX deficiency (COXI–III). As mt tRNAs are involved in the maturation of mtRNA transcripts (Ojala et al. 1981), we hypothesized that mutations in the flanking tRNAs ($tRNA^{Ser}$, $tRNA^{Asp}$, $tRNA^{Lys}$, $tRNA^{Gly}$) could account for isolated COX deficiency. Sequencing these tRNA genes failed to detect any point mutation in our patients. In addition, owing to the observation of an isolated COX deficiency in a patient with the MELAS syndrome harboring the 3243 $tRNA^{Leu}$ point mutation (Goto et al. 1992), we also sequenced the $tRNA^{Leu}$ gene and failed to find any abnormality in our series. Finally, patients presenting with Leigh syndrome or cardiomyopathy were screened for the NARP mutation (MITOMAP 1995) and the cardiomyopathy mutations, respectively (MITOMAP 1995). None of these mutations could be detected in our series and none of our patients presented large-scale mtDNA deletions.

Alignment of the nucleotide sequences with the Cambridge reference sequence (Anderson et al. 1981) led us to identify four homoplasmic base changes, resulting in amino acid substitutions. First, an A→G substitution at nucleotide 6663, in patient 15, changed an isoleucine into a valine in the COXI subunit. This transition was absent in 200 control DNAs and alignment of 93 COXI sequences from different species showed that the isoleucine at codon 254 was replaced by a valine in 10/93 species. Various amino acids were found at this codon in nine other species (methionine, phenylalanine, leucine, alanine). Second, a G→A transition at residue 9477, changing a valine into an isoleucine, was found in the COXIII gene of two unrelated patients (patients 4 and 16). This base change has been previously reported as a rare polymorphism (MITOMAP 1995). Similarly, the G→C transversion in the COXIII gene (nucleotide 9559) observed in all patients and two unrelated controls and changing an arginine into a proline has been previously reported as a polymorphism (MITOMAP 1995). Finally, a homoplasmic T→C transition at residue 9903 (patient 10), changing a phenylalanine into a leucine in the COXIII subunit of patient 10 and in her unaffected mother, led us to regard this base substitution as a rare polymorphism as well. Sequence analysis of mt tRNA and COX genes revealed numerous conservative polymorphisms, including seven previously reported and

**Table 2** Polymorphic nucleotide substitutions in patients with COX deficiencies

| Nucleotide substitutions | Patient |
|---|---|
| C5922T | 5 |
| T5999C | 17 |
| A6047G | 17 |
| A6146G | 17 |
| C6164T | 17 |
| T6221C[a] | 1, 10 |
| G6260A | 14 |
| T6293C | 17 |
| C6371T | 1, 10 |
| G6528A | 17 |
| A6663G | 15 |
| T6680C[a] | 2, 5 |
| T6734A | 6 |
| T7022C | 9 |
| C7028T[a] | 1, 3–7, 9, 10, 13–18 |
| T7175C | 5 |
| C7256T | 5, 15 |
| C7274T | 5 |
| C7277T | 15 |
| G7337A | 12 |
| A7768G | 4, 16 |
| A7771G | 5, 15 |
| G7831C | 10 |
| C7915T | 2 |
| C7927G | 16 |
| G8206A[a] | 5 |
| G8251A[a] | 6 |
| G9206A | 15 |
| A9221G | 5 |
| G9477A[a] | 4, 16 |
| T9482C | 1, 3 |
| T9540C[a] | 2, 15 |
| T7541C | 5 |
| A9545T | 17 |
| G9559C[a] | 1–18 |
| T9688C | 14 |
| T9716C | 25 |
| C9788T | 5 |
| T9899C | 1 |
| T9903C | 10 |

[a] Polymorphism already described (MITOMAP 1995)

28 unreported polymorphisms (Table 2). All these polymorphisms appeared to be homoplasmic by sequence.

## Discussion

The mt COX genes represent obvious candidate genes in COX deficiency as they encode the catalytic site of the enzyme in eukaryotes. For this reason and despite the absence of maternal inheritance in our patients, we sequenced the COXI–III genes of 18 COX-deficient patients and failed to detect any deleterious mutation or microdeletion in our series. Similarly, sequencing of the flanking tRNA genes did not reveal deleterious mutations either.

Although one cannot formally exclude disease-causing mutations in other tRNAs or in rRNAs of the mt genome, such mutations seem unlikely as they are expected to produce multiple or generalized respiratory chain deficiency. Similarly, mtDNA mutations/deletions altering complexes I–III and V genes are unlikely as they usually result in defects of the corresponding complex, as shown for the ATPase deficiency observed in the NARP mutation (Tatuch and Robinson 1993) or the COX deficiency in the 15-bp deletion of the COXIII gene (Keightley et al. 1996). For these reasons, the present results support the view that the disease-causing mutations in our patients do not lie in the mt genome but, rather, in the nuclear genes encoding either the COX subunits or the proteins involved in assembly of the complex. Recurrence of the disease and consanguinity in several sibships add to the view that an autosomal recessive mode of inheritance could be involved in several cases. Alternative hypotheses in non-inbred families include X-linked recessive inheritance and, possibly, de novo dominant mutations, especially as an advanced paternal age at the time of conception has been occasionally observed in our series (not shown). Almost all respiratory enzyme nuclear genes in humans are known and sequenced. Up till now, however, little is known regarding the genes involved in respiratory chain assembly. In yeast, 11 genes have already been shown to be involved in assembly of the respiratory chain components. Among them, OXA1 plays a role in complex IV and V assembly (Altamura et al. 1996). Its human counterpart has been identified and mapped to chromosome 14 (Molina-Gomes et al. 1995) but its mutations are expected to cause a multiple enzyme deficiency rather than an isolated COX deficiency.

Whatever the disease-causing nuclear genes, the present study is highly relevant to genetic counseling. Indeed, absence of mtDNA mutations/deletions in our series strongly supports a recurrent risk of 25% rather than other modes of inheritance in COX deficiencies (including maternal or sporadic). In the absence of reliable enzymatic or molecular prenatal diagnoses, this study should therefore prompt sperm donation rather than oocyte donation for couples eliciting medically assisted procreation.

## References


Altamura N, Capitanio N, Bonnefoy N, Papa S, Dujardin G (1996) The *Saccharomyces cerevisiae* OXA1 gene is required for the correct assembly of cytochrome *c* oxidase and oligomycin-sensitive ATP synthase. FEBS Lett 382:111–115

Anderson S, Bankier AT, Barrell BH, Bruijn MHL de, 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

Chabrol B, Mancini J, Chretien D, Rustin P, Munnich A, Pinsard N (1994) Cytochrome *c* oxidase defect, fatal hepatic failure and valproate: a case report. Eur J Pediatr 153:133–135

Cormier V, Rustin P, Bonnefont JP, Rambaud, Vassault A, Rabier D, Parvy P, Couderc S, Parrot-Roulaud F, Carré M, Risse JC, Cahuzac C, Saudubray JM, Rötig A, Hubert P, Munnich A (1991) Hepatic failure in disorders of oxidative phosphorylation with neonatal onset. J Pediatr 6:951–954

Cormier-Daire V, Rustin P, Rötig A, Chretien D, Le Merrer M, Belli D, Le Goff A, Hubert P, Ricour C, Munnich A (1996) Craniofacial anomalies and malformations in respiratory chain deficiency. Am J Med Genet 66:457–463

DiMauro S, Lombès A, Nakase H, Mita S, Fabrizi GM, Tritschler HJ, Bonilla E, Miranda AF, DeVivo DC, Schon EC (1990) Cytochrome *c* oxidase deficiency. Pediat Res 28:536–541

Edery P, Gérard B, Chretien D, Rötig A, Cerrone R, Rabier D, Rambaud C, Fabre M, Saudubray JM, Munnich A, Rustin P (1994) Liver cytochrome *c* oxidase deficiency in a case of neonatal-onset hepatic failure. Eur J Pediatr 1533:190–194

Goto Y, Horai S, Matsuoka T, Koga Y, Nihei K, Kobayashi M, Nonaka I (1992) Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS): a correlative study of the clinical features and mitochondrial DNA mutation. Neurology 42:545–550

Keightley JA, Hoffbuhr KC, Burton MD, Salas VM, Johnston WSW, Penn AMW, Buist NRM, Kennaway NG (1996) A microdeletion in cytochrome *c* oxidase (COX) subunit III associated with COX deficiency and recurrent myoglobinuria. Nat Genet 12:410–416

MITOMAP (1995) Human mitochondrial genome database. Center for Molecular Medicine, Emory University, Atlanta, Ga, USA. (http://www.gen.emory.edu/mitomap.html)

Molina-Gomes D, Bonnefoy N, Nguyen VC, Viegas-Péquignot E, Rötig A, Dujardin G (1995) The OXA1Hs gene which controls cytochrome *c* oxidase assembly maps to the 14q11.2 region of the human genome. Genomics 30:396–398

Ogier H, Lombès A, Scholte HR, Poll-The BT, Fardeau M, Aicardi J, Vignes B, Niaudet P, Saudubray JM (1988) de Toni-Fanconi-Debré syndrome with Leigh syndrome revealing severe muscle cytochrome *c* oxidase deficiency. J Pediatr 112: 734–739

Ojala D, Montoya J, Attardi G (1981) tRNA punctuation model of RNA processing in human mitochondria. Nature 290:470–474

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

Saunier P, Chretien D, Wood C, Rötig A, Bonnefont JP, Saudubray JM, Rabier D, Munnich A, Rustin P (1995) Cytochrome *c* oxidase deficiency presenting as recurrent neonatal myoglobinuria. Neuromuscul Disord 5:285–289

Tatuch Y, Robinson BH (1993) The mitochondrial DNA mutation at 8993 associated with NARP slows the rate of ATP synthesis in isolated lymphoblast mitochondria. Biochem Biophys Res Commun 192:124–128