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Cytochrome c Oxidase Deficiency Associated with the First Stop-Codon Point Mutation in Human mtDNA

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Summary

We have identified the first stop-codon point mutation in mtDNA to be reported in association with human disease. A 36-year-old woman experienced episodes of encephalopathy accompanied by lactic acidemia and had exercise intolerance and proximal myopathy. Histochemical analysis showed that 90% of muscle fibers exhibited decreased or absent cytochrome c oxidase (COX) activity. Biochemical studies confirmed a severe isolated reduction in COX activity. Muscle immunocytochemistry revealed a pattern suggestive of a primary mtDNA defect in the COX-deficient fibers and was consistent with either reduced stability or impaired assembly of the holoenzyme. Sequence analysis of mtDNA identified a novel heteroplasmic G→A point mutation at position 9952 in the patient's skeletal muscle, which was not detected in her leukocyte mtDNA or in that of 120 healthy controls or 60 additional patients with mitochondrial disease. This point mutation is located in the 3′ end of the gene for subunit III of COX and is predicted to result in the loss of the last 13 amino acids of the highly conserved C-terminal region of this subunit. It was not detected in mtDNA extracted from leukocytes, skeletal muscle, or myoblasts of the patient's mother or her two sons, indicating that this mutation is not maternally transmitted. Single-fiber PCR studies provided direct evidence for an association between this point mutation and COX deficiency and indicated that the proportion of mutant mtDNA required to induce COX deficiency is lower than that reported for tRNA-gene point mutations. The findings reported here represent only the second case of isolated COX deficiency to be defined at the molecular genetic level and reveal a new mutational mechanism in mitochondrial disease.

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Introduction

A large number of primary defects of mtDNA have been described in association with a group of human diseases known as the mitochondrial myopathies and encephalomyopathies (Morgan-Hughes 1994). This is a group of clinically and biochemically heterogeneous disorders that frequently have the common feature of mitochondrial proliferation at the level of the single muscle fiber (Morgan-Hughes 1994). The primary mtDNA defects described fall broadly into two groups: large-scale rearrangements and point mutations (Holt et al. 1988; Nelson et al. 1989; Moraes 1996; Schon et al. 1997). Large-scale rearrangements, which include deletions and duplications, are frequently associated with chronic progressive ophthalmoplegia, Pearson syndrome, and Kearns-Sayre syndrome (Morgan-Hughes 1994; Poulton et al. 1989). Point mutations may occur either in tRNA genes or in structural genes. There are now >50 tRNA-gene point mutations that have been described (Moraes 1996), the most common being at nucleotide positions 3243 and 8344, and these frequently are associated with MELAS (mitochondrial encephalomyopathy with lactic acidosis and strokelike episodes) syndrome and MERRF (myoclonus epilepsy and ragged red fibers) syndrome, respectively. Missense point mutations in protein-coding genes are associated with Leber hereditary optic neuropathy (Harding and Sweeney 1994). However, unlike observations made in nuclear-genetic disorders, point mutations leading to premature stop codons (null mutations) in protein-coding genes have not been described in human mtDNA. This is perhaps surprising, in view of the high polymorphic rate of human mtDNA (Brown et al. 1979). Point mutations are inherited in the matrilineal line, whereas single deletions of mtDNA are sporadic and are rarely, if ever, transmitted (Morgan-Hughes 1994).

Cytochrome c oxidase (COX), complex IV of the respiratory chain, catalyzes the transfer of electrons from reduced cytochrome c to molecular oxygen, forming water, and has proton-pumping activity (Capaldi 1990). It comprises 13 subunits, 3 encoded by mtDNA and the

remainder encoded in the nucleus. Isolated complex IV deficiency is not infrequently associated with pediatric disorders such as subacute necrotizing encephalomyelopathy, fatal infantile myopathy, myopathy with cardiomyopathy, and benign reversible COX deficiency (DiMauro et al. 1994). These disorders generally develop during the first few years of life and are thought to be autosomal recessive, but, to date, no nuclear-gene defect has been identified (Adams et al. 1997; Munaro et al. 1997).

We now report an adult patient with recurrent encephalopathy, myopathy, and exercise-induced myalgia who had isolated COX deficiency demonstrated biochemically and histochemically. A new stop-codon point mutation in the COX III gene has been identified, and we provide evidence that, uniquely, this point mutation is not inherited in the matrilineal line.

Patients and Methods

Patients

The proband is a 36-year-old woman who was a normal full-term delivery and who had normal early motor and cognitive development. She was good at sports at school and excelled in middle-distance running. At the age of 17 years she collapsed at the end of a 1.5-km race and was found to be confused and complaining of a headache. She recovered during the next few hours and remained well for the next 4 years. In retrospect, this may have been an episode of exercise-induced lactic acidosis. At the age of 21 years she began to experience symptoms of fatigue after exertion and, soon after exertion, would develop generalized muscle aches that could last for $\leqslant$24 h but were not accompanied by pigmenturia. She stopped drinking alcohol, which exacerbated her myalgia, gave up athletics, and became less active. Her symptoms became much worse during her three pregnancies, although her labors were uneventful. At the age of 28 years, 3 mo after her third child was born, she developed a marked increase in fatigue, with headaches, over the course of 1 wk. She subsequently became drowsy and confused and was admitted to hospital in a stuporous state.

On clinical examination she had a reduced level of consciousness. She exhibited decorticate posturing on painful stimulation, but there was no spontaneous movement. There were roving eye movements. All four limbs exhibited increased tone in a spastic fashion, and there was generalized hyperreflexia with extensor-plantar responses. Laboratory investigations showed an increased anion gap and metabolic acidosis that were due to an elevated level of plasma lactate (7 mmol/liter). Serum creatine kinase was elevated, at 860 IU (normal <150 IU/liter). An electroencephalogram showed widespread slow-wave abnormalities, which resolved over the next 72 h. Results of both computed-tomography brain scan on admission and subsequent magnetic-resonance–imaging brain scans have been normal. She recovered from this episode but had two further identical presentations over the next year. There was no evidence of intercurrent illness preceding any of these episodes. Since the age of 29 years her symptoms and signs have been static. She experiences fatigue and myalgia and frequent common migraine-type headaches. Current clinical examination shows symmetrical proximal (mild) muscle weakness involving all four limbs. Results of electromyogram and nerve conduction studies have been normal. Exercise testing by a bicycle ergometer, as described elsewhere (Petty et al. 1986), has demonstrated an abnormal lactate response to sub–anaerobic-threshold exercise, consistent with a defect in respiratory-chain function. The patient has been unable to tolerate phosphorous magnetic-resonance spectroscopy (MRS) studies of her muscle or proton MRS of her brain.

Her mother had a hypertensive intracerebral hemorrhage at the age of 50 years, from which she recovered, and she is otherwise well. The patient has three sons, ages 16, 15, and 10 years. The youngest son is well. The two older sons describe episodes of myalgia lasting $\leqslant$24 h that are not clearly related to exertion, but they have normal muscle function and, indeed, are both good athletes. The eldest son has a history of predominantly nocturnal episodes in which he becomes confused and describes vivid visual hallucinations. Despite extensive investigation, no abnormality has been identified in these two boys, and, in particular, there is no evidence of respiratory-chain dysfunction (see Results section). Our current hypothesis is that these two sons are exhibiting psychologically driven symptoms reinforced by their mother's illness behavior; they are currently undergoing family therapy.

Methods

Histochemical and immunocytochemical analysis.—Ten-micron cryostat sections of frozen muscle were stained to demonstrate the activities of COX and succinate dehydrogenase (SDH). COX activity was determined in medium containing 4 mM 3,3′-diaminobenzamidine and 100 mM cytochrome c in 0.1 mM phosphate, pH 7.0, at 25°C. SDH activity was determined by 1.5 mM nitroblue tetrazolium, 130 mM sodium succinate, 0.2 mM phenazine methosulfate, and 0.1 mM sodium azide in 0.1 mM phosphate, at pH 7.0 at 25°C. Sections were also stained with the Gomori trichrome stain (Engel and Cunningham 1963).

A battery of subunit-specific mouse monoclonal antibodies was used to identify COX subunits immunohistochemically in serial muscle sections (Taanman et al.

1996). The antibodies were directed against the following subunits: I and II (mtDNA encoded) and IV, Va, and VIc (nuclear-DNA encoded). Visualization was by the Strept ABC technique, by use of 3,3′-diaminobenzidine (Rahman et al. 1997).

Biochemical analysis.—Mitochondria were isolated from skeletal muscle removed from the left quadriceps. Polarographic determination of mitochondrial function, spectrophotometric analysis of respiratory-chain–enzyme activities, and low-temperature analysis of cytochrome content were performed as described elsewhere (Morgan-Hughes et al. 1977; Mann et al. 1992).

mtDNA analysis.—Total DNA was extracted from blood, muscle, skin, fibroblast, and myoblast cultures, by standard techniques described elsewhere (Hanna et al. 1995*b*). Southern blotting was performed on total muscle DNA (Holt et al. 1988). Oligonucleotide primers with M13 tails at the 5′ end were designed to amplify all mitochondrial tRNA genes and all three mitochondrially encoded COX subunits (sequences of primers are available, on request, from the authors). DNA extracted from muscle was used for DNA sequencing. The PCR products generated were cleaned by Centricon filters, and both strands were sequenced by a Dye Primer *Taq* cycle sequencing kit (Applied Biosystems [ABI]). The sequencing products were then separated on 10% polyacrylamide-urea gels in a 373A automated DNA sequencer (ABI). The sequence data generated were analyzed by Seq Ed software (ABI).

A mismatch PCR was designed to screen for the 9952 point mutation identified, in which a restriction site for the endonuclease, *Dra*I, was created in the presence of the mutant mtDNA. The forward primer was light strand (L) 9817–9836; and the reverse mismatch primer was heavy strand (H) 9973–9944 ATG GAG ACA TAC AGA AAT A<u>T</u>T (the mismatch nucleotide is underlined). Conditions for the mismatch PCR were as follows: an initial denaturing step at 94°C for 3 min, followed by 30 cycles of the following: 92°C for 30 s, 53°C for 30 s, and 72°C for 20 s. A final extension step of 72°C for 10 min was used. After digestion with *Dra*I, the fragments were separated on a 3.2% agarose gel stained with ethidium bromide and were visualized on a UV-light box. The 157-bp product was digested into two fragments—of 135 bp and 22 bp—in mutant mtDNA but not in wild-type mtDNA.

An adaptation of the same mismatch PCR was used to quantitate the proportion of mutant mtDNA in total muscle and single fibers from the patient. Fluorescently labeled deoxynucleotide was added prior to the last cycle of the mismatch PCR, followed by digestion with *Dra*I. The products were then separated on a 6% nondenaturing polyacryalmide gel on a 373A DNA sequencer (ABI) and quantified by GENESCAN software (ABI).

Single-fiber PCR.—Single-fiber PCR was performed according to methods described elsewhere (Sciacco et al. 1994; Moraes and Schon 1996). Thirty-micron transverse muscle sections were prepared and were reacted for COX. Freshly stained sections were immersed in 50% ethanol. COX-positive fibers and fibers with no visually detectable COX activity were selected. Single-fiber segments were removed by a borosilicate microcapillary tube under an inverted-light microscope. The removed segments were put into 10 μl water and immediately were centrifuged for 10 min. The water was removed, and 5 μl lysis solution was added (200 mM potassium hydroxide and 50 mM DTT). The fibers were then incubated in this lysis solution for 1 h at 65°C, and then 5 μl neutralizing solution was added (900 mM Tris-HCl, pH 8.3, and 200 mM HCl). The fluorescent mismatch PCR described above was then performed with this 10-μl product.

Human myoblast and fibroblast culture.—Mass myoblast cultures were established from the diagnostic muscle-biopsy sample, by methods described elsewhere (Hanna et al. 1995*a*). Standard growth medium for myoblasts comprised DMEM (Dulbecco's modified Eagle medium) with GLUTAMAX-1, 4.5 g glucose/liter, 110 mg sodium pyruvate/liter, 20% FCS, 2% detoxified chick-embryo extract, 50μg uridine/ml, and 10μg gentamycin/ml. Fibroblast cultures were established from skin-punch biopsy samples, by the explant method (Martin 1973). Growth medium for fibroblasts differed from that used for myoblasts, in that 10% FCS was used and chick-embryo extract was not used.

Results

Histochemical analysis of the patient's muscle-biopsy sample showed a marked reduction in COX activity, in a mosaic distribution. Ten percent of the fibers exhibited normal COX activity, whereas the remainder exhibited reduced or absent activity (fig. 1). There were no ragged red fibers, as determined by staining using the modified Gomori trichrome method. Staining for SDH activity revealed that <2% of the fibers exhibited a slightly increased staining pattern, consistent with a mild degree of mitochondrial proliferation. There were no SDH-intense blood vessels. Biochemical analysis confirmed an elevated citrate synthase activity in the muscle homogenate, compared with the control value, a result consistent with some mitochondrial proliferation (table 1). Results of histochemical analysis of the muscle-biopsy samples from the patient's two sons and from the patient's mother were entirely normal. Immunocytochemical studies using monoclonal antibodies directed against both nuclear and mitochondrially encoded COX subunits were abnormal in the patient's COX-negative fibers. There was a selective reduction in staining with antibodies against subunits I, II, and VIc. The staining pat-

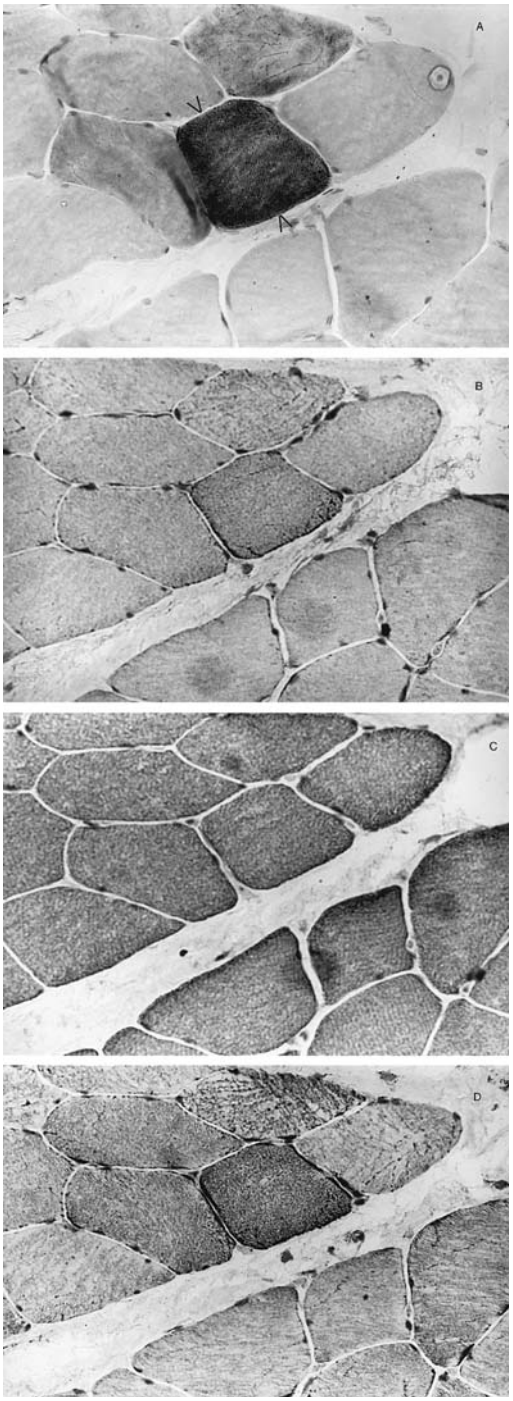

Figure 1 Muscle-biopsy serial sections from the proband, stained for COX activity (*A*) and immunostained with antibodies directed against subunits II (*B*), Va (*C*, and VIc (*D*). Panel *A* shows one type I fiber (*center*) with normal COX activity (*arrows*). The remaining fibers have markedly reduced COX staining. Fibers with reduced COX activity have reduced immunostaining with antibodies directed against COX subunits II and VIc but have normal immunostaining with antibodies directed against subunit Va. Fibers with reduced COX activity also showed reduced immunostaining with antibodies directed against subunit I and showed normal immunostaining with antibodies against subunit IV (data not shown).

tern with antibodies directed against subunits IV and Va did not differ from either that in normal controls or that of COX-positive fibers in the patient's biopsy sample (fig. 1). Biochemical analysis of respiratory-chain enzymes in freshly isolated mitochondria of the patient revealed a marked isolated decrease in both complex IV activity (20% of the control value) and cytochrome aa_3 content (17% of the control value) (table 1). Polarographic studies showed decreased oxygen consumption with the natural substrates—pyruvate + malate, glutamate + malate, and succinate—all of which require a fully functional COX. Oxygen consumption with the artificial substrate ascorbate and tetramethyl-p-phenylenediamine dihydrochloride reduces cytochrome aa_3 directly, therefore bypassing cytochrome a. Results of respiratory-chain–enzyme studies of the patient's sons and of her mother were normal (data not shown).

A large-scale rearrangement was excluded by Southern blotting. DNA sequence analysis of all 22 mitochondrial tRNA genes and of the genes for subunits I, II, and III of COX was undertaken. Three changes were identified, compared with the Anderson et al. (1981) sequence; these were T14766C in the cytochrome b gene, A1438G in the 12SrRNA gene, and G9952A in the gene for subunit III of COX. The first two changes are recognized to be neutral polymorphisms (Kobayashi et al. 1990; Tanaka and Ozawa 1994). The G→A transition at position 9952 has not been described elsewhere (fig. 2). This mutation results in the wild-type TGA codon for tryptophan being converted into a TAA stop codon. This premature stop codon is predicted to result in the loss of the last 13 amino acids of the highly conserved C-terminal region of subunit III of COX. This mutation was heteroplasmic in the patient's muscle but was not detected in her skin or blood or in the blood or muscle samples taken from her two sons and from her mother. The G9952A mutation was not present in either 120 healthy control samples or 60 additional patients with mitochondrial disease but with no known mtDNA mutation. The proportion of mutant mtDNA was the same (57%) in the patient's muscle-biopsy samples in 1990 and in 1997. Early-passage myoblasts and fibroblasts from the proband and myoblasts from her mother and her two sons were grown in medium, to support respiratorily deficient cells, as described in the Methods subsection. However, none of these cell lines harbored the G9952A mutation.

Single-fiber PCR studies were performed to investigate the relationship between the mutation identified and COX deficiency at the level of the single muscle fiber. The mean proportion of mutant mtDNA in the COX-negative fibers ($n = 24$) was 56.2% (standard error of the mean [SEM] 5.1%), and that in the COX-positive fibers ($n = 21$) was 10.1% (SEM 2.3%) ($P < .0002$, by *t*-test).

Discussion

Isolated COX deficiency as observed in this case is rare after the 1st decade of life, and there are few reported. Haller et al. (1989) identified a 27-year-old woman with life-long exercise intolerance and a mild proximal limb weakness. A muscle-biopsy sample showed no frank ragged red fibers by the Gomori trichrome stain but did show that >90% of the fibers had virtually absent COX activity. This defect was confirmed spectophotometrically. There was no family history, and no molecular-genetic data were reported. A recent case reported by Keightley et al. (1996) is a 15-year-old female who experienced recurrent episodes of myoglobinuria and muscle cramps with no muscle weakness on examination. A muscle-biopsy sample in this patient did show typical ragged red fibers and 64% COX-negative fibers. Results of immunoblotting and immunocytochemistry were consistent with either a lack of assembly or instability of the COX holoenzyme. A heteroplasmic in-frame 15-bp microdeletion was identified in the COX III gene. Although the patient described by Haller et al. had some clinical features in common with the patient whom we have reported, neither of these cases had evidence of CNS disease, which is the most likely cause of our patient's recurrent encephalopathy. Adult-onset Leigh syndrome has been reported in association with COX deficiency, but our patient does not have the imaging features seen in this condition (Adams et al. 1997). COX deficiency in combination with other respiratory-chain–complex deficiencies is much more commonly seen in adults, and, in such cases, a primary defect in mtDNA, resulting in impaired intramitochondrial protein synthesis, is frequently identified (Hanna et al. 1995*b*).

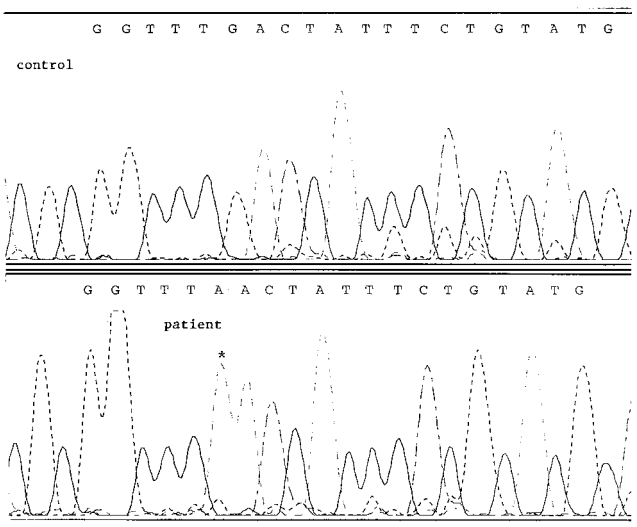

Figure 2 Electropherograms showing wild-type G nucleotide at position 9952 in control muscle mtDNA (*top*) and mutant A nucleotide (*asterisk* [*]) in the proband's muscle mtDNA (*bottom*).

Immunocytochemical studies in our patient demonstrated a decreased staining pattern in COX-negative fibers, with antibodies directed against subunits I, II, and VIc. The staining patterns with antibodies directed

Table 1

Biochemical Analyses in Isolated Muscle Mitochondria from the Proband

	Proband[a]	Mean ± SD in Controls ($n = 5$)
Mitochondrial state–3 respiration rates for substrates (nmol O_2/min/mg mitochondrial protein):		
Pyruvate + malate	61	106 ± 17.6
Glutamate + malate	54	107 ± 25.6
Succinate + rotenone	79	147 ± 49.6
Ascorbate +TMPD	468	305 ± 109.6
Respiratory-chain–enzyme activities:		
Rotenone-sensitive NADH CoQ reductase (nmol/min/mg mitochondrial protein)	126	182 ± 64
Succinate cytochrome c reductase (nmol/min/mg mitochondrial protein)	333	242 ± 134
COX (1st-order rate constant/min/mg/mitochondrial protein)	7.66	44.4 ± 14.6
V_{max} (nmol/min/mg mitochondrial protein) using 50 μM cytochrome c	383	2,189 ± 774
Citrate synthase:		
nmol/min/mg mitochondrial protein	1,719	1,180 ± 186
mmol/min/gm muscle homogenate	13.35	9.5 ± 1.7
Cytochrome concentrations (nmol/mg mitochondrial protein):		
b	.494	.41 ± .09
c	.646	.69 ± .10
c_1	.486	.54 ± .07
aa_3	.10	.42 ± .09

NOTE.—For methods, see Morgan-Hughes et al. (1977); Mann et al. (1992).

[a] Abnormal results are underlined.

against subunits IV and Va did not differ from those in controls. We have observed this pattern of immunostaining in COX-negative fibers in patients with a number of primary defects of mtDNA (Rahman et al. 1997). In contrast, different staining patterns are observed in COX-negative fibers from patients in whom no mtDNA defect has been identified and who are suspected to have nuclear-gene mutations as the basis of their COX deficiency (Rahman et al. 1997). The explanation for these different immunostaining patterns remains to be determined. However, it is possible that primary mtDNA defects that result in either impaired synthesis or altered amino acid sequence of subunits may impair holoenzyme assembly. This may render certain subunits more susceptible to degradation. Recent X-ray crystallographic studies in bovine heart COX suggests that subunit VIc is intimately related to subunit II, which may explain why, in patients with primary mtDNA defects, the immunostaining pattern of this nuclear subunit mirrors the other mtDNA-encoded COX components (Tsukihara et al. 1996). It is possible that some of the normally synthesized nuclear COX subunits in patients with primary mtDNA defects may be able to associate into a subcomplex that is resistant to degradation.

In view of both the mosaic pattern of COX deficiency and the pattern of immunostaining abnormalities described, we have analyzed the muscle mtDNA in this patient. Sequencing of all 22 tRNA genes and of the genes for subunits I and II of COX showed two changes from the published mtDNA sequence (Anderson et al. 1981), which are recognized polymorphisms (Kobayashi et al. 1990; Tanaka and Ozawa 1994). However, sequence analysis of the gene for subunit III of COX showed a G→A transition mutation at position 9952. In keeping with previously described mtDNA mutations associated with disease, there were two features that suggested that this mutation was likely to be pathogenic. First, it was heteroplasmic, a feature generally associated with disease-associated mtDNA mutations rather than with neutral polymorphisms. Second, it did not occur in 120 healthy control individuals. However, unlike any other mtDNA point mutation described, this mutation results in a premature stop codon being introduced at the 3′ end of the COX III gene. This predicts the loss of the final 13 amino acids of the COX III subunit. This region of COX III has been highly conserved during evolution, suggesting that it is likely to have functional importance (Desjardin and Morais 1989). Within this same region, a point mutation at position 9957, resulting in an amino acid change, has been described in a patient with MELAS, indicating that defects in this region may associate with disease (Manfredi et al. 1995).

The mutant load in our patient's most recent muscle biopsy, performed in 1997, was 57%; that in an earlier muscle biopsy, performed in 1990, was identical. This is in contrast with observations made in relation to a recently identified tRNA-gene point mutation, in which increases in both mutant load and proportion of ragged red fibers were documented over a 12-year period (Weber et al. 1997). Despite a severe decrease in COX activity in our patient's muscle, there was minimal evidence of mitochondrial proliferation, and, therefore, there were no true ragged red fibers. The absence of true ragged red fibers may, at least in part, account for the stability of the mutant load over 7 years. The precise cellular trigger for mitochondrial proliferation remains unknown. The case reported by Keightley et al. (1996), which also harbored a mutation in the COX III gene, did exhibit marked mitochondrial proliferation and ragged red fibers, indicating that it is unlikely that COX deficiency itself is the trigger for mitochondrial proliferation.

To study the relationship between the 9952 mutation and COX deficiency, we performed single-fiber PCR. We observed a clear relationship between COX deficiency and the proportion of mutant mtDNA, at the level of the single fiber. The mean proportion of mutant mtDNA in the 24 COX-negative fibers analyzed was 56.2%, whereas that in 21 COX-positive fibers was 10.1% This suggests that the threshold for expression of this mutation is lower than that reported for other mtDNA point mutations. For example, the threshold for expression of tRNA-gene point mutations is generally reported to be >85%, although this may differ in different cell types, possibly depending on mtDNA copy number (Boulet et al. 1992; Chomyn et al. 1994; Hanna et al. 1995*a*). However, there is no a priori reason why the behavior of this unique stop-codon mutation should mirror the behavior of other, previously identified point mutations. Indeed, our data suggest that the rules governing the threshold for expression of this mutation are quite different from those influencing the threshold for expression of tRNA-gene point mutations. The high threshold for expression of mtDNA tRNA-gene point mutations is thought to be due, at least in part, to intramitchondrial complementation (Boulet et al. 1992). It is possible that such complementation does not occur for defective COX subunits. Alternatively, the lower threshold may occur because functional COX is a dimer (Tsukihara et al. 1996). At a mutant level of 50%, 75% of COX holoproteins would be defective, and this may be enough to produce the biochemical phenotype observed. A functional threshold of 75% (i.e., 75% of a COX subunit is defective) is similar to that observed in some deletions of mtDNA (Hanna et al. 1998).

Although the C-terminal region of the COX III subunit is highly conserved, its precise function is not known. Indeed, the function of the entire COX III subunit remains to be elucidated. It is now established that it is not involved in the proton-pumping capability of

COX, but there is some evidence, from studies in *Paracoccus dentrificans,* that it may have a role in either energy conservation or assembly of the holoenzyme (Haltia et al. 1991; Wu et al. 1995). We were unable to study the consequences of this mutation in a cell-culture system, since neither fibroblasts nor early-passage myoblasts from the patient harbored any detectable mutation. The absence of this mutation in early-passage myoblasts may suggest that the mutation is in fact not present in the satellite-cell population from which such myoblast cultures are derived. This possibility has been suggested in relation to another tRNA-gene mutation recently identified (Weber et al. 1997), and it may have therapeutic implications (Clark et al. 1997; Shoubridge et al. 1997).

mtDNA has been shown to be virtually exclusively maternally inherited, since the spermatozoon contributes no mitochondria to the zygote (Giles et al. 1980). Primary mtDNA point mutations, as well as their associated diseases, have been shown to exhibit strict matrilineal inheritance. In contrast, deletions of mtDNA are sporadic in the vast majority of cases, and matrilineal inheritance is rarely observed (Larsson et al. 1992; Bernes et al. 1993). The absence of this COX mutation in any of the tissues examined in the maternal relatives indicates that, in our patient, it probably arose as a sporadic event. The lack of transmission to her sons is surprising and, in this respect, resembles the observations described in relation to single deletions of mtDNA. There are at least three ways in which the observed lack of transmission may be explained: (1) the mutation may not be present in the germ line; (2) it may be present in very low amounts in oocytes and be filtered out by the bottleneck thought to exist during oogenesis (Hauswirth and Laipis 1985); or (3) it may be lost during either oogenesis or early embryogenesis, possibly because of a selective disadvantage of cells harboring high proportions of the mutant molecule. Since germ-line tissues from the proband are not available for study, it is not possible to pursue this question.

In conclusion, we have identified the first stop-codon mutation in human mtDNA and have provided evidence that it is associated with adult-onset isolated COX deficiency. This mutation is likely to induce COX deficiency by a novel mechanism, possibly by affecting the assembly of complex IV. This point mutation, in contrast to others described, is not transmitted in the maternal line.

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