Differential Features of Patients with Mutations in Two COX Assembly Genes, *SURF-1* and *SCO2*

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We screened 41 patients with undiagnosed encephalomyopathies and cytochrome *c* oxidase (COX) deficiency for mutations in two COX assembly genes, *SURF-1* and *SCO2;* 6 patients had mutations in *SURF-1* and 3 had mutations in *SCO2*. All of the mutations in *SURF-1* were small-scale rearrangements (deletions/insertions); 3 patients were homozygotes and the other 3 were compound heterozygotes. All patients with *SCO2* mutations were compound heterozygotes for nonsense or missense mutations. All of the patients with mutations in *SURF-1* had Leigh syndrome, whereas the 3 patients with *SCO2* mutations had a combination of encephalopathy and hypertrophic cardiomyopathy, and the neuropathology did not show the typical features of Leigh syndrome. In patients with *SCO2* mutations, onset was earlier and the clinical course and progression to death more rapid than in patients with *SURF-1* mutations. In addition, biochemical and morphological studies showed that the COX deficiency was more severe in patients with *SCO2* mutations. Immunohistochemical studies suggested that *SURF-1* mutations result in similarly reduced levels of mitochondrial-encoded and nuclear-encoded COX subunits, whereas *SCO2* mutations affected mitochondrial-encoded subunits to a greater degree. We conclude that patients with mutations in *SURF-1* and *SCO2* genes have distinct phenotypes despite the common biochemical defect of COX activity.

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Cytochrome *c* oxidase (COX; complex IV of the mitochondrial respiratory chain) catalyzes the transfer of reducing equivalents from cytochrome *c* to molecular oxygen and pumps protons across the inner mitochondrial membrane.[1] The 3 largest subunits (I, II, and III) of the 13-subunit enzyme are encoded by mitochondrial DNA (mtDNA) whereas the 10 smaller subunits are encoded by nuclear DNA (nDNA). In addition, several other nuclear genes are required for the correct assembly and function of the holoenzyme. These ancillary genes are referred to as the COX assembly genes.

COX deficiency has been associated with a variety of human disorders. There are two main subgroups—those due to genetic defects in mtDNA and those due to nDNA gene defects. Mutations in each of the three mtDNA-encoded COX subunits have been associated with tissue specific disorders such as myopathy[2,3] and sideroblastic anemia,[4] or multisystemic disorders such as MELAS syndrome,[5] an amyotrophic lateral sclerosis–like condition,[6] and encephalomyopathies.[7,8] However, no mutations have yet been identified in any of the nDNA-encoded COX subunits.[9–12] Recently, however, mutations in two different nuclear-encoded COX assembly genes, *SURF-1* and *SCO2,* have been reported. The aim of this study was to analyze a large series of patients with COX deficiency and encephalomyopathy for mutations in *SURF-1* and *SCO2* in order to glean the relative frequency and characterize the clinical phenotypes associated with each mutation.

Patients and Methods

Patient Selection

Forty-one patients referred to Columbia-Presbyterian Medical Center with undiagnosed encephalomyopathies and COX deficiency were entered into the study. Using the previously described diagnostic criteria,[13] patients were divided into two groups—those with typical neuroradiological or neuropathological features of Leigh syndrome (LS), and those with

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Leigh-like clinical presentation but without typical brain lesions (LL). The clinical histories of some of the patients in this study (*SURF-1* Patients 2 to 4) have previously been published as Patients 5, 11, and 12.[14]

Morphological Studies

Frozen muscle sections, 6 to 8 μm thick, were stained for COX activity using standard laboratory methods.[15] When samples were available, we performed additional immunohistochemical studies on 4-μm-thick sections using antibodies to both mtDNA-encoded and nDNA-encoded subunits. Paraffin sections from autopsy studies were reviewed when possible.

Biochemistry

Respiratory chain enzyme assays were performed at 30°C using previously described methods[16] in the following tissues: muscle from 19 patients (8 from the LS group, 10 from the LL group, and 1 from the *SCO2* group); cultured fibroblast cell lines from 15 patients (6 from the LS group, 5 from the LL group, and 4 from the *SURF-1* group); and both muscle and fibroblasts in 9 patients (4 from the LS group, 1 from the LL group, 2 from the *SURF-1* group, and 2 from the *SCO2* group). Samples of brain, heart and liver obtained at autopsy (3 patients) were also tested. Tissues were stored in liquid nitrogen or at −70°C until analysis. Mitochondrial enzyme assays were normalized to citrate synthase and compared with normal controls. When clinically indicated, measurements of pyruvate dehydrogenase complex and pyruvate carboxylase activities in skin fibroblast cell cultures (n = 15) were performed using standard laboratory techniques.[17]

DNA Analysis

Total DNA was extracted from muscle in 19 patients and from cultured fibroblast cell lines in 22 patients. The DNA fragments for *SURF-1* and *SCO2* gene were amplified by polymerase chain reaction (PCR) using intronic primers.[18,19] Direct sequencing of purified PCR products was performed in an ABI Prism 310 Genetic analyzer using the Big Dye Terminator Cycle sequencing reaction kit (Perkin-Elmer Applied Biosystems, Foster City, CA). To determine the exact breakpoint of heterozygous rearrangements in the *SURF-1* gene, purified PCR products were subcloned (Topo TA Cloning, Invitrogen) before they were sequenced. To confirm point mutations within the *SCO2* gene, restriction fragment-length polymorphism analysis was performed as previously described.[20] When possible, the parents of affected children were tested. To exclude other known causes of LS, we screened all patients for the T8993G mutation in mtDNA using previously described methods.[21]

Results

Clinical Features

The clinical features of our patients are summarized in Table 1. Six patients had mutations in *SURF-1*, and 3

Table 1. Summary of Clinical Features of the Patients

	Patients with Leigh Syndrome	Patients with Leigh-Like Disease	Patients with *SURF-1* Mutations[a]	Patients with *SCO2* Mutations[b]
Total number	17	15	6	3
Age onset (mo)	13.3 (23.2)	7.3 (7.2)	13.4 (18.0)	1.7 (0.8)
Age death (mo)	42.8 (37.9) (n = 6)	44.3 (49.7) (n = 6)	42.2 (16.7) (n = 5)	6.7 (5.0) (n = 3)
Sex (M:F)	8:9	8:7	4:2	1:2
Failure to thrive (early stage)	9	4	5	0
Regression of motor development	13	8	6	0
Cognitive delay	7	9	4	0
Poor feeding (early stage)	5	2	5	0
Respiratory difficulties	6	1	6	2
Vomiting	6	3	2	0
Seizures	5	5	1	1
Hypotonia	10	6	5	0
Spasticity	4	0	0	0
Hyporeflexia	10	5	4	1
Hyperreflexia	9	2	3	0
Ataxia	6	1	5	0
Involuntary movements	8	1	2	1
Optic atrophy	5	1	2	0
Abnormal extraocular movements	4	1	1	1
Cardiomyopathy	4	4	0	3
Basal ganglia lesions on neuroimaging	17	0	6	0
Lactic acidosis	17	8	6	3

[a]All patients with *SURF-1* mutations had Leigh syndrome.
[b]All patients with *SCO2* mutations had Leigh-like features (see text for details).

Standard deviations are in parentheses.

patients had mutations in *SCO2*. The mean age at presentation was 13.4 months (SD 18.0) for patients with *SURF-1* mutations and the mean age at presentation was 1.7 months (SD 0.8) for patients with *SCO2* mutations.

Patients with mutations in *SURF-1* were usually normal at birth and showed the first symptoms between 6 and 12 months of age. All patients failed to thrive and had delayed early motor development with further regression of motor milestones in early childhood. Hypotonia with hyporeflexia was present in the earlier stages of disease, but this later evolved to generalized hyperreflexia, with muscle weakness and ataxia in most patients. All patients had metabolic acidosis. On neuroimaging, there were extensive basal ganglia lesions, but occasionally, the midbrain, dentate nucleus and cerebral peduncles were also involved. Two of the 6 patients with *SURF-1* mutations had involuntary movements (tremor or dystonic posturing), but this was not a distinguishing feature when compared with other patients with LS. Seizures, while occasionally present in patients with *SURF-1* mutations, were more common in patients with unidentified causes of LS.

In contrast, patients with *SCO2* mutations presented in the neonatal period, and none of them had typical clinical or radiological features of LS. These infants presented with severe respiratory difficulties accompanied by metabolic acidosis, and all required ventilatory support in the first 10 weeks of life. All had marked hypotonia, one had nystagmus and limb dystonia, and another had dysmorphic facial features and electromyographic evidence of myopathy and peripheral neuropathy. However, the most distinctive feature was a rapidly progressive, severe hypertrophic cardiomyopathy, which resulted in the terminal event of cardiac failure and subsequent cardiorespiratory arrest. No patients with *SURF-1* mutations had similar cardiac involvement. Although 4 other patients with LS and unknown gene mutations also had cardiomyopathy, this was less severe and usually later in onset.

Muscle Pathology

Muscle biopsy specimens from 25 patients were studied; 3 of these patients had *SURF-1* mutations and 2 patients had *SCO2* mutations. The morphological features of 4 of the 5 five patients with identified mutations have been described in detail elsewhere.[9,20] Briefly, patients with *SURF-1* mutations showed a partial reduction in COX activity, affecting both type I and type II fibers. Immunohistochemistry showed similarly decreased staining for both mitochondrial (COX II) and nuclear-encoded (COX IV and VIIa) subunits. In contrast, the 2 patients with *SCO2* mutations showed a profound reduction of COX activity in all fibers, and immunohistochemical studies showed that the reduction in mitochondrial-encoded subunits (COX I and II) was more severe than the reduction in nuclear-encoded subunits (COX IV and Va). Succinate dehydrogenase stains in patients with both *SURF-1* and *SCO2* mutations showed slightly increased subsarcolemmal staining suggestive of incipient mitochondrial proliferation, but there were no typical ragged red fibers (Fig).

Neuropathology

One patient with *SURF-1* mutations and all patients with the *SCO2* mutations had autopsy studies. The patient with *SURF-1* mutations had changes consistent with subacute necrotizing encephalomyelopathy (LS), including bilateral symmetrical involvement of the basal ganglia, midbrain, pons, medulla, and cerebellum. None of the patients with *SCO2* mutations had lesions typical of LS,[20] although 1 had early capillary proliferation that was suggestive of LS. The second patient had necrosis of the globus pallidus and atrophy of the hippocampus, deep gray nuclei, white matter, and cerebellum, and the third had changes suggestive of a migrational disorder, with patches of cortical dysplasia in the left temporal lobe and focal heterotopia in the cerebellum.

DNA Analysis

All *SURF-1* mutations predicted a truncated protein (Table 2). The most common mutation in our patients was the 312Del10, insAT in exon 4, which has been also observed by others.[18] All patients with mutations in *SCO2* were compound heterozygotes for nonsense or missense mutations. All parents of affected children were heterozygous for one of the mutations. No patient had the T8993G mutation in mtDNA, commonly associated with maternally inherited LS.[22]

Biochemistry

Respiratory enzymes were analyzed in 28 muscle samples, 24 fibroblast cell lines, and in autopsy samples from 3 patients. COX deficiency in muscle was less severe in patients with *SURF-1* mutations than in patients with *SCO2* mutations, whereas COX deficiency in skin fibroblasts was more severe in patients with *SURF-1* mutations (Table 3). Additional differences in the severity of COX deficiency in patients with different COX assembly gene mutations emerged from the postmortem studies. In 1 patient with *SURF-1* mutations (*SURF-1* Patient 4), COX activity was reduced in all tissues tested, most severely in liver (6%), and less so in heart (27%) and kidney (36%). In comparison, COX deficiency in autopsy samples from 2 patients with *SCO2* mutations (*SCO2* Patients 1 and 3) was more marked in heart (8 and 8%) and less in liver (16 and 52%).

All other respiratory chain enzyme activities were generally normal, although occasional low values of

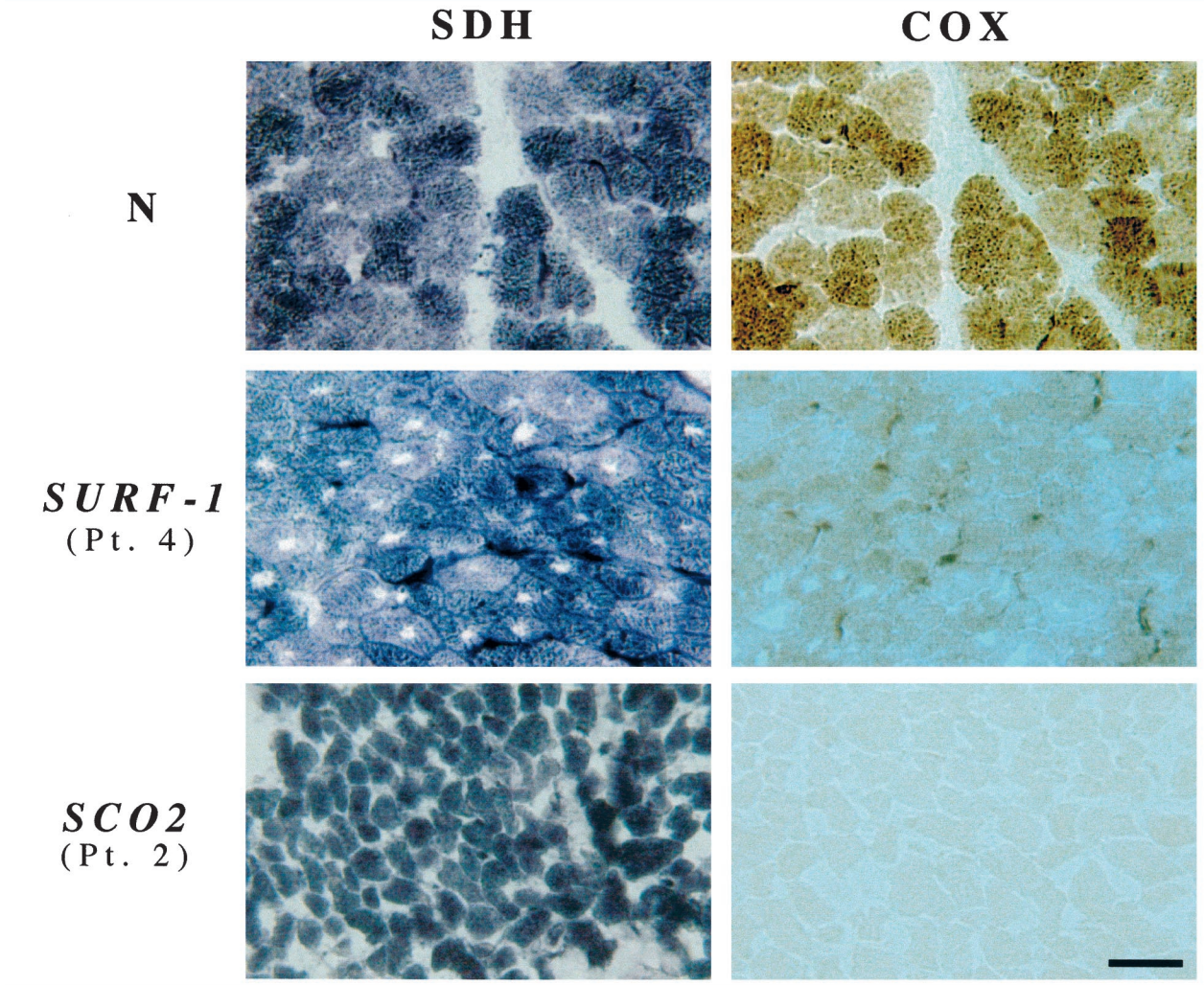

Fig. Morphology of muscle sections of SURF-1 *Patient 4,* SCO2 *Patient 2, and control (N). Succinate dehydrogenase (SDH) and cytochrome* c *oxidase (COX) enzyme studies are shown. COX staining was reduced in both patients, but the muscle sample from* SCO2 *Patient 2 was more severely affected. Bar = 100 mm.*

single enzymes in individual patients were recorded. Pyruvate dehydrogenase complex and pyruvate carboxylase activities were normal in all patients tested.

Discussion

We found that 9 of 41 patients with encephalomyopathy and COX deficiency had mutations in one of the two COX assembly genes that we screened for, *SURF-1* and *SCO2*. Six patients with typical LS had mutations in *SURF-1*, and 3 patients with an infantile encephalopathy associated with fatal hypertrophic cardiomyopathy had mutations in *SCO2*. In contrast to other causes of LS, such as pyruvate dehydrogenase complex deficiency,[23] there was no male predominance in either subgroup of patients.

Clinical presentations were different in patients with *SURF-1* and *SCO2* mutations. Similar to others, we found that patients with *SURF-1* mutations presented with a progressive loss of developmental milestones in early infancy but usually following a well period of several months to a year.[18,19,24] After their motor regression, these children would usually experience respiratory and feeding difficulties, with failure to thrive and death in the first decade of life. All children had the neuroradiological features of LS, and 1 child studied postmortem also had the signature neuropathological features of this disease. In contrast to patients with *SURF-1* mutations, all 3 patients with *SCO2* mutations presented at a much earlier age, within the first 3 months of life. These infants had respiratory difficulties and required ventilatory support. However, the most striking clinical feature in all these patients was the severe and rapidly progressive hypertrophic cardiomyopathy that led to cardiac failure and death during early

Table 2. List of Mutations Identified in Patients

Patient ID[a]	Clinical Features	Mutation
SURF-1		
Patient 1	Leigh syndrome	312Del10, insAT Ex 4/4
Patient 2	Leigh syndrome	312Del10, insAT Ex 4/4
Patient 3	Leigh syndrome	312Del10, insAT Ex4/587insCAGG Ex 6
Patient 4	Leigh syndrome	312Del10, insAT Ex4/587insCAGG Ex 6
Patient 5	Leigh syndrome	828DelCT Ex9/855 Del CT Ex 9
Patient 6	Leigh syndrome	855DelCT Ex9/Ex9
SCO2		
Patient 1	Infantile encephalopathy, hypertrophic cardiomyopathy	Q53X/E140K
Patient 2	Infantile encephalopathy, hypertrophic cardiomyopathy	Q53X/E140K
Patient 3	Infantile encephalopathy, hypertrophic cardiomyopathy	E140K/S222F

infancy. Although 4 of our LS patients also had cardiomyopathy, this was usually of later onset and of lesser severity. None of the 6 patients with *SURF-1* mutations in this study had cardiomyopathy. Therefore, we suggest that hypertrophic cardiomyopathy should be considered as the clinical hallmark of patients with SCO2 mutations, rather than the neuroradiological picture associated with patients with LS and SURF-1 mutations.

COX deficiency in muscle was more severe in patients with *SCO2* mutations than in patients with *SURF-1* mutations. Although the number of patients with *SCO2* mutations was small, this finding was evident in both biochemical and histochemical studies. In contrast, COX deficiency in fibroblasts was more severe in patients with *SURF-1* mutations than in those with *SCO2* mutations. This is important because biochemical assays in cultured skin fibroblasts are commonly used for the diagnosis of LS or LS-like syndromes. Other differences in the tissue distribution of COX deficiency were noted in liver and heart and are probably due to differences in the tissue expression of each gene.[20]

The immunohistochemical studies suggested a qualitative difference in the reduction of COX activity between patients with *SURF-1* mutations and patients with *SCO2* mutations. In patients with *SURF-1* mutations, both nDNA- and mtDNA-encoded subunits were similarly affected, whereas in patients with *SCO2* mutations, the mtDNA-encoded subunits were more severely involved. This difference may be due to the fact that mtDNA-encoded subunits COX I and COX II contain copper and all known *SCO*-like proteins seem to bind copper[25] and also suggests that the pathogenetic mechanisms differ in the two conditions.

Although the clinical presentations of our patients with *SURF-1* mutations were similar to those reported by others, the incidence of mutations in our study group was lower than observed in one other large cohort of patients.[19] Tiranti and colleagues[19] found *SURF-1* mutations in 75% of their population of patients with LS and COX deficiency, whereas we found *SURF-1* mutations in only 6 of the 23 (26%) patients with LS. Two main reasons may explain this discrepancy. First, in Tiranti's study, patients belonged to a single complementation group[26] and were probably more homogeneous than ours. Second, this may reflect regional differences between the two populations. One rearrangement in exon 4 (312del10, insAT) was found in both studies. This may indicate a founder effect, or, more likely, it may mean that this locus is a "hotspot" for mutations in the *SURF-1* gene.

All the mutations identified in the *SURF-1* gene in this study were small-scale rearrangements, predicting a truncated protein. This is similar to what was found in two other studies,[18,24] which also reported a few nonsense and splice-junction mutations. In contrast, thus far we have encountered only nonsense and missense

Table 3. Mean Percentage of COX Activity in Patient Tissues

	Leigh Syndrome Group	Leigh-Like Group	Patients with *SURF-1* Mutations	Patients with *SCO2* Mutations
Muscle[a]	23.4% (17.7)[c] (range, 2.9–53.9) (n = 11)	22.5% (22.1) (range, 2.3–65.1) (n = 11)	25.8% (29.7) (range, 4.8–46.8) (n = 2)	8.7% (8.1) (range, 4.0–18.0) (n = 3)
Fibroblasts[b]	21.1% (18.9)[c] (range, 1–57.2) (n = 9)	37.0% (15.8) (range, 13.9–52.8) (n = 6)	11.3% (6.7) (range, 3.3–19.6) (n = 6)	21.0% (12.7) (range, 12.0–30.0) (n = 2)

Values of COX activity are in [a]μmol/min/g protein or [b]μmol/min/ml homogenate, referred to citrate synthase activity and expressed as a percentage of control values.

[c]± SD are in parentheses.

n = number of patients.

mutations in *SCO2*. Even though the number of patients with *SCO2* mutations is very small, these differences suggest that mutations in the two COX assembly genes may arise by different mechanisms and have different degrees of pathogenicity.

Mutations in COX assembly genes are an important cause of LS and other neurological disorders associated with COX deficiency. Patients with *SURF-1* mutations present with typical LS associated with partial COX deficiency. Children usually are normal at birth, experience the first symptoms in infancy or early childhood, and die within the first decade of life. In contrast, patients with *SCO2* mutations present in the neonatal period with encephalopathy and respiratory difficulties. They experience a severe, fulminant hypertrophic cardiomyopathy and usually die in early infancy. The cardiomyopathy distinguishes patients with *SCO2* mutations from those with *SURF-1* mutations. COX deficiency in patients with *SCO2* was more severe in the clinically affected tissues such as muscle, brain, and heart than that observed in patients with *SURF-1* mutations. While mutations in *SURF-1* extend the genetic heterogeneity LS, mutations in *SCO2* broaden the clinical spectrum of disorders associated with COX assembly genes, since the clinical phenotype is not that of typical LS. Future studies identifying other mutations in COX assembly genes are required to further classify the COX deficiency disorders.

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References

1. Michel H, Behr J, Harrenga A, Kannt A. Cytochrome *c* oxidase: Structure and spectroscopy. Annu Rev Biophys Biomol Struct 1998;27:329–356
2. Keightley J, Hoffbuhr K, Burton M, et al. A microdeletion in cytochrome *c* oxidase (COX) subunit III associated with COX deficiency and recurrent myoglobinuria. Nat Genet 1996;12: 410–415
3. Hanna M, Nelson I, Rahman S, et al. Cytochrome *c* oxidase deficiency associated with the first stop-codon point mutation in human mtDNA. Am J Hum Genet 1998;63:29–36
4. Gatterman N, Retzlaff S, Wang Y, et al. Heteroplasmic point mutation of mitochondrial DNA affecting subunit I of cytochrome *c* oxidase in two patients with acquired idiopathic sideroblastic anemia. Blood 1997;90:4961–4972
5. Manfredi G, Schon E, Moraes C, et al. A new mutation associated with MELAS is located in a mitochondrial DNA polypeptide-encoding gene. Neuromusc Disord 1995;5:391–398
6. Comi G, Bordoni A, Salani S, et al. Cytochrome *c* oxidase subunit I microdeletion in a patient with motor neuron disease. Ann Neurol 1998;43:110–116
7. Clark K, Taylor R, Johnson M, et al. An mtDNA mutation in the initiation codon of the cytochrome *c* oxidase subunit II gene results in lower levels of the protein and a mitochondrial encephalopathy. Am J Hum Genet 1999;64:1330–1339
8. Bruno C, Martinuzzi A, Tang Y, et al. A stop-codon mutation in the human mtDNA cytochrome *c* oxidase I gene disrupts the functional structure of complex IV. Am J Hum Genet 1999; 65:611–620
9. Lombes A, Nakase H, Tritscher H-J, et al. Biochemical and molecular analysis of cytochrome *c* oxidase deficiency in Leigh's syndrome. Neurology 1991;41:491–498
10. DiMauro S, Hirano M, Bonilla E, et al. Cytochrome *c* oxidase deficiency: progress and problems. In: Shapira A, DiMauro S, eds. Mitochondrial disorders in neurology. Oxford: Butterworth-Heinemann, 1994:91–115
11. Adams P, Lightowlers R, Turnbull D. Molecular analysis of cytochrome *c* oxidase deficiency in Leigh's syndrome. Ann Neurol 1997;41:268–270
12. Jaksch M, Hofmann S, Kleinle S, et al. A systemic mutation screen of 10 nuclear and 25 mitochondrial candidate genes in 21 patients with cytochrome *c* oxidase (COX) deficiency shows $tRNA^{Ser(UCN)}$ mutations in a subgroup with syndromal encephalopathy. J Med Genet 1998;35:895–900
13. Rahman S, Blok R, Dahl H-H, et al. Leigh syndrome: clinical features and biochemical and DNA abnormalities. Ann Neurol 1996;39:343–351
14. Van Coster R, Lombes A, DeVivo D, et al. Cytochrome *c* oxidase–associated Leigh syndrome: phenotypic features and pathogenetic speculations. J Neurosci 1991;104:97–111
15. Sciacco M, Bonilla E. Cytochemistry and immunocytochemistry of mitochondria in tissue sections. In: Attardi G, Chomyn A, eds. Methods in enzymology. Vol 264. New York: Academic Press, 1996:509–521
16. DiMauro S, Servidei S, Zeviani M, et al. Cytochrome *c* oxidase deficiency in Leigh syndrome. Ann Neurol 1987;22:498–506
17. De Vivo D, Hammond M, Obert K, Nelson J, Pagliara A. Defective activation of the pyruvate dehydrogenase complex in subacute necrotizing encephalomyopathy (Leigh disease). Ann Neurol 1979;6:483–494
18. Zhu Z, Yao J, Johns T, et al. SURF1, encoding a factor involved in the biogenesis of cytochrome *c* oxidase, is mutated in Leigh syndrome. Nat Genet 1998;20:337–343
19. Tiranti V, Jaksch M, Hofmann S, et al. Loss-of-function mutations of SURF-1 are specifically associated with Leigh syndrome with cytochrome *c* oxidase deficiency. Ann Neurol 1999; 46:161–166
20. Papadopoulou LC, Sue CM, Davidson M, et al. Fatal infantile cardioencephalomyopathy with cytochrome *c* oxidase (COX) deficiency and mutations in SCO2, a human COX assembly gene. Nat Genet 1999;23:333–337
21. Santorelli F, Shanske S, Jain K, et al. AT→C mutation at nt8993 of mitochondria DNA in a child with Leigh syndrome. Neurology 1994;44:972–974
22. Santorelli F, Shanske S, Macaya A, et al. The mutation at nt 8993 of mitochondrial DNA is a common cause of Leigh's syndrome. Ann Neurol 1993;34:827–834
23. De Vivo D, Hirano M, DiMauro S. Mitochondrial disorders. In: Moser H, ed. Handbook of clinical neurology. Vol 22, New York: Elsevier Science, 1996:389–446
24. Tiranti V, Hoertnagel K, Carrozzo R, et al. Mutations of

SURF-1 in Leigh disease associated with cytochrome oxidase deficiency. Am J Hum Genet 1998;63:1609–1621
25. Glerum D, Shtanko A, Tzagoloff A. SCO1 and SCO2 act as high copy suppressors of mitochondrial copper recruitment defect in Saccharomyces cerevisae. J Biol Chem 1996;271:20531–20535
26. Munaro M, Tiranti V, Sandona D, et al. A single cell complementation class is common to several cases of cytochrome *c* oxidase–defective Leigh's syndrome. Hum Mol Genet 1997;6:221–228
27. Lennard A, Gaston K, Fried M. The SURF-1 and SURF-2 genes and their essential bidirectional promotor elements are conserved between mouse and human. DNA Cell Biol 1994;13:1117–1126