Cytochrome c oxidase (COX) deficiency is found in patients with different clinical phenotypes primarily affecting organs with high energy demand such as the brain, skeletal muscle, heart, and kidney. Pedigree studies suggest an autosomal recessive inheritance in most cases; however, mutations in nuclear genes coding for the COX subunits themselves have so far been reported. Several nuclear genes are known from yeast studies to be essential factors for assembly and maintenance of the COX complex. The human homologues of four such assembly genes—SURF1, SCO2, COX10, and SCO1—have now been identified in human COX deficiency disorders. Mutations in SCO2 have so far been described in six infants with a fatal disorder with hypertrophic...
obstructive cardiomyopathy (HCMP) as the predominant symptom.7,8 As all patients with SCO2 mutations carried a G1541A mutation on one allele with different mutations, including stop and missense mutations, on the other allele, it was suggested that the G1541A mutation might represent an ancient founder allele or a mutational hotspot.8 The G1541A mutation is located next to the CxxxxC copper binding motif of Sco2, changing a conserved glutamate to lysine (E140K). This amino acid substitution was proposed to alter the copper binding properties of the protein, but the exact mechanism of pathogenesis remains unknown. Evidence for a functional COX impairment due to this mutation in humans was obtained via restoration of COX activity after introducing a normal chromosome 22 into COX deficient skin fibroblasts of an index patient with compound heterozygous mutations at nucleotide positions G1541A and C1634T.8 In contrast, SCO2 mutations engineered in yeast failed to demonstrate an effect on respiratory function.13,14

Here we present clinical, biochemical, functional, and MRI and MRS data in two infants carrying a homozygous G1541A transition (E140K) and briefly report on a third case presenting with similar clinical symptoms.

**Patient and methods.** Patient A. This girl was the second child of nonconsanguineous parents (mother from Poland, father from Hungary). There was no family history of neurologic disease. Her older sister, an extremely premature baby, died after 3 days. The patient was born by spontaneous delivery in the 38th week of gestation. Apgar scores were 9/10/10. Persistent feeding difficulties prompted a pediatric consultation at the age of 4 months, when muscular hypotonia was noted. At age 7 months, weight was 6200 g (<3rd percentile), length was 65 cm (<3rd percentile), and head circumference was 42.5 cm (10th percentile). She was unable to lift her head when prone and at rest was hypotonic, but when upset she assumed an opisthotonic posture. Clinical examinations of the respiratory, cardiac, and abdominal organs were normal. A severe low frequency resting and action tremor of the upper extremities and trunk were noted. Tongue fasciculations and ptosis developed, and her cognitive development plateaued. She developed breath-holding spells and inspiratory stridor when upset. Abdominal ultrasound, EEG, and echocardiography were normal. Specific laboratory tests and metabolic screening in blood and urine were first performed at age 7 months. Copper concentrations were found repeatedly decreased at age 7 and 8 months (Cu: 0.38, 0.45; reference range 1.1–2.5 mg/L) but ceruloplasmin, iron, transferrin, and ferritin were normal. Lactate was elevated (2.6; reference range 0.5–2.2 mmol/L) and free serum carnitine was slightly decreased. CSF was normal except for a slightly elevated lactate (2.3; reference range up to 2 mmol/L). Electroencephalography (EMG) and nerve conduction studies at age 7 and 10 months revealed a neurogenic pattern and active denervation. Serum copper improved but did not normalize (0.61 [at age 9 months], 0.57 [9.5 months], 0.47 [10 months], 1.23 [11 months], 0.66 [12 months], 0.63 [12.5 months]) after starting oral copper-orotate (930 μg copper/day) supplementation at age 9 months and subcutaneous copper-histidinase (500 μg/day) supplementation at age 12 months. Urine copper excretion was normal at the beginning of the oral copper supplementation. Therapy with carnitine, ascorbate, coenzyme Q10, copper, riboflavin, L-valine, aspartate, vitamin K, histidine, and diazepam was also instituted. At age 8 months she was admitted twice to the pediatric intensive care unit. Intubation was required for worsening respiratory embarrassment and metabolic acidosis. During this period HCMP developed and repeated echocardiographies were done. At 11 months, left ventricular myocardial thickening and a hypomobile intraventricular septum was noted. At that time, lactate was highly elevated in blood (7.8 mmol/L) and in CSF (8.0 mmol/L).

She was treated with propranolol and furosemide, but the HCMP progressed rapidly. Two weeks later, echocardiogram demonstrated obstructive cardiomyopathy with a thickness of the ventricular septum of 1.0 to 1.3 cm. At age 13 months, she died of cardiac failure.

Patient B. This girl is the second child born to nonconsanguineous German parents with no family history of neurologic disease. Delivery was normal. Birthweight was 3430 g and Apgar scores were 10/10/10. Feeding difficulties were noted shortly after birth and were persistent. At age 6 months developmental delay was first noted. At age 9 months she presented with dystrophy and severe generalized muscle hypotonia; she was unable to sit unsupported. Impaired ocular movements, progressing bilateral ptosis, and facial weakness were described. Chest was clear and heart sounds were normal. EEG showed reduced background amplitude and generalized nonspecific cortical activity changes. Echocardiography, MRI, EMG, and nerve conduction velocities (NCV) were all normal at that time. Laboratory investigation and metabolic screening in blood and urine showed normal values except for mildly increased lactate values.

At age 11 months she developed respiratory failure necessitating pediatric intensive care unit admission and intubation, from which she cannot be weaned. Several periods of lactic acidosis with maximum lactate peaks of 12 mmol/L have been observed, most of which have been associated with respiratory infections.

At age 18 months mild HCMP was first noted (septum thickness 0.8 cm), which progressed over the following 4 months (septum thickness 1.2 cm). After the diagnosis of SCO2 mutations at age 21 months subcutaneous copper-histidinase (500 μg/day) supplementation was started, but clinically no beneficial effect was observed. At age 23 months EMG did not detect any significant potentials and NCV examination showed no conduction at all. During the next 4 weeks (up to her current age of 25 months) her HCMP worsened (septum thickness 1.5 cm); however, blood pressure was normal or slightly elevated. Currently, she has no detectable active movements except for very discrete perioral contractions.

Patient C. This girl was the second child born to Hungarian nonconsanguineous parents who had no family history of neurologic disease. Her older sibling is healthy. She was small for gestational age (weight 2650 g, length 50 cm at term). At 3 months of age, her parents noted feeding difficulties and developmental delay. The developmental delay was documented at pediatric consultation at 4
months. MRI of the brain, EMG, and NCV were normal at that time. She underwent physiotherapy; however, her symptoms progressed.

At 7 months she had limited extraocular movements, bilateral ptosis, inspiratory stridor, axial hypotonia, and limb spasticity more of the lower than the upper extremities. Laboratory investigation and metabolic screening in blood and urine showed normal values except for increased lactate values both in blood (5.26 mmol/L) and in CSF (3.4 mmol/L). EEG demonstrated generalized high amplitude slow waves. NCV revealed decreased motor conduction velocities in both peroneal nerves and EMG showed polyphasic potentials.

At age 8 months severe generalized muscle hypotonia occurred and she needed intensive care for respiratory insufficiency. Symptoms of cardiac failure developed promptly and 5 days later she died. At autopsy there was severe HCMP, which was believed to be the immediate cause of death.

MRI and 1H-MRS. Patient A. Two MR examinations (1.5 T Signa Echospeed, GE Medical Systems, Milwaukee, WI) were performed at age 7 and 13 months including standard imaging protocols (T1- and T2-weighted). In addition, localized proton spectroscopy (PRESS: repetition time [TR] = 2000/echo time [TE] = 144 msec, 128 averages) of the left putamen and right periventricular parietal white matter (6.5 and 8 mL voxel sizes) were obtained and analyzed using a prior knowledge fitting procedure (LCModel).15

Patient B. The second patient was examined at age 20 months with the protocol as above. 1H-MRS was obtained from left putamen, white matter, and parietal cortex using short echo and long echo times (PRESS: TR = 2000/TE = 144 msec or 35 msec, 128 averages).

Copper uptake in cultured skin fibroblasts. Copper uptake and retention in fibroblasts of Patient A were measured using JFK's standard protocol.16 In brief, the cells were pulsed for 20 hours in 10µM 64CuCl2 added to medium F12 supplemented with 20% fetal calf serum followed by a 24-hour pulse chase in nonlabeled medium.

Muscle histology and biochemistry. At age 7 and 11 months (Patient A), at age 9 months (Patient B), and at age 7 months (Patient C), open muscle biopsies were performed and used for histochemistry and biochemistry. Six-micrometer serial cross-sections were obtained for histochemical stainings according to standard procedures.17 A frozen part of each biopsy was used for biochemistry.

Respiratory chain (RC) complexes I–IV activities were determined in skeletal muscle and in fibroblasts (Patient A only) as described.18 Skin and a second muscle biopsy in Patient B were refused by the parents.

DNA analysis. DNA was extracted from skeletal muscle and leukocytes according to standard purification protocols (Qiagen, Hilden, Germany). The SCO2 gene was amplified and sequenced as described.19 The G1541A mutation was analyzed by additional restriction fragment length polymorphism (RFLP) analysis in the index patient and her parents as well as in 400 control subjects. SURF1, as well as COX17 and SCO1 mutations19 and frequent mitochondrial DNA mutations at nucleotide positions 3243, 3250, 3271, 7512, 7472, 8344, 8356, and 8993, were analyzed by standard RFLP, Southern blot, and sequence analysis. We did not sequence the ATP7A gene because of the normal copper retention in fibroblasts in Patient A.

Results. MRI and MRS. Patient A. Initially (7 months), nonspecific MRI findings with moderate frontal atrophy, delay of myelination, and rare irregular T2 hyperintensities (HI) in the white matter were noted. 1H-MRS investigations showed moderately elevated lactate in the left putamen only with otherwise unremarkable metabolites (figure 1, a and b). At 13 months, the atrophy had markedly progressed and there were multiple, symmetric T2 hyper- and T1 hypointense lesions in the periventricular white matter, rostral mesencephalon, internal pallida, and medial thalami, as well as diffuse T2 hyperintensities of both striata, the dorsal pons, and large parts of the white matter with sparing of the u-fibers (figure 1e). Lactate had further increased in the putamen and was similarly elevated in the partially necrotic appearing parietal white matter in addition to mildly elevated choline-containing compounds and reduced N-acetylaspartate (NAA) (figure 1, c and d).

Patient B. MRI at 20 months showed marked ventricular enlargement and bilateral HI in atrophic basal ganglia and thalami as well as in periventricular white matter and diffuse subcortical HI, predominantly in the frontal lobes. There was neither necrosis nor brainstem involvement (figure 1f). MRS revealed severe changes with high lactate increase and NAA reduction most pronounced in the parietal cortex, but also in white matter and basal ganglia. In addition, choline and alanine were mildly elevated and glucose was markedly elevated (data not shown).

Muscle histology and biochemistry. Patient A. At age 7 months, some degenerating, myopathic fibers were found in a scattered distribution. In addition, a few small and angular fibers were present, indicating additional neurogenic changes (figure 2B1). Histochemistry showed an overall reduction of COX activity in the majority of fibers; however, no COX-negative or ragged red fibers were seen (figure 2B2). Biochemical measurements of a muscle homogenate showed reduced COX activity with 37 U (reference range: 90–281 U, U/gram noncollagen protein) and to citrate synthase (CS) with 85.5 U resulting in a ratio of 0.46 (reference range: 0.9–4.7). Other complexes of the RC were normal. Residual activity of COX in fibroblasts was 68% compared to the lowest reference range. At age 11 months, a strong progression of the neurogenic changes was noted compared to the first biopsy. The majority of fibers showed an angular shape and there was a severe reduction in fiber size (figure 2C1). On histochemical staining, COX activity was not detectable in most fibers (figure 2C2). Biochemically, COX activity was further reduced when related to noncollagen protein (NCP) (12 U) and to CS (37) with a ratio of 0.3. Other complexes of the RC were normal.

Patient B. At age 9 months most of the fibers were small and some degenerating fibers were also found in a scattered distribution (figure 2D1). On histochemistry all fibers were COX negative (figure 2D2). Biochemically, COX activity was severely reduced when related to NCP (16 U) and to CS (111) with a ratio of 0.14. Other complexes of the RC were normal.
Figure 1. (a–d) $^1$H-MRS of the left putamen (a, c) and right parietal white matter (b, d) at 7 (a, b) and 13 months (c, d). Note the increase of the inverted doublet at 1.3 ppm representing lactate. (e) Patient A. Axial T2-weighted MRI scans at 13 months show marked atrophy and a chronic subdural hematoma on the left. Note the bilateral necrotic lesions in the rostral mesencephalon, internal globi pallida, and periventricular white matter (arrows), and diffuse hyperintensities in the basal ganglia and white matter. (f) Patient B. Axial T2-weighted MRI scans at 20 months. Note the marked ventricular enlargement and bilateral hyperintensities in atrophic basal ganglia, thalami, and in periventricular white matter.
Patient C. Muscle biopsy at age 7 months showed generalized atrophic fibers and some normal size fibers and also some degenerating fibers in a scattered distribution (data not shown). On histochemistry an overall reduction of COX activity was observed (data not shown).

**Copper uptake in cultured fibroblasts.** The copper uptake was performed on fibroblasts from Patient A and was significantly increased in repeated experiments (patient 45.0; Menkes patients \[n = 47\] median 82.6, range 42.6–127.6 ng, \[^{64}\text{Cu}\] per mg protein per 20 hours; controls \[n = 18\] median 19.9, range 8.2–30.8); however, the retention value was completely normal (patient 18.7%; Menkes patients median 65.8%, range 40.3–94.5%; controls median 16.3%, range 9.7–22.7%), indicating that she did not have Menkes disease. Increased copper uptake has now been confirmed in other patients with compound heterozygous mutations in \(\text{SCO2}\) (unpublished data, 2001).

**DNA analysis.** A homozygous G1541A transition mutation, predicting an E140K substitution, was identified on both alleles of \(\text{SCO2}\) in all three patients (figure 3A). The parents of all patients were heterozygous for the G1541A mutation (figure 3B, data not shown for Patient C). No heterozygous carriers of the 1541 mutation were identified in 400 controls.

**Discussion.** We here describe a distinctive (and probably underdiagnosed) phenotype in three nonrelated patients with a homozygous E140K mutation. All patients so far reported with \(\text{SCO2}\) mutations are compound heterozygotes for the E140K mutation, the other allele being either a nonsense (Q53X, R90X) or missense mutation (S225F, R171W).\(^7\) These mutations result in a severe reduction in COX activity.

**Figure 2.** (A–D) Muscle histology. Magnification \(\times 100\). Panels A–D show H-E staining (\(A_1-D_1\)) and COX staining (\(A_2-D_2\)) for each biopsy. \(A_1\), Normal control H-E staining; \(A_2\), COX staining. Patient A with biopsies taken at age 7 months (panel B) and 11 months (panel C), and Patient B (panel D). Panel B (Patient A): At age 7 months, some degenerating fibers show loss of myofibrils, or invasion of mononuclear cells was noted (\(B_1\)). Residual COX activity (\(B_2\)). Panel C (Patient A): At age 11 months, the majority of fibers showed an angular shape and were severely reduced in fiber size, indicating a strong progression of the neurogenic changes (\(C_1\)). No COX activity (\(C_2\)). Panel D (Patient B): At age 9 months, some degenerating fibers show loss of myofibrils or invasion of mononuclear cells and small, angular-shaped fibers were noted (\(D_1\)). No COX activity (\(D_2\)).

**Figure 3.** (A–B) Sequence and restriction fragment length polymorphism analysis. Sequencing of \(\text{SCO2}\) in the patients showed a homozygous G1541A mutation (A). Restriction fragment length polymorphism analysis (B) confirmed the homozygosity in the index Patients A and B (lanes 1 and 2) and heterozygosity in both parents of index Patients A and B (lanes 3–6) and the negative control (lane 7). (Data not shown for Patient C.)
activity and early onset HCMP (table). The clinical course and the predominant symptoms differ significantly from all previously reported SCO2 cases in all three of our patients. First, the onset of symptoms occurred later and the clinical course was less progressive. Second, predominant involvement of the peripheral nervous system, including demyelination and denervation, was observed. In both Patients A and C, a spinal muscular atrophy (SMA) variant was considered histologically. We are therefore currently investigating more samples genetically tested to be negative for SMA for the occurrence of the common SCO2 mutation. Third, there was an unusual pattern of cerebral lesions, with predominant involvement of white matter and late appearance of moderate lesions in the basal ganglia and thalamus (Leigh-like syndrome). Finally, the fatal HCMP occurred late at age 10 months (Patient A), 18 months (Patient B), and 8 months (Patient C). Thus, the progressive cardiomyopathy in SCO2 patients is not necessarily an early symptom of this disorder, but it is indicative of a specific aerobic energy supply threshold for cardiac function. The complex neurologic phenotype observed in our patients suggests that there may be significant clinical heterogeneity associated with particular SCO2 mutations. Defects of SCO2 should therefore be considered in patients with COX deficiency and a Leigh-like syndrome, or with prominent peripheral neuropathy and CNS involvement, and also in patients with a muscle histology suggestive for a SMA variant.

Patient A showed a mild specific COX deficiency (~50% of the lowest reference in repeated experiments) in muscle at age 7 months that declined to about 30% on reinvestigation at 11 months. Although such a decrease in COX could result from the long immobilization of the patient, a 50% decrease in the mitochondrial marker-enzyme CS was also observed at 11 months and as COX activity was normalized to CS, this excludes an artificial deficiency of COX. A severe COX deficiency was detected in Patient B at age 9 months, although she presented with mild symptoms at that time. In support of the different biochemical findings, histochemistry showed a similar picture with higher levels of COX activity in Patient A compared to Patient B. Thus, no exact correlation can be found between COX activity and clinical symptoms in our cases, which could reflect genetic background or environmental influences. On the other hand, our reference range for COX activity assumes a normal fiber distribution and the progressive neurogenic atrophy could influence the interpretation of COX biochemistry.

The mechanism by which the E140K affects Sco2 function is unknown; however, its close proximity to the putative CxxxC copper binding site suggests that it may impair copper binding or copper release to COX. It is thought that the cysteine residues in the CxxxC motif are directly involved in the insertion of copper into the CuA center of COX subunit 2 and this may occur through an enzymatic function involving direct metal ion exchange between the two CxxxC domains.20,21 Yeast functional studies on the homologous mutation (E155K) failed to demonstrate a deficiency in COX activity in haploid yeast.14 In contrast, another mutation homologous to S225F in human Sco2 produced a defect in COX assembly that resulted in respiratory incompetence in these cells. These data predict that the functional consequences of E140K mutation in humans are less severe than the other mutations described in SCO2, and this is supported by the clinical outcome in our patients.

### Table: Genotype-phenotype correlation in patients with SCO2 mutations

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Onset/death, mo</th>
<th>COX residual activity related to CS, %</th>
<th>Main symptoms occurring in chronological order</th>
<th>Onset of HCMP</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>E140K/R90X</td>
<td>0/1</td>
<td>10</td>
<td>MH, RI, seiz, HCMP</td>
<td>3 wk</td>
<td>8</td>
</tr>
<tr>
<td>E140K/R90X</td>
<td>0/1</td>
<td>12</td>
<td>MH, RI, seiz, HCMP</td>
<td>3 wk</td>
<td>8</td>
</tr>
<tr>
<td>E140K/Q53X</td>
<td>0/2</td>
<td>18</td>
<td>MH, HCMP, RI</td>
<td>Neonatal</td>
<td>7</td>
</tr>
<tr>
<td>E140K/Q53X</td>
<td>ND/3</td>
<td>4</td>
<td>RI, HCMP, MH</td>
<td>6 wk</td>
<td>7</td>
</tr>
<tr>
<td>E140K/R173W</td>
<td>1/4</td>
<td>0</td>
<td>MH, RI, seiz, HCMP</td>
<td>8 wk</td>
<td>8</td>
</tr>
<tr>
<td>E140K/S225F</td>
<td>2.5/6</td>
<td>4</td>
<td>HCMP, RI, MH, NP, BA</td>
<td>10 wk</td>
<td>7</td>
</tr>
<tr>
<td>E140K/E140K</td>
<td>4/13</td>
<td>51</td>
<td>MH, LLS, ptosis, NP</td>
<td>Patient A (at age 7 mo)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>MH, LLS, ptosis, NP, RI, BA, HCMP</td>
<td>8 mo</td>
<td>Patient A (between age 8–11 mo)</td>
</tr>
<tr>
<td>E140K/E140K</td>
<td>6/alive, 24 mo</td>
<td>16</td>
<td>MH, ptosis, RI, NP</td>
<td>Patient B (at age 9 mo)</td>
<td></td>
</tr>
<tr>
<td>E140K/E140K</td>
<td>3/8</td>
<td>ND</td>
<td>Ptosis, MH, LLS, NP, RI, HCMP</td>
<td>8 mo</td>
<td>Patient C</td>
</tr>
</tbody>
</table>

MH = muscle hypotonia; RI = respiratory insufficiency; seiz = seizures; HCMP = hypertrophic cardiomyopathy; NP = neuropathy; LLS = Leigh-like syndrome; BA = brain atrophy.
(see the table). The very severe COX defect that is seen in some compound heterozygotes, however, remains unexplained. Although the sample size is not large, the extent of the deficiency in these cases is usually greater in those in which the E140K allele occurs with another missense allele. Such a result might be explained if the Sco2 protein functions as a dimer or higher order oligomer.

The observation of an increased copper uptake in fibroblasts of Patient A in the face of normal retention values demonstrates that function of the copper export pump ATP7A is unchanged compared to controls and thus may be viewed as a compensatory mechanism to overcome impaired Sco2 function. A higher copper turnover in cells might also explain the low serum copper values found in Patient A. Detailed functional analyses on patient cells with different SCO2 mutations are needed to investigate their role in the intramitochondrial copper transport.

Patients A and B presented here were the first cases with SCO2 mutations being treated with copper starting in a late stage of the disease at age 9 months and 21 months, respectively. As expected, no beneficial effects on clinical progression were observed, probably owing to irreversible damage on heart and nervous system. Earlier treatment might be considered in other patients with SCO2 mutations.

SCO2, like the other COX assembly factors that have been implicated in human disease (Surf1, Sco1, Cox10), is ubiquitously expressed in human tissues, and there remains no satisfactory explanation for the tissue-specific differences in COX deficiency, or the clinical involvement of particular cell types that characterize mutations in this gene. The development of the fatal HCMP seems to be relatively specific for SCO2 defects as it has been reported only in a single patient with SURF1 mutations so far, and the reported cases suggest that the onset of this symptom reflects the severity of the mutation (see the table). The fact that two nonsense mutations in SCO2 have not been observed suggests that a Sco2 null phenotype might be associated with prenatal lethality. Analysis of patient cells and other models may reveal further insights in the biochemical consequences of the common E140K mutation and the pathogenesis of Sco2 defects.

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