ampullopetal deflection and inhibition of the superior canal.1,5,9 Clockwise direction of the slow phase indicates predominant inhibition of the right superior canal that might be more affected than the left one.

The permanent oscillopsia in our patient might be explained by abnormal visual inhibition of VOR, which in association with gaze-evoked nystagmus and the abnormal clinical aspect of smooth pursuit might be linked to cerebellar lithium toxicity. Indeed, these different cerebellar oculomotor symptoms are known to occur with lithium therapy even within the range of that drug’s therapeutic blood level.11

In conclusion, this article adds important data to the clinical description and pathophysiological understanding of superior canal dehiscence syndrome. Vertical oscillopsia and pulse-synchronous nystagmus may be observed in bilateral symptomatic forms as a result of an abnormal communication between the inner ear and intracranial space.

We thank Dr G. Rambaud for referring the patient described in this article.

References

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We report a cytochrome c oxidase (COX)–deficient patient, clinically affected with Leigh-like disease, with a homozygous mutation in the COX10 start codon. Two-dimensional gel electrophoresis showed a decrease of fully assembled COX without the accumulation of partially assembled COX subcomplexes. Western blot analysis with antibodies directed to COX subunits I, II, and IV showed a decrease of these subunits in this patient compared with control. Overexpression of the COX10 protein in the patient’s fibroblasts proved that the detected mutation was indeed the disease cause.

Human cytochrome c oxidase (COX) consists of 13 subunits; three of these are encoded by the mitochondrial DNA. Because of the bigenomic origin of the complex, isolated COX deficiencies can be caused by mutations in either the mitochondrial or the nuclear genome. In contrast to complexes I, II, and III, no mutations have yet been described in any nuclear-encoded structural subunit of COX.1–3 However, six genes involved in COX biogenesis have been linked to COX deficiency in humans (SURF1, SCO1 and SCO2, COX10, COX15, and LRPPRC).4–13 The COX10 and COX15 proteins play a role in the mitochondrial heme biosynthetic pathway. COX10 catalyzes the conversion of protoheme to heme O. COX15 exerts its role in the next step, in which heme O is converted to heme A, an essential group for the functioning of complex IV.10 To date, three patients harboring mutations in COX10

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have been described. Here we report a new patient, phenotypically classified as suffering from a Leigh-like disease, with a mutation in the start codon of the COX10 gene. The effect of this mutation on the assembly or stability of mitochondrial COX has been analyzed by two-dimensional blue-native electrophoresis.

Case Report
The male patient was born at term as the first child of consanguineous parents. At 5 months’ age, he developed progressive failure to thrive, and pronounced motor agitation was noted. Gross motor development was severely delayed at 7 months. At this age, the patient showed generalized muscular hypotonia with persistent head lag at traction, ataxia, hypermetria, exaggerated tendon reflexes with enlarged reflex zones, low-amplitude nystagmus, and saccadic eye movements. Ocular fixation was weak. He was not able to grasp.

Laboratory evaluation showed metabolic acidosis with elevated serum and cerebrospinal fluid lactate concentrations. Magnetic resonance imaging of the brain showed slight atrophy and hyperintense lesions in the thalamus, olives, and the nucleus ruber, a pattern comparable to Leigh-like disease. Biochemical COX activity was significantly reduced in muscle and fibroblasts (0.15 COX/citrate synthase [CS]; control range, 0.52–2.08; and 0.22 COX/CS; control range, 0.68–1.19 COX/CS, respectively). The boy died at 9 months of age of acute pneumonia and cardiorespiratory failure. Prenatal diagnosis was performed in a later pregnancy. Normal COX activity was found in chorionic villi, and the mother gave birth to a healthy girl.

Materials and Methods

Cell Culture and Biochemical Measurements
Human skin fibroblasts were cultured in M199 (Life Technologies, Bethesda, MD) supplemented with 10% fetal calf serum and antibiotics. Mitochondrial OXPHOS complex activities were measured in skin fibroblasts and muscle (slightly modified from the method described previously).

Analysis of COX10 DNA
A group of 11 patients with an isolated COX deficiency at least expressed in cultured skin fibroblasts were included in this study. DNA was isolated from patients’ fibroblasts and parents’ blood. The oligonucleotide primers used for the amplification of COX10 genomic DNA were described previously. The DNA sequence was analyzed on an ABI 377 sequencer (Perkin-Elmer, Oak Brook, IL). To confirm the presence of the mutation, we performed restriction fragment length polymorphism analysis with BstZI (Promega, Madison, WI).

COX10 Complementary DNA Construct, Virus Production, Infection, and Measurement of Enzyme Activity
The retroviral vector was created as described previously. COX activities were measured before and after overexpression of COX10 protein (as described by Capaldi and colleagues and Srere).

Protein Electrophoresis
One- and two-dimensional blue-native electrophoresis were performed with digitonin-isolated mitochondria. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Schagger and von Jagow. Proteins were transferred to a PROTRAN nitrocellulose membrane (Schleicher & Schuell, Keene, NH). Western blotting was performed using anti-COXI, anti-COXII, anti-COXIV (all from Molecular Probes, Eugene, OR), anti–mitochondrial HSP70 (Alexis, Molecular Probe, Eugene, OR), and peroxidase-conjugated anti–mouse immunoglobulin G (Molecular Probes). The signal was detected by enhanced chemiluminescence with ECL Plus (Amersham Biosciences, Arlington Heights, IL).

Results
Eleven patients with decreased COX activity established in cultured fibroblasts (data not shown) were an-
alyzed for mutations in the COX10 gene. A homozygous mutation in the COX10 start codon was detected in one patient. The T→C transition of the second base of the start codon (ATG) results in the abolition of the start site for protein translation (Fig 1A). Besides this mutation, two polymorphisms were detected in control and COX-deficient patient material (complementary DNA G476A [dbSNP rs8077302] and A699G [dbSNP rs2230354]). The mutation present in the COX10 start codon was confirmed by restriction enzyme analysis (Fig 1B).

To investigate the functional significance of the described mutation, we infected patient and control fibroblasts with a retrovirus containing human COX10 complementary DNA. COX activity was established before and after infection of the cells. Before infection, COX activity in the patient's fibroblasts was 0.09 COX/CS. In the patient cell lines, the COX activity rose to 0.51 COX/CS. Control fibroblasts infected with COX10 showed a COX-to-CS ratio of 0.64. This experiment suggests that the deficiency in the patient's fibroblasts has been mainly rescued by overexpression of wild-type COX10.

One-dimensional blue-native gel electrophoresis was performed to investigate the effect of the mutation on the formation of COX. A reduction in the amount of COX could be detected in patient material compared with that in control (Fig 2A). Blue-native electrophoresis showed that overexpression of COX10 results in an increase in the amount of COX holoenzyme complex (Fig 2A).

A two-dimensional gel (blue native/SDS) was also performed, as this method is more sensitive for the detection of COX subcomplexes. The patient’s fibroblasts display a general decrease of all subcomplexes as well as a lesser amount of holo COX (Fig 2B). This general decrease of the subcomplexes was also detected with an antibody directed to COXII (data not shown).

SDS-PAGE showed hardly detectable mitochondrial-encoded COXI and COXII levels in the patient's fibroblasts (Fig 2C). The nuclear-encoded subunit COXIV steady state level was also reduced compared with control levels. The effect of COX10 overexpression resulted in elevated levels of COXI and COXII in patient and control material. COXIV level was increased after overexpression of COX10 but to a lesser extent than the COXI and COXII levels (Fig 2C). Taken together, these results demonstrate that the mutation in the COX10 gene is responsible for the COX defect observed in the patient.

Discussion

This article describes the results of a mutational analysis study in the COX assembly gene COX10. In 1 of 11 patients who suffered from Leigh-like disease, a mutation in the start codon of COX10 was found. Both parents were heterozygous for this mutation. Two additional in-frame ATG codons are present, 72bp downstream and 174bp upstream of the predicted start codon. These may serve as alternative translational start sites. However, these start sites will probably not lead to a fully functional protein, as the COX enzyme activity was substantially increased after overexpression of the COX10 protein.

The effect of the mutation on the formation of

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**Fig 2.** (A) Mitochondria were extracted from fibroblasts, and the protein complexes were separated on a 5–12% blue-native acrylamide gel. The gel was blotted. An antibody directed to COXI was used to detect COX in control (C) and patient (COX10) fibroblasts and patient's fibroblasts overexpressing COX10 (COX10*). (B) For the separation of the individual subunits of control (upper panel) and patient (lower panel) fibroblasts, a 10% tricine–sodium dodecyl sulfate (SDS) gel was run in the second dimension, followed by western blotting. Lanes 1–3 indicate the previously described COX assembly intermediates; lane 4 indicates holo-COX. (C) For SDS polyacrylamide gel electrophoresis, 30µg of whole-cell lysates isolated from control (C) and patient (COX10) fibroblasts and patient's fibroblasts overexpressing COX10 (COX10*) was used. The proteins were separated on a 10% tricine–SDS gel. The gel was blotted and incubated with antibodies raised against COX subunits COXI, COXII, COXIV, and against mitochondrial HSP70 as a loading control.
COX was studied using blue-native gel electrophoresis, which demonstrated that the mutation leads to a lesser amount of holo COX when compared with control material. This finding was also confirmed by two-dimensional gel electrophoresis, in which a general decrease in COX assembly could be detected in patient material. Similar observations were obtained in other patients harboring a mutation in COX10. It is clear from both approaches that a general decrease in COX assembly occurs when COX10 is mutated.

The effect of the mutation in COX10 on COX protein levels resulted in a severe reduction in the steady state levels of COXI, COXII, and COXIV subunits in the patient’s fibroblasts. This result is in line with western blot experiments performed on samples from another patient with a COX10 mutation. It is clear from both approaches that a general decrease in COX assembly occurs when COX10 is mutated.

The increase in COX activity after COX10 overexpression was also reflected in COX protein levels. SDS-PAGE shows that COXI and COXII protein levels are increased by overexpression of COX10 in the patient’s fibroblasts. The levels were higher than those in the control cell line. One explanation is that overexpression of COX10 might lead to higher than normal levels of heme. The heme groups can stabilize COXI, which might lead to higher COXI steady state levels. In contrast to COXI and COXII, the COXIV levels do not reach control levels after COX10 overexpression. These results together suggest that COX10 overexpression might lead to an accumulation of the subcomplexes, which might result in a slightly disturbed assembly of complex IV. This is also reflected in the enzyme activity of complex IV.

All COX10 patients described so far showed different clinical features (Table). However, the disease progression is very fast; after the first clinical symptoms, all patients died within a few months. Other general features are hypotonia and elevated blood lactate levels, which could be detected in all patients. All patients showed a residual activity of COX, suggesting that COX10 is not essential for the formation of complex IV but is essential for the maintenance of wild-type levels. In the previously described patients, COX10 could have some residual COX activity, as all patients have missense mutations; in the patient described here, the residual activity could be explained by the use of alternative start codons. However, it may also be possible that another mechanism converts some heme O to heme A. These observations show that it is difficult to establish a general clinical picture for COX10-deficient patients.

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References

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease associated with the death of motor neurons in the spinal cord and brainstem. The cause of ALS is unknown and there is no cure. This study demonstrates, for the first time, that vascular endothelial growth factor (VEGF) delays progression of symptoms and prolongs survival in a Cu/Zn superoxide dismutase (SOD1) transgenic mouse model of ALS. These observations suggest that VEGF or related compounds, might be of value in the treatment of ALS patients.

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Vascular Endothelial Growth Factor Prolongs Survival in a Transgenic Mouse Model of ALS

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Amyotrophic lateral sclerosis (ALS) is a late-onset neurodegenerative disease characterized by progressive muscle weakness and paralysis, leading to death within 1 to 5 years of diagnosis. The cause of ALS is largely unknown; however, Cu/Zn superoxide dismutase (SOD1) mutations are seen in some cases of familial ALS and produce an animal model of the disease. Similarly, recent studies have shown that deletion of the hypoxia-response element in the vascular endothelial growth factor (VEGF) promoter in mice causes muscle weakness and motor neuron degeneration reminiscent of ALS. The latter findings support the notion that motor neurons are especially vulnerable to insufficiencies in blood supply and attendant hypoxia; conversely, it is conceivable that the administration of VEGF may protect against motor neuron stress or degeneration. To address the potential therapeutic role of VEGF in ALS, we analyzed motor performance and...

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