circumstances (in our patient, psychosocial and behavioral problems and a disturbed parental relationship). Although scalp EEG is not always able to detect ictal activity in the limbic system, it can verify the propagation to neocortical brain areas. Additional video recording can help to uncover signs essential for differential diagnosis such as associated tonic posturing or postictal anomia, which may be overseen by the patient's relatives, as in our case.

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A Novel Mutation in the *SURF1* Gene in a Child With Leigh Disease, Peripheral Neuropathy, and Cytochrome-*c* Oxidase Deficiency

ABSTRACT

We report a 16-month-old boy with psychomotor regression, muscle hypotonia, peripheral neuropathy, and lactic acidosis. Brain magnetic resonance imaging showed a bilateral abnormal signal in the substantia nigra and in the subthalamic nucleus, suggestive of Leigh disease. Histochemical analysis of skeletal muscle showed decreased cytochrome-*c* oxidase activity. Biochemical analysis of respiratory chain enzymes in muscle homogenate and in cultured fibroblasts showed isolated cytochrome-*c* oxidase deficiency. Western blot analysis in fibroblasts showed the absence of Surf1 protein. Genetic analysis of the *SURF1* gene revealed that the patient was compound heterozygous for a previously reported mutation at the splice-junction site of intron 3 (240 + 1G > T), and for a novel 4-bp deletion in exon 6 (531_534delAAAT). Our data further enlarge the spectrum of mutations in *SURF1* gene in patients with Leigh disease and cytochrome-*c* oxidase deficiency, contributing to better characterization of the clinical and neuroradiologic features of this group of patients for genotype-phenotype correlations. (*J Child Neurol* 2002;17:233–236).

Leigh disease (McKusick 256000) is a progressive neurodegenerative disorder of infancy and childhood characterized by developmental delay or psychomotor regression, signs of brainstem dysfunction such as incoordination of ocular movements and abnormal respiration, and lactic acidosis. Brain magnetic resonance imaging (MRI) reveals focal symmetric lesions in the brainstem, basal ganglia, thalamus, and spinal cord. The disease has a relapsing and remitting course, and the final outcome is poor.[1,2]

Biochemical or molecular defects in the pyruvate dehydrogenase complex and in the mitochondrial respiratory chain complexes have been identified in up to 75% of patients, making both mendelian and maternal patterns of inheritance possible in Leigh disease.[3,4] An isolated, generalized defect in cytochrome-*c* oxidase (complex IV of the mitochondrial respiratory chain) plays an important pathogenetic role in Leigh disease. Mutations in the nuclear *SURF1* gene, encoding a protein involved in cytochrome-*c* oxidase assembly, have been identified in patients with cytochrome-*c* oxidase–deficient Leigh disease.[5,6] We report a child with cytochrome-*c* oxidase–deficient Leigh disease and peripheral neuropathy who is compound heterozygous for a previously reported missense mutation at the splice-junction site of intron 3 and for a novel 4-bp deletion in exon 6 of the *SURF1* gene.

Case Report

A 16-month-old boy was referred from another hospital to the Gaslini Institute for further investigation because of hypotonia, metabolic acidosis, and a brain MRI suggestive of a brainstem tumor. He was born at term after an uneventful pregnancy from unrelated healthy parents. The neonatal period was unremarkable. At 12 months of age, he was able to sit and roll around. At 14 months of age, his developmental milestones slowed, and he pre-

sented with muscle hypotonia. Examination at 16 months of age revealed weight, height, and head circumference below the second percentile. He had neurodevelopmental regression, generalized symmetric hypotonia, and absent deep-tendon reflexes. Horizontal and vertical eye movements were normal, and no ptosis was noted. Laboratory investigations revealed elevated blood lactate at rest (58 mg/dL; normal = 8–22 mg/dL) and increased lactate/pyruvate ratio (61.6; normal = 6–25). Electrocardiography and cardiac ultrasonography were normal. Funduscopy showed no retinal changes. Electromyography of the anterior tibialis muscle was suggestive of a neurogenic lesion. A motor nerve conduction study showed a slowing of the conduction velocities along the peroneal and medial nerves (26.6 m/seconds, and 22.3 m/seconds, respectively). Brain MRI showed a bilateral hyperintense signal on T_2-weighted images in the substantia nigra and in the subthalamic nucleus of Luys, and no masses were identified. Electroencephalogram revealed mild, nonspecific abnormalities. In the following months, the patient experienced severe respiratory difficulties, and he died of cardiopulmonary arrest at 22 months of age. An autopsy was not performed.

Materials and Methods

Biopsies of the patient's skin and muscle of the left quadriceps were performed at the age of 16 months by an open surgical technique under local anesthesia. Morphologic analysis of skeletal muscle and staining for cytochrome-*c* oxidase and succinic dehydrogenase activities in muscle and cultured fibroblasts were performed as described.[7,8] Activities of the respiratory chain complexes and of citrate synthase were assayed in muscle homogenate and in fibroblasts, as described.[8,9]

Isolation of total genomic DNA and Southern blot analysis of DNA from the patient's leukocytes and muscle were performed according to standard procedure.[10,11] The common mitochondrial DNA mutations associated with Leigh disease were ruled out by polymerase chain reaction (PCR)/restriction fragment length polymorphism screening, as described.[12] The coding sequence region of the *SURF1* gene was amplified by PCR from genomic DNA and sequenced, in both directions, with the same set of primers as per amplification,[5] using the ABI Prism Big-dye terminator cycle sequencing kit and an ABI Prism 310 Genetic Analyzer (Applied Biosystem, Foster City, CA). Western blot analysis was performed as described by Tiranti and collaborators.[13] To identify the 4-bp deletion, PCR product of exon 6 was cloned into the PCR-cloning vector pGEM-T (Promega) according to the manufacturer's protocol. After transformation in bacteria (Topoisomerase cloning kit, Invitrogen, Carlsbad, CA), the clone insert was amplified directly by PCR from each colony, purified, and sequenced in both directions, according to the manufacturer's instructions.

Results

Histochemical analysis of skeletal muscle showed lipid accumulation and virtually absent cytochrome-*c* oxidase activity. Biochemical analysis of the respiratory chain complexes in muscle homogenate and in cultured fibroblasts confirmed an isolated reduction of cytochrome-*c* oxidase. The common mitochondrial DNA mutations were ruled out by PCR/restriction fragment length polymorphism screening. Southern blot of the proband's muscle mitochondrial DNA excluded the presence of large-scale deletion or duplication. Western blot analysis performed in fibroblasts showed that Surf1 protein was absent (data not shown). By sequencing the entire coding region of the *SURF1* gene, the patient was shown to be compound heterozygous for a previously reported mutation at the splice-junction site of intron 3 (240 + 1G > T) and for a novel heterozygous deletion in exon 6. The deletion involves 4 bp after nucleotide 530 (531_534delAAAT), predicting a truncated Surf1 protein (Figure 1).

Discussion

We have identified a novel mutation in the *SURF1* gene in a 16-month-old boy with Leigh disease and peripheral neuropathy, associated with cytochrome-*c* oxidase deficiency.

The patient was referred to the Gaslini Institute for further investigation because of an abnormal brain MRI indicative of a cerebral tumor. Brain MRI indeed showed bilaterally hyperintense substantia nigra and subthalamic nucleus, and no masses were identified. In addition, the clinical course (psychomotor regression) and the laboratory investigations (lactic acidosis), together with the neuroimaging, were suggestive of Leigh disease. Histochemical analysis of skeletal muscle and biochemical analysis of

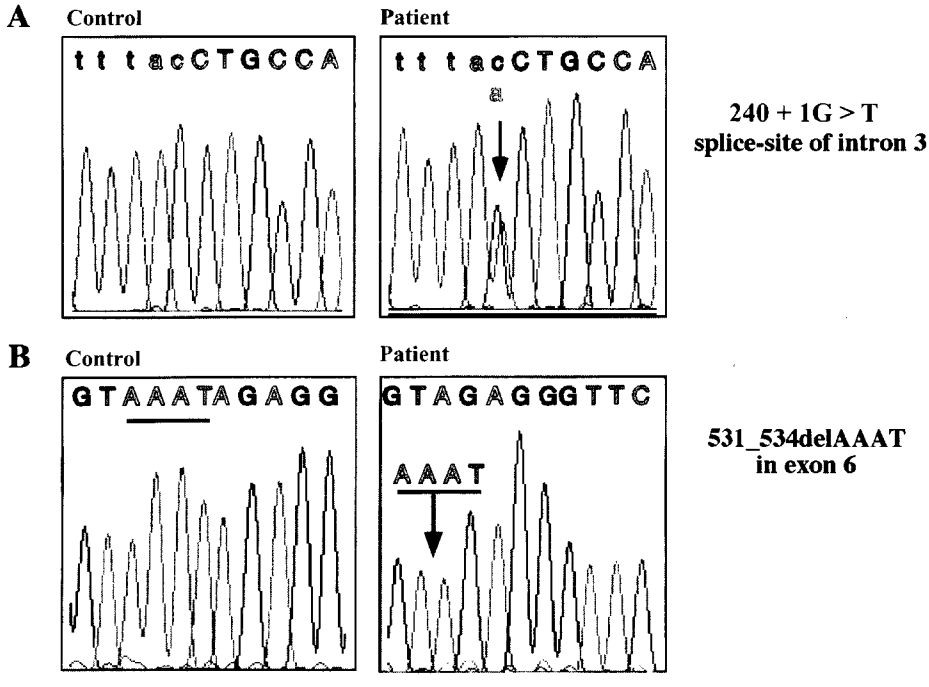

Figure 1. *SURF1* gene DNA sequence analysis of the two mutations founded in the patient. *A*, G→T transition at the boundary site of intron 3. The reverse complementary strand is displayed. *B*, A 4-bp deletion at the nucleotide 531 in exon 6. The amplified fragment was inserted into plasmid vectors, and nucleotide sequences of the subcloned plasmids were determined.

muscle and cultured fibroblasts revealed an isolated decrease of cytochrome-*c* oxidase activity. Western blot analysis revealed the absence of the Surf1 protein. Sequence analysis of the *SURF1* coding region identified two alterations, a previously reported missense mutation at the splice-junction site of intron 3 (240 + 1G→T), and a novel 4-bp deletion in exon 6 (531_534delAAAT), according to the nomenclature proposed by Pequignot et al,[14] based on the complementary DNA sequence described by Lennard and collaborators.[15]

To date, 30 different mutations of the *SURF1* gene have been reported in 40 patients with Leigh disease with isolated severe cytochrome-*c* oxidase deficiency.[14] Tiranti and collaborators reported the 240 + 1G→T missense mutation in a homozygous way in a typical patient with Leigh syndrome with 5% and 15% of residual cytochrome-*c* oxidase activity in muscle and fibroblasts, respectively.[16] Pequignot and collaborators then reported a 1-year-old girl with cytochrome-*c* oxidase–deficient Leigh disease, who was compound heterozygous for a mutation at the splice-junction site of intron 6 (588 + 1delG) and for the 240 + 1G→T, showing that the latter causes the retention of the mutated intron 3.[17]

The 4-bp deletion in exon 6, which has never been previously reported, introduces a frameshift in the open reading frame creating a premature stop codon. In addition, it affects a very conservative area of the gene, and it was not found in 50 controls.

Our patient presented with the classic clinical feature of Leigh disease associated with peripheral neuropathy. Peripheral neuropathy has rarely been reported in patients with Leigh disease[18–20] and was not listed in the original description of the disease by Dr Leigh[1] and in the inclusive criteria for cytochrome-*c* oxidase–deficient Leigh disease proposed by Rahman and collaborators.[3] In addition, there are few reports on nerve conduction studies and on morphologic examinations of neuropathy in Leigh disease. Goebel and collaborators described primary demyelination and remyelination in the sural nerve biopsies of four unrelated children with Leigh disease with cytochrome-*c* oxidase deficiency and electrophysiologic documentation of peripheral neuropathy,[21] whereas in three other affected children, sural nerve biopsies revealed hypomyelination.[22] Recently, Santoro and collaborators reported a novel nonsense mutation in the *SURF1* gene in a child with cytochrome-*c* oxidase-deficient Leigh disease with demyelinating neuropathy and observed a higher frequency of peripheral neuropathy in patients with cytochrome-*c* oxidase–deficient Leigh disease as compared to patients with Leigh disease with mitochondrial DNA mutations or pyruvate dehydrogenase deficiency.[23] Thus, peripheral neuropathy seems to be a more frequent clinical feature among children with cytochrome-*c* oxidase–deficient Leigh disease than was previously supposed.

Significantly, our patient was referred because of brain MRI evocative of a brainstem tumor, but no tumoral masses were identified. Moreover, there was no evidence of basal ganglia and thalami involvement, which represent the most commonly reported neuroradiologic abnormalities of Leigh disease.[24] Instead, T_2-weighted images showed a bilateral hyperintense signal in the substantia nigra and in the subthalamic nucleus of Luys, which are areas rarely reported to be affected in patients with cytochrome-*c* oxidase–deficient Leigh disease.

Interestingly, a *SURF1* mutation has recently been associated with a neuroradiologic pattern of leukodystrophy,[25] indicating that *SURF1* mutations may be associated with more than one neuroradiologic pattern and underlining the importance of an extensive MRI analysis of the brain in the differential diagnosis of neurodegenerative disorders in childhood.

In conclusion, our data further enlarge the spectrum of mutations in the *SURF1* gene in patients with cytochrome-*c* oxidase–deficient Leigh disease, contributing to better characterization of the clinical and neuroradiologic features of this group of patients for genotype-phenotype correlations.

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Neonatal Hypocalcemic Seizures: Case Report and Literature Review

ABSTRACT

Seizures during the neonatal period have a broad differential diagnosis, many with a specific treatment and prognosis. In the case reported, a combination of dietary and endocrinologic abnormalities resulted in hypocalcemic seizures, which continued despite aggressive correction of serum ionized calcium levels. Serial electroencephalograms (EEG) performed during the hospitalization were markedly abnormal, and treatment with anticonvulsant drugs was considered given the persistence of seizures despite normalization of serum calcium levels. After 4 days of intravenous calcium administration, the seizure activity resolved, and the patient returned to his normal baseline level of functioning. This case highlights the clinical course of neonatal hypocalcemic seizures, EEG findings in several cases, and possible mechanisms for both hypocalcemic precipitation of seizures and anticonvulsant ineffectiveness. (*J Child Neurol* 2002;17:236–239).

The incidence of hypocalcemic seizures in the neonatal population has decreased dramatically since the improvement in infant formulas over the past few decades. Most infants that present with seizures owing to hypocalcemia have underlying endocrinologic etiologies rather than dietary insufficiencies.[1] There is a universal clinical description of infants with hypocalcemia reporting underlying irritability and jitteriness between seizures that are frequently focal and clonic.[1–8] Standard anticonvulsants are often described as ineffective, with intravenous calcium leading to clinical and electrographic improvement within days.[1,4–5,9] We describe a 1-month-old infant who presented with hypocalcemic seizures. Etiologies of how hypocalcemia causes seizure activity, why anticonvulsants might be ineffective, and a review of the previously reported cases are discussed.

Case Report

A 34-day-old infant boy presented to the emergency department with a 1-day history of jerking episodes and poor feeding. The episodes were described as stiffening and flexing of both the upper and lower extremities, "foaming at the mouth" with eye deviation to the right, and apnea with facial pallor. These events occurred approximately every 10 minutes and lasted several seconds to minutes, followed afterward by a period of crying and irritability. There was rarely a return to normal alertness between episodes.

Past medical history was significant for neonatal jaundice. Birthweight was 2440 g. Pregnancy had been complicated by Class A gestational diabetes. Maternal prenatal serologies were negative, and two routine newborn screens for metabolic disease were normal. Family history was significant for maternal neurofibromatosis type 1 (cutaneous lesions only).