Leigh syndrome caused by mutations in the flavoprotein (Fp) subunit of succinate dehydrogenase (SDHA)

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Detailed clinical, neuroradiological, histological, biochemical, and genetic investigations were undertaken in a child suffering from Leigh syndrome. The clinical symptoms started at age five months and led to a severe progressive neurodegenerative disorder causing epilepsy, psychomotor retardation, and tetraspasticity. Biochemical measurement of skeletal muscle showed a severe decrease in mitochondrial complex II. Sequencing of SDHA revealed compound heterozygosity for a nonsense mutation in exon 4 (W119X) and a missense mutation in exon 3 (A83V), both absent in normal controls. In six additional patients—five with Leigh or Leigh-like syndrome and one with neuropathy and ataxia associated with isolated deficiency of complex II—mutations in SDHA were not detected, indicating genetic heterogeneity.

CASE REPORT

The nine year old girl was first child of non-consanguineous German parents. Family history was negative. The patient was born healthy at 38 weeks after normal pregnancy (weight 2700 g, length 49 cm, head circumference 34 cm). At five months of age developmental delay with axial hypotonia and gaze fixation difficulties were noted. At eight months of age (weight 7780 g (50th centile), length 65 cm (3rd centile), head circumference: 41 cm (3rd centile)), neurological examination showed generalised muscular hypotonia with axial predominance. She could not fix her gaze and bilateral horizontal nystagmus was noted. Deep tendon reflexes were bilaterally increased, with Achilles clonus and Babinski sign. Neither grasping nor eye contact were possible. Focal and generalised seizures were noted. Over recent years the child has shown an arrest of psychomotor functions with recurrent seizures and further development of severe contractures. At the current age of nine years she is tetraparetic, unable to speak, has complete visual loss, and has seizures without involvement of other organs. A muscle biopsy was done at age of nine years because of possible prenatal consequences.

METHODS

Morphology and biochemistry of skeletal muscle

Serial cross sections (8 μm) were obtained for histochemical stains according to standard procedures. Activities of the respiratory chain (RC) complexes I–IV were determined in skeletal muscle of the patient as described.8

DNA analysis

DNA and RNA extraction was done according to standard purification protocols (Qiagen, Hildesheim, Germany). Direct sequencing of fibroblast cDNA as well as the promoter region was carried out according to Parfait et al.4 We constructed intronic primers for amplification of the nuclear gene (online material). Polymerase chain reaction (PCR) for sequencing the nuclear gene was done by using the HotStar Taq kit (Qiagen, Hildesheim, Germany) with 50°C annealing temperature.

Denaturing high performance liquid chromatography

Denaturing high performance liquid chromatography (DHPLC) analysis was carried out with the PCR product of

Abbreviations: DHPLC, denaturing high performance liquid chromatography; RC, respiratory chain; SDH, succinate dehydrogenase
exon 3 of SDHA as described on an automated HPLC device equipped with a DNA separation column (WAVE™ DNA Fragment Analysis System: Transgenomic, San Jose, California, USA). All fragments were separated by an universal gradient, starting at 40% and ending at 70% of buffer B (Transgenomic), over a period of seven minutes at temperatures of 56°C, 58°C, and 59°C.

Immunoblotting
Twenty five micrograms of protein from skeletal muscle homogenates were loaded on 12% polyacrylamide gels. The following antibodies were used according to the recommendations of the supplier: mouse monoclonal anti-SDHA antibody (anti-complex II Fp 70 kDa, Molecular Probes, 1:2000), anti-chicken actin (Developmental Studies Hybridoma Bank, University of Iowa, 1:2000) and secondary anti-mouse IgG coupled to HRP (Dako) 1:2000. Signals were visualised with enhanced chemiluminescence (ECL plus; Amersham Pharmacia Biotech) according to the manufacturer’s recommendations.

RESULTS
Magnetic resonance imaging
Brain magnetic resonance imaging (MRI) at age eight months showed bilateral cortical atrophy with massive T2 weighted signal intensities in the basal ganglia involving the thalamus, pallidum, substantia nigra, nucleus ruber, and nucleus caudatus; however, the brain stem and the cerebellum were normal.

At age three years, cortical atrophy and ventricular enlargement had markedly progressed. The T2 weighted signal hyperintensities in the putamen and nucleus caudatus were still present; however, the bilateral thalamus lesions had disappeared. Defect myelination was described predominantly in the periventricular white matter. Cerebellum and pons were normal.

Morphology and biochemistry of skeletal muscle
Muscle biopsy of the left tibialis anterior muscle showed normal results on light and electron microscopy. Histochernistry for SDH showed equal staining of all fibres. Biochemical measurements of the respiratory chain enzymes revealed a significantly decreased activity of complex II (3.7 U/gNCP; normal range 16 to 42 U/gNCP, 23% residual activity) but no abnormalities in the activities of other RC enzymes (pyruvate dehydrogenase and citrate synthase).

DNA analysis
Direct sequencing of SDHA revealed two compound heterozygous mutations in the patient: one nonsense mutation in exon 4 (W119X) inherited from the father, and a missense mutation in exon 3 (A83V) inherited from the mother. Both mutations were also present in the patient’s cDNA; however, the W119X allele was difficult to detect, possibly because of nonsense mediated decay. A83V was not present in 100 normal control DNA samples screened by WAVE. Sequencing of all exons, the exon–intron boundaries, and the SDHA promoter region revealed several polymorphisms, present in the patient as well as in control DNA samples (online-only material).

By sequencing the cDNA of SDHA, we detected an additional cDNA species expressed by the previously described SDHA pseudogene on chromosome 3 that showed a few differences compared with the “true” SDHA gene on chromosome 5, including a frameshift mutation leading to a stop codon in exon 2.

Immunoblotting
Immunoblotting with anti-SDHA antibody (CII Fp) showed a significantly diminished signal in the patient compared with controls, thus indicating a reduced steady state level of this protein. As a control for the equal loading, an immunodetection of the same blot was performed using actin antibody (fig 1).

DISCUSSION
Isolated complex II deficiency is a very rare condition, occurring in approximately 2–4% of all respiratory chain enzyme deficiencies. Mutations of SDHA have been reported in only five families so far. Three of these presented with autosomal recessive Leigh syndrome,1 3 and one child died suddenly at the age of five months from a severe deterioration of neuromuscular, cardiac, and hepatic symptoms after an intermittent infection.9 All of these children were carrying missense mutations in the C-terminal domain. In the fifth family, two siblings suffered from late onset optic atrophy, ataxia, and myopathy, and a heterozygous mutation was identified, suggesting a possible role of SDHA mutations in autosomal dominant neurodegenerative disorders.10 Further studies showed that several patients with complex II deficiency do not have mutations of SDHA. This suggested a role of additional nuclear genes involved in synthesis, assembly, or maintenance of SDH.11 Interestingly, dominant mutations of the three other SDH subunit genes (SDHB, SDHC, and SDHD) were only described in association with tumours, such as hereditary paraganglioma and abdominal phaeochromocytoma.12

The biochemical complex II defect in our patient was confirmed by a significantly reduced level of SDHA protein in muscle and compound heterozygote mutations in the SDHA gene. Although the activity of complex II was markedly reduced (23% rest activity), SDHA was still detectable by histochemistry. We hypothesise that partial SDH decrease may be overlooked if histochemistry only is performed. The heterozygote missense mutation (A83V) inherited from the mother occurred in a region of the protein which is conserved over a wide range of species (online-only material). It was not found in more than 200 control alleles. W119X is the first stop mutation described in SDHA; all other previously described mutations were missense mutations. Both parents are healthy and carry one of the mutations heterozygously, supporting a recessive trait in this family.

Parfait et al8 reported two almost identical SDHA gene copies encoding SDHA (Chr 5p15 and 3q29) with preserved genomic organisation (15 exons for both copies). The SDHA copy on chromosome 3q29 contains a 1 base pair (bp)
deletion in exon 2 which is predicted to cause a frameshift and premature termination of the amino acid chain. Thus the SDHA copy on Chr 3q29 probably represents a pseudogene. However, by analysing cDNA as described we found that both SDHA copies are expressed, which is in contrast to a previous report. Thus by screening cDNA only, heterozygous mutations might easily be missed as they represent 25% (one in four alleles) or fewer. This may cast doubt on an SDHA mutation detected by cDNA sequencing which was thought to have a “dominant” effect. We therefore designed intronic primers to specifically amplify and sequence the SDHA gene on chromosome 5.

Six additional patients of our cohort similarly showed a severe isolated complex II deficiency—five presented with Leigh or Leigh-like syndrome and one with neuropathy and ataxia (table 1).

No pathogenic mutations in SDHA were found, suggesting genetic heterogeneity that confirms previous studies. Further investigation using cells or tissues of these patients are warranted in order to identify additional genes and thus far unknown mechanisms involved in the maintenance and assembly of mitochondrial complex II.

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**REFERENCES**