Leukoencephalopathy due to Complex II Deficiency and Bi-Allelic *SDHB* Mutations: Further Cases and Implications for Genetic Counselling

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Abstract Isolated complex II deficiency is a rare cause of mitochondrial disease and bi-allelic mutations in *SDHB* have been identified in only a few patients with complex II deficiency and a progressive neurological phenotype with onset in infancy. On the other hand, heterozygous *SDHB* mutations are a well-known cause of familial paraganglioma/pheochromocytoma and renal cell cancer. Here, we describe two additional patients with respiratory chain deficiency due to bi-allelic *SDHB* mutations. The patients' clinical, neuroradiological, and biochemical phenotype is discussed according to current knowledge on complex II and *SDHB* deficiency and is well in line with previously described cases, thus confirming the specific neuroradiological presentation of complex II deficiency that recently has emerged. The patients' genotype revealed one novel *SDHB* mutation, and one *SDHB* mutation, which previously has been described in heterozygous form in patients with familial paraganglioma/pheochromocytoma and/or renal cell cancer. This is only the second example in the literature where one specific *SDHx* mutation is associated with both recessive mitochondrial disease in one patient and familial paraganglioma/pheochromocytoma in others. Due to uncertainties regarding penetrance of different heterozygous *SDHB* mutations, we argue that all heterozygous *SDHB* mutation carriers identified in relation to *SDHB*-related leukoencephalopathy should be referred to relevant surveillance programs for paraganglioma/pheochromocytoma and renal cell cancer. The diagnosis of complex II deficiency due to *SDHB* mutations therefore raises implications for genetic counselling that go beyond the recurrence risk in the family according to an autosomal recessive inheritance.

Introduction

Mitochondrial respiratory chain diseases are inborn errors of metabolism that are caused by defective oxidative phosphorylation. Oxidative phosphorylation is the primary mechanism of ATP production in eukaryotic cells, utilizing the five transmembrane complexes (complex I–V), which constitute the respiratory chain (RC) in the inner mitochondrial membrane. The RC consists of at least 90 protein subunits encoded by nuclear and mitochondrial genes (DiMauro et al. 2013). Clinically, deficiency of the RC with onset in childhood is associated with a variable spectrum of manifestations, including Leigh syndrome, mitochondrial encephalomyopathy with multisystem involvement, and leukoencephalopathy (Goldstein et al. 2013). Brain imaging with magnetic resonance (MRI) may show affection of basal ganglia, cerebellum, brain stem, white matter, and/or cortical structures. Different patterns of MRI abnormalities can support the diagnostic process (Bricout et al. 2014; Helman et al. 2015). Even though involvement of the basal ganglia is a frequent and well-known feature of mitochondrial disease, white matter involvement has been increasingly recognized in more recent patient descriptions (Wong 2012; Morato et al. 2014).

The four subunits of complex II, which is also termed succinate dehydrogenase (SDH), are exclusively encoded by the nuclear genome. Complex II has a dual role in mitochondrial metabolism: it functions in the citric acid cycle as well as in the RC, transferring electrons to ubiquinone (Jain-Ghai et al. 2013). Two subunits, SDHA and SDHB, have a catalytic function, while SDHC and SDHD are considered anchoring proteins. In addition, a number of proteins, including SDHAF1, SDHAF2 (SDH5), SDHAF3, and SDHAF4, are required for correct assembly and functioning of the protein complex (Jain-Ghai et al. 2013). Overall, complex II deficiency is a rare cause of RC deficiency and was primarily identified in cases with bi-allelic *SDHA* and *SDHAF1* mutations, while heterozygous mutations of *SDHA*, *SDHB*, *SDHC*, *SDHD*, and *SDHAF2* were found to cause familial paraganglioma/pheochromocytoma (Ghezzi et al. 2009; Rutter et al. 2010; Jain-Ghai et al. 2013). Familial paraganglioma/pheochromocytoma is a hereditary tumor susceptibility disorder with development of paraganglioma and pheochromocytoma with high penetrance, and with an increased risk for malignancy and renal cell carcinoma in the case of *SDHB* mutations (Ricketts et al. 2010). Complex II-related RC deficiencies show a wide clinical spectrum including presentations with isolated myopathy/cardiomyopathy as well as severe multisystem disorders with neurological manifestations. Recently, the

first patients with complex II deficiency due to bi-allelic mutations in *SDHB* and *SDHD* were described and patients presented with progressive mitochondrial encephalomyopathy (Alston et al. 2012; Jackson et al. 2014; Helman et al. 2015).

Here, we present two additional patients with RC deficiency due to *SDHB* mutations. The patients' phenotype including their leukoencephalopathic MRI pathology is described and compared to the phenotypic spectrum associated with complex II deficiency. The implications of identifying complex II deficiency for genetic counseling are discussed in the context of current knowledge regarding familial paraganglioma/pheochromocytoma.

Subjects and Methods

Patient 1 Patient 1 was the first common female child of consanguineous parents of Lebanese descent. The mother's sister deceased at the age of 18 months due to an undiagnosed progressive neurological disease with onset at the age of 10 months; the pedigree is presented in Fig. 1. There was no family history of cancer or paraganglioma/pheochromocytoma. The patient was born by cesarean section in gestational week 35 because of intrauterine growth retardation; birth weight was 1,663 g (−34% SGA), length 41 cm (−2 SD), head circumference 30 cm (−1.1 SD), and Apgar score normal. The pregnancy had been complicated by preeclampsia. The neonatal course was uncomplicated. The patient's development during the first 12 months was slightly delayed with truncal hypotonia and instable sitting position, but the patient crawled at 8 months and pulled herself into a standing position at 10 months of age. From the age of 12 months, a progressive loss of these previously acquired abilities was noted and the deterioration was more pronounced during infections. At 15 months of age, she had pronounced truncal hypotonia, increased muscle tone and pathologically increased deep tendon reflexes in upper and lower extremities, extensor plantar response, and intermittent vertical nystagmus. Weight, length, and head circumference had developed along −1.8, −1.4 SD, and −1.6 SD, respectively. Initial laboratory analysis showed normal blood counts and routine biochemistry parameters. Blood gas analysis showed compensated metabolic acidosis with a lactate level of 3.5 mmol/L (ref < 2.1 mmol/L). Lactate, glucose, and protein concentration, as well as cell count, and amino acid profile in cerebrospinal fluid were normal. Urine metabolic screening showed a normal lactate concentration, but increased levels of ketone bodies and Krebs cycle metabolites, especially succinate. All additional metabolic

Fig. 1 Pedigree of the family of patient 1 (arrow). Affected family members are shown with *filled symbols*, and heterozygotes for the p.Leu257Val variant are shown with *half-filled symbols*

examinations and array CGH analysis were without abnormalities. Eye examination revealed slight atrophy of the optic nerves bilaterally, reduced vision (6/40), and horizontal nystagmus with elements of opsoclonus. Cerebral MRI at 16 months of age was pathological and is presented in Fig. 2a–h. A muscle biopsy was performed at the age of 18 months. In the further course, the patient's neurological deterioration continued, and she became dependent on gavage feeding. Her truncal hypotonia with an opisthotonic neck position and her spasticity increased. At the age of 25 months, the patient deceased in the course of a severe respiratory infection causing multi-organ failure.

Patient 2 Patient 2 was the third male child of healthy and unrelated parents of Turkish and Swedish descent (mother and father, respectively). There was no family history of cancer or paraganglioma/pheochromocytoma. Pregnancy was complicated by preeclampsia. The patient was born by elective cesarean section in gestational week 35 because of intrauterine growth retardation. Birth weight was 1,200 g (< -3 SD), length was 38.5 cm (< -3 SD), and head circumference was 30 cm (-2 SD). Apgar values were normal. The neonatal course was uncomplicated. The early psychomotor development was normal, but from age 6 months a progressive loss of previously acquired skills was noted with episodic deterioration during infections. At 9 months of age, he had pneumonia with respiratory failure necessitating assisted ventilation and lost the ability to babble, grasp, turn around, and needed tube

feeding. Neuropediatric examination showed complete head lag with pronounced truncal hypotonia, increased muscle tone in upper and lower extremities as well as increased tendon reflexes. Weight, length, and head circumference had developed along -3.5 , -3 SD, and -1.5 SD, respectively. Initial laboratory analysis showed increased blood lactate levels up to 9 mmol/L (ref < 1.8), serum levels of ALAT and ASAT up to 40 (ref < 0.6) and 30 μ kat/L (ref < 0.8), respectively, creatine kinase level to 4,970 U/L (ref < 150), and the INR level up to 2.4. A cardiomyopathy was found with severe dilatation and hypertrophy of the septum and posterior wall of the left ventricle. Upon treatment with enalapril, metoprolol, and furosemide, the biochemical parameters normalized except for lactate, which remained slightly increased. Cerebral MRI and MR spectroscopy at 11 months of age showed leukoencephalopathy, detailed in Fig. 2i–o. Cerebrospinal fluid analysis showed a normal cell count and glucose level, while the lactate level was 3.0 mmol/L (ref < 1.7). Urine organic acid screening showed slightly increased lactate, but no succinate. Eye examination revealed strabismus and anisometropia with abnormal pursuit and saccades. EEG was normal. A muscle biopsy was performed at the age of 11 months (Fig. 3a). The patient was put on treatment with riboflavin and coenzyme Q10. In the further course, the patient's condition was stable with some improvement until the age of 1 year when he had a severe relapse with multi-organ failure and cardiac arrest leading to death.

Fig. 2 Magnetic resonance imaging (MRI) of patient 1 (a–h) at 15 months of age and patient 2 (i–n) at 10 months of age including

T2-weighted (T2W) (a–e; i–k, n) and diffusion-weighted (DWI) (f–h; l, m) imaging. MRI of patient 1 demonstrates increased T2W signal

Methods

Analysis of RC Enzyme Activity

Patient 1 Spectrophotometric analysis of RC enzyme activity in frozen muscle tissue and cultured fibroblasts was performed as previously described (Larsen et al. 2012).

Patient 2 Isolation of fresh muscle mitochondria, polarographic measurements, and spectrophotometric enzyme analyses were performed essentially as previously described (Tulinius et al. 1991).

Genetic Investigations

Patient 1 DNA from patient 1 was used for a genomewide search for homozygosity with the Affymetrix GeneChip 6.0 array (Affymetrix Inc., Santa Clara, CA), essentially as described (Ostergaard et al. 2011). *SDHB* and *SDHAF1* were PCR-amplified using intronic primer pairs flanking each exon. PCR products were sequenced using an ABI 3730 DNA analyzer (Applied Biosystems).

Patient 2 Whole Exome Sequencing (WES) and WES data analysis were carried out as described (Sofou et al. 2015). Sanger sequencing of DNA from the patient and his parents verified the mutations identified by WES. Sequencing analysis was performed using an ABI PRISM 3100 Genetic analyzer and the BigDye Terminator v.1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). All *SDHB* variants are numbered according to GenBank accession number NM_003000.2. The strategies for identification of the genetic background for the disorder differed, since the two patients were investigated in different laboratories.

Western Blot Analysis

Western blot analysis was performed essentially as previously reported (Ostergaard et al. 2007). Primary antibodies were against SDHB (Atlas Antibodies, Stockholm, Sweden;

1:1,000), SDHA (Thermo Fischer Scientific, Waltham, MA; 1:1,000), and against porin (Proteintech, Chicago, IL; 1:1,000) as loading control. The secondary antibody was goat anti-rabbit at a 1:1,000 dilution (Dako, Glostrup, Denmark).

Results

Muscle Histology

Histological analysis revealed normal fiber diameter variation with only few centralized nuclei in patient 1, while there was slightly increased fiber diameter variability and normally placed nuclei in patient 2. Both had normal amount of connective tissue and no vacuoles, inclusion bodies, or ragged red fibers in trichrome staining (not shown). Both patients had positive COX staining in all fibers, while there was a diffuse and severe lack of SDH staining (shown for patient 2 in Fig. 3a, I). Oil-red-O staining showed some fibers with lipid content, but within the normal range (patient 1, not shown). Sudan Black staining revealed some fibers with increased lipid content in patient 2 (not shown). Electron microscopy was performed in patient 2 only and was found pathological as presented in Fig. 3a, III.

RC Enzyme Activity

Polarographic (patient 2) and spectrophotometric (patient 1 and 2) analyses revealed a severe complex II deficiency in muscle from both patients (spectrophotometric 14 and 7% of control mean for patient 1 and 2, respectively; polarographic 17% of control mean for patient 2). Complex I activity was mildly affected, whereas the other enzyme activities were practically normal. The results were confirmed in fibroblasts of patient 1 with complex II activity at 6% of control mean (supplementary Table S1). Together with the finding of increased succinate concentration in urine of patient 1 and an abnormal succinate peak on ¹H MR spectroscopy of patient 2, this constellation confirmed complex II deficiency.

Fig. 2 (continued) intensity involving frontal, parietooccipital, and posterior temporal white matter sparing juxtacortical fibers (a–c), lateral geniculate bodies (b), corpus callosum with sparing of the inner and outer blades (c), middle cerebellar peduncles, corticospinal tracts in the anterior pons (d, e), and extensive involvement of the cervical medulla (e). Diffusion restriction is demonstrated in the periventricular white matter and corpus callosum (f), and particularly evident in the lateral geniculate bodies (g). Minimal changes are seen in the thalamic pulvinar (h) and bilateral ventral nuclei (f). MRI of patient 2 likewise reveals extensive T2 hyperintensities involving frontal, parietooccipital, and posterior temporal white matter with sparing of the

juxtacortical fibers (i, k). T2 hyperintensity and restricted diffusion of the bilateral pulvinar and ventral thalamic nuclei (i, l) was prominent. Infratentorially, the pons including crossing fibers, middle cerebellar peduncles, and deep white matter of the cerebellar hemispheres (j, k) as well as medullary pyramids and central cervical medulla (n) are affected. Decreased diffusivity is demonstrated in the dorsal pons and cerebellar peduncles (m). (o) shows ¹H MR spectroscopy with short echo time and voxel of interest placed over centrum semiovale with an abnormal succinate peak at 2.4 ppm detected in patient 2

Fig. 3 Muscle histology, sequence analysis, and Western blot analysis in patients with *SDHB* mutations. **(a)** Muscle histology of patient 2: Muscle biopsy of the vastus lateralis muscle at age 1 year demonstrated reduced histochemical activity of succinate dehydrogenase (SDH) (*I*) compared to an age-matched control (*II*). Electron microscopy (*III*) demonstrated a few lipid droplets (L), some enlarged mitochondria with partly disrupted cristae (arrows), and abundant glycogen between the myofibrils. **(b)** DNA sequence analysis of *SDHB* in patient 1 indicates the position of homozygous missense

mutation c.769C>G (p.Leu257Val) (*top*) compared to wild-type sequence in a control individual (*bottom*). **(c)** Alignment of a relevant stretch of amino acid sequence of *SDHB* in different species shows that leucine 257 (black rectangle) is evolutionary conserved. **(d)** Immunoblot analysis of fibroblast cells from patient 1, patient 2, and three controls with antibodies against *SDHA*, *SDHB*, and porin as loading control reveals reduced amount of *SDHB* and a lesser extent of *SDHA* in both patients

Molecular Genetic Analyses

Patient 1 Homozygosity mapping showed several homozygous regions, and *SDHB* and *SDHAF1* were the only complex II-related genes located in regions of homozygosity. Sequencing of the *SDHAF1* gene was normal while sequencing of the *SDHB* gene showed a homozygous missense mutation c.769C>G (p.Leu257Val), not previously reported in the literature (Fig. 3b). It has been reported in 1 out of 121,292 alleles in the ExAC database (<http://exac.broadinstitute.org/>), but it has to be noted that this database does not include any significant representation of persons of Lebanese descent. This mutation affects a highly conserved amino acid (Fig. 3c). According to in silico analysis with SIFT (<http://sift.jcvi.org/>), MutationTaster (<http://www.mutationtaster.org/>), and PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), we assessed this mutation as likely pathogenic. Both parents as well as several other family members were heterozygous carriers of the mutation (Fig. 1).

Patient 2 The variants identified by WES were sequentially filtered according to four criteria: (1) variants located in genes encoding mitochondrial proteins in MitoCarta (Pagliarini et al. 2008), (2) low allele frequency in general population, as described previously (Sofou et al. 2015), (3) variants listed in Human Gene Mutation Database (HGMD), and (4) variants compatible with an autosomal recessive pattern of inheritance. This strategy rendered only two variants in one candidate gene, *SDHB*, namely c.143A>T (p.Asp48Val) and c.689G>A (p.Arg230His). The variants were verified by Sanger sequencing and the parents each carried one of the mutations (p.Asp48Val maternal and p.Arg230His paternal). Information on genetic testing of other family members was not available. Both mutations have been reported previously; c.143A>T (p.Asp48Val) has been found homozygous and compound heterozygous in patients with leukodystrophy and complex II deficiency (Alston et al. 2012; Helman et al. 2015), whereas the c.689G>A (p.Arg230His) mutation has been reported in heterozygous form in two patients with

apparently sporadic paraganglioma/pheochromocytoma (Amar et al. 2005), in a family with paraganglioma/pheochromocytoma and renal cell cancer (Ricketts et al. 2012), as well as in patients with head and neck paraganglioma (Cerecer-Gil et al. 2010; Peterson et al. 2014).

Western Blot Analysis

Western blot analysis of fibroblast cells showed a clearly decreased amount of SDHB protein in patient 1 and 2. The level of SDHA protein was only slightly reduced (Fig. 3d).

Discussion

While heterozygous *SDHB* mutations are a well-known cause of familial paraganglioma/pheochromocytoma (Ricketts et al. 2010), bi-allelic mutations in *SDHB* have been identified in only a few patients with complex II deficiency and a progressive neurological phenotype with onset in infancy (Alston et al. 2012; Helman et al. 2015). Here, we present two further patients with bi-allelic *SDHB* mutations causing complex II deficiency and a progressive neurological disease with leukoencephalopathy. Altogether three mutations were found, of which two have been reported previously. The novel missense mutation in *SDHB* (c.769C>G, p.Leu257Val) was assessed as most likely pathogenic since it was found in a patient with complex II deficiency as assessed by biochemical measurement, *SDHB* was one of the two complex II genes in the homozygous regions according to homozygosity mapping, the amino acid is highly conserved, and database analysis (in silico analysis and ExAC database) supported this assessment. More significantly, Western blot analysis showed decreased amount of SDHB protein in patient fibroblasts. The p. Asp48Val variant has been found in six out of seven reported patients with complex II deficiency due to *SDHB* mutations (Alston et al. 2012; Helman et al. 2015), whereas the p.Arg230His variant has been reported in heterozygous form in patients with familial paraganglioma/pheochromocytoma (Amar et al. 2005) and/or renal cell cancer (Ricketts et al. 2012). To our knowledge, this is only the second example in the literature where one specific *SDHx* mutation is associated with both recessive mitochondrial disease in one patient and familial paraganglioma/pheochromocytoma in others; previously, this has exclusively been described for the c.91C>T (p.Arg31*) variant in *SDHA* (Renkema et al. 2015).

Clinically, the phenotypic presentation of both *SDHB* patients in this report lies well within the phenotypic spectrum of complex II deficiency (Jain-Ghai et al. 2013) as well as of *SDHB* deficiency (Alston et al. 2012; Helman

et al. 2015). Onset at age 6–12 months, progressive neurological involvement with spasticity, truncal hypotonia, and pathological brain imaging as in patient 1 and 2 are typically described for the more severely affected patients, and cardiomyopathy as found in patient 2 is likewise reported in about 25% of 37 complex II deficient patients reviewed by Jain-Ghai et al. (2013). Time of death at age 25 months (patient 1) and 12 months (patient 2) is relatively early compared to mortality in the complex II deficient cohort where only 5 of 37 patients reportedly deceased before the age of 2.5 years. On the other hand, outcome data were not available on at least 15 of the patients in this study. Of the six patients with *SDHB* deficiency, one patient died at age 1 year, whereas the remaining five patients are alive, with current ages from 19 months to 9 years (Alston et al. 2012; Helman et al. 2015). The ophthalmological features of patient 1 including optic atrophy, decreased vision, and nystagmus are in line with the expected phenotype of mitochondrial encephalomyopathy including previously reviewed patients with complex II deficiency (Jain-Ghai et al. 2013). No specific eye manifestations have been described in previous reports of complex II-related leukoencephalopathy (Alston et al. 2012; Jain-Ghai et al. 2013; Helman et al. 2015).

Regarding neuroradiological findings, both patients presented with encephalomyelopathy (Fig. 2) with extensive involvement of white matter in frontal, parietooccipital, and posterior temporal lobes with sparing of the U-fibers, involvement of corpus callosum, the middle cerebellar peduncles, and cerebellar deep white matter. In addition, the corticospinal tracts and cervical medulla were affected extensively, and pulvinar and ventral thalamic nuclei showed pathological signal in both patients. MR spectroscopy was only performed in patient 2 and revealed a succinate peak, a specific finding in complex II deficiency (Brockmann et al. 2002; Ghezzi et al. 2009). Recently, a distinct MRI pattern was defined for patients with *SDH* deficiency-related leukoencephalopathy (Helman et al. 2015), with a typical progression of MRI appearance during the course of disease, allowing differentiation from other mitochondrial leukoencephalopathies. It can be added to the list of recognizable MRI patterns, which serve as valuable diagnostic tools in the evaluation of genetic leukoencephalopathies (Schiffmann and van der Knaap 2009; Parikh et al. 2015). MRI pathologies of the patients in this study fit into this MRI pattern description, probably corresponding to early or intermediate stage of the disease. As MRI pattern recognition, MR spectroscopy as well as next generation sequencing technologies get more readily available, the diagnostic approach in patients with suspected mitochondrial leukoencephalopathy will lead to the establishment of diagnosis without the need for muscle biopsy.

The diagnosis of complex II deficiency due to *SDHB* mutations raises implications for genetic counseling that go beyond the recurrence risk in the family according to an autosomal recessive inheritance. Heterozygous mutations in *SDHB* have been described in familial paraganglioma/pheochromocytoma with a higher rate of malignancy and earlier onset than with the other *SDH* genes. *SDHB* mutations have also been associated with gastrointestinal stromal tumors, and renal cell carcinoma (Neumann et al. 2004; Ricketts et al. 2010). In one study, the lifetime risk (at age 70 years) of *SDHB*-related symptoms was ~80%, with a penetrance of ~60% for pheochromocytoma and ~45% for paraganglioma. The risk of renal cell cancer by age 70 years was 15–20% (average age-specific cumulative risk for cancer 50% by age 50) (Ricketts et al. 2010). Later studies have questioned this high penetrance and have suggested an overestimation because no correction was made for the ascertainment of mutation carriers. Substantially lower average penetrance was estimated when adjusting for ascertainment (average penetrance 13% at age 50; average lifetime penetrance (to age 80) 30%) (Schiavi et al. 2010). These results were confirmed in a more recent study based on a large pedigree with paraganglioma and pheochromocytoma with a Dutch founder mutation in *SDHB* (9% by age 50). The bias in this study is expected to be low, but an effect of this specific mutation cannot be excluded (Rijken et al. 2016). Also with these lower estimates, the need for expanded family testing and referral of heterozygous mutation carriers to a surveillance program remains unchanged. This should also be considered in the families of patients with bi-allelic mutations in *SDHD* and *SDHA* that also have been described with a dual role, with mitochondrial disease in patients with bi-allelic mutations and risk of paraganglioma/pheochromocytoma and cancer in heterozygotes (Ricketts et al. 2010; Kunst et al. 2011; Welander et al. 2011; Renkema et al. 2015).

There were no known cases of paraganglioma/pheochromocytoma in either of the two families reported here. In the family of patient 1, there are at least eight healthy carriers (five shown to be carriers and three obligate carriers) of the p.Leu257Val variant, and thus the penetrance of this variant can be assumed to be low. The p.Arg230His variant found in patient 2 has previously been reported in patients with paraganglioma/pheochromocytoma (Amar et al. 2005; Ricketts et al. 2012), but the penetrance is unknown. Due to the uncertainties regarding penetrance of different mutations, all heterozygous *SDHB* mutation carriers from family 1 were referred for a surveillance program comprising an annual clinical examination with measurement of blood pressure, urine catecholamines, and plasma metanephrines. In addition, MRI of the cranial basis, neck, thorax, abdomen, and pelvis was done every second year, alternating with MIBG scintigraphy. Children were tested

for the mutation from age 10 years, and mutation carriers were referred to surveillance. Attention should especially be warranted in the case of the father of patient 2 who carries the *SDHB* mutation p.Arg230His that previously has been described to be associated with familial paraganglioma/pheochromocytoma and at least two cases of renal cell cancer. The parents were referred to a surveillance program consisting of a clinical examination and CT scan of the neck, thorax, and abdomen as well as screening with plasma metanephrines.

In this report, we present two additional patients with complex II deficiency with leukoencephalopathy caused by recessive *SDHB* mutations. We suggest that, in addition to *SDHA* and *SDHAF1*, also sequencing of *SDHB*, *SDHD*, and possibly *SDHC* should be included into the genetic work-up of patients with multisystem mitochondrial disorder and complex II deficiency, not least because of the potential implications regarding cancer surveillance in family members.

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Synopsis

Diagnosing leukoencephalopathy due to complex II deficiency and bi-allelic *SDHx* mutations has important implications for genetic counseling going beyond the recurrence risk in the family.

Author Contributions

S.G., N.D., and E.Ø. have planned the work; all authors have contributed to the evaluation of the patients, including analysis and interpretation of clinical, neuroradiological, biochemical, histological, and molecular results; all authors have contributed pertinent aspects to the reporting of this work.

Sabine Grønborg serves as a guarantor for the manuscript.

Conflict of Interest

Sabine Grønborg, Niklas Darin, Maria J. Miranda, Bodil Damgaard, Jorge Asin Cayuela, Anders Oldfors, Gittan Kollberg, Thomas V.O. Hansen, Kirstine Ravn, Flemming Wibrand, and Elsebet Østergaard declare that they have no conflict of interest.

For this Case Report ethics approval was not required at the participating authors' institutions.

The patients' families have given informed consent for the publication of these case reports.

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