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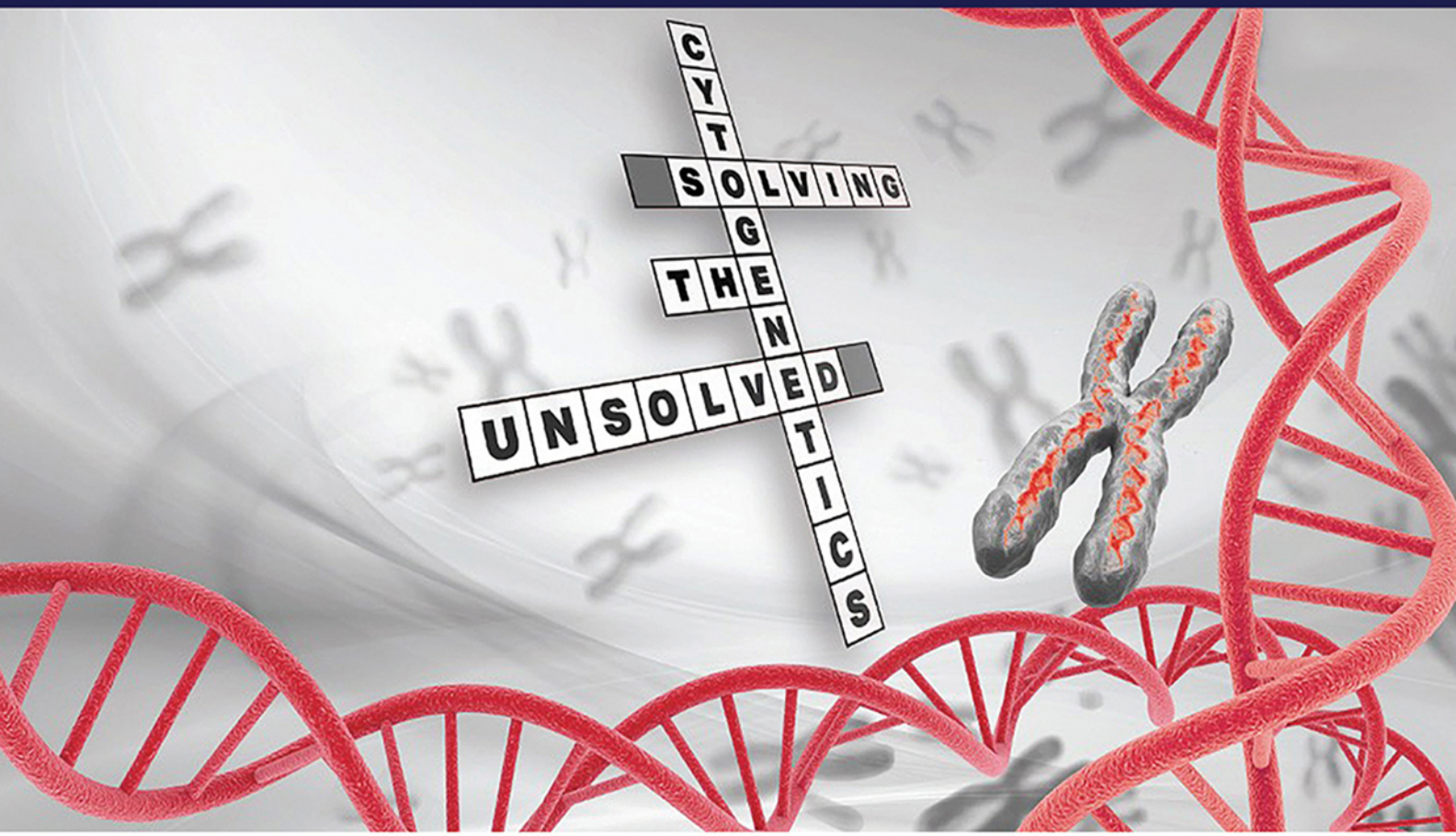


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CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

# *SDHA* Mutation with Dominant Transmission Results in Complex II Deficiency with Ocular, Cardiac, and Neurologic Involvement

**Carolina Courage,[1] Christopher B. Jackson,[2,3] Dagmar Hahn,[2] Liliya Euro,[3] Jean-Marc Nuoffer,[2] Sabina Gallati,[1] and André Schaller[1]***

[1]Division of Human Genetics, Department of Pediatrics, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

[2]Division of Inherited Metabolic Diseases, Institute of Clinical Chemistry, Inselspital, Bern, Switzerland

[3]Biomedicum Helsinki, Research Program for Molecular Neurology, University of Helsinki, Helsinki, Finland

Manuscript Received: 22 October 2015; Manuscript Accepted: 7 September 2016

**Isolated defects of the mitochondrial respiratory complex II (succinate dehydrogenase, SDH) are rare, accounting for approximately 2% of all respiratory chain deficiency diagnoses. Here, we report clinical and molecular investigations of three family members with a heterozygous mutation in the large flavoprotein subunit SDHA previously described to cause complex II deficiency. The index patient presented with bilateral optic atrophy and ocular movement disorder, a progressive polyneuropathy, psychiatric involvement, and cardiomyopathy. Two of his children presented with cardiomyopathy and methylglutaconic aciduria in early childhood. The daughter deceased at the age of 7 months due to cardiac insufficiency. The 30-year old son presents with cardiomyopathy and developed bilateral optic atrophy in adulthood. Of the four nuclear encoded proteins composing complex II (SDHA, SDHB, SDHC, SDHD) and currently known assembly factors SDHAF1 and SDHAF2 mainly recessively inherited mutations have been described in *SDHA*, *SDHB*, *SDHD*, and *SDHAF1* to be causative for mitochondrial disease phenotypes. This is the second report presenting autosomal dominant inheritance of a *SDHA* mutation.**



**Key words:** complex II deficiency; SDHA; mitochondrial disorder; cardiomyopathy; optic atrophy

**How to Cite this Article:**
Courage C, Jackson CB, Hahn D, Euro L, Nuoffer J-M, Gallati S, Schaller A. 2017. *SDHA* mutation with dominant transmission results in complex II deficiency with ocular, cardiac, and neurologic involvement.
Am J Med Genet Part A 173A:225–230.

## INTRODUCTION

Complex II (CII; succinate-ubiquinone oxidoreductase; EC 1.3.5.1) of the respiratory chain is composed of four nuclear-encoded proteins [Bourgeron et al., 1995]. The catalytic core is formed by the large flavoprotein (SDHA, 70 kDa) and the iron-sulfur cluster containing SDHB (30 kDa), whereas the hydrophobic subunits SDHC (15 kDa) and SDHD (17 kDa) contain a single heme group, ubiquinone binding sites, and are anchoring the complex within the inner mitochondrial membrane. Complex II couples oxidation of succinate to fumarate with reduction of electron carrier ubiquinone to ubiquinol thus directly connecting mitochondrial electron transfer system (ETS) with Krebs cycle [Saraste, 1999].

Complex II deficiency (OMIM #252011) presents with a wide clinical variability ranging from early onset encephalomyopathies to optic atrophy and tumor formation in adulthood. SDH deficiency itself has been associated with progressive encephalomyopathy with dementia, myoclonic seizures, and short stature, Kearns–Sayre syndrome with conduction defects, myopathy and encephalopathy, isolated hypertrophic cardiomyopathy plus skeletal muscle myopathy, generalized muscle weakness and easy fatigability [Rustin and Rötig, 2002]. All four *SDH* genes

Carolina Courage and Christopher B. Jackson contributed equally.
Ethical Approval: Informed consent was obtained from all participating individuals and the study was approved by the local ethical committee of the Canton of Bern.
All authors have reviewed the final manuscript and approved it.
Conflict of interest: None.
Grant sponsor: SNF Postdoc.
*Correspondence to:
André Schaller, Division of Human Genetics, Department of Pediatrics, Inselspital, Bern University Hospital, University of Bern, Freiburgstrasse, CH-3010 Bern, Switzerland.
E-mail: andre.schaller@insel.ch
Article first published online in Wiley Online Library (wileyonlinelibrary.com): 28 September 2016
DOI 10.1002/ajmg.a.37986

*(SDHx)* and one of its known assembly factors *SDHAF2* have tumor suppressor function, with numerous germline and somatic mutations reported in association with hereditary cancer syndromes [Burnichon et al., 2010; Hoekstra and Bayley, 2013].

Up to date only *SDHA* (OMIM #600857), *SDHB* (OMIM #185470), *SDHD* (OMIM #602690) and *SDHAF1* (OMIM #612848] have been described as cause of a predominantly recessively inherited mitochondrial disorder [Bourgeron et al., 1995; Alston et al., 2012; Jackson et al., 2014]. Furthermore, complex II deficiencies in children have been reported to cause Leigh syndrome, infantile and nonspecific leukoencephalopathies, and isolated neonatal cardiomyopathy [Levitas et al., 2010a; Hoekstra and Bayley, 2013; Jain-Ghai et al., 2013]. Manifestations in adulthood include mitochondrial myopathy, exercise intolerance, neurodegeneration in combination with optic atrophy and ataxia [Jain-Ghai et al., 2013]. We describe a family with a heterozygous *SDHA* mutation involved in complex II deficiency causative of a mitochondrial disorder.

## CLINICAL REPORTS

The index patient (Fig. 1A; II:5) presented in early childhood with clumsiness, nystagmus in one eye, cramps in the foot and speech impediment as the first symptoms. He was referred to the children's hospital at the age of 15 years due to progressing symptoms with worsening ocular paresis with dissociated spontaneous nystagmus, pyramidal signs (present Babinski sign), and ataxia. Concomitantly the diagnosis of a cardiomyopathy with cardiomegaly was made. At the age of 47 years a myocardial biopsy with pericardial tamponade as complication revealed a discrete increase of mitochondria with an increased lipofuszinosis. In the same year, bilateral optic atrophy was diagnosed. Over the years the optic atrophy, the eye movements and gait disturbances slowly progressed (last ophthalmologic investigation at the age of 60 years). At 54 years of age the heart problems progressed to a combined hypertrophic and dilative cardiomyopathy with diminished ejection fraction of 48%. Due to the unstable gait, he uses a walker frame since the age of 60 years. His neurological symptoms as well as ocular and cardiac involvement are still progressing. A brain MRI at the age of 60 years was normal. No dementia, stroke-like episodes or epilepsy did occur but the clinical course was further complicated by recurrent episodes of depression with two attempted suicides by intake of analgesics at 40 and 59 years of age.

## Family History

The index patient's wife had a miscarriage at gestational age 10 weeks (III:1). The first child, a boy (III:2), presented with generalized fatigue at the age of 8 months, when the diagnosis of his father was still unknown. Echocardiogram showed slight repolarization dysfunction with the dilative cardiomyopathy being attributed to fibroelastosis resulting in reduced myocardial function. The cardiomegaly showed no signs of heart insufficiency and was well controlled with digoxin and diuretics. At 2.5 years of age, he was hospitalized related to recurrent vomiting due to gastroenteritis. Investigations revealed slight blood lactate elevation (2.4 mmol/L) with repeatedly elevated urinary 3-methylglutaconic and 3OH-methylglutaric excretion. Now, at 30 years of age, the patient presents with cardiomyopathy and previously diagnosed bilateral optic atrophy with apparent progression. Cardiomyopathy is stable with normal output. A cranial MRI was normal except for the optic atrophy and unspecific alterations in the white substance. Beside the progressive optic atrophy, the overall clinical status is stable.

A female child (Fig. 1A: III:3) was stillborn at 26 weeks of gestation with no additional investigations done. The second child, a girl (Fig.1A: III:4), deceased at the age of 7 months due to cardiac insufficiency. Laboratory investigation showed marginally elevated lactate in the blood as well as urinary 3-methylglutaconic and 3OH-methylglutaconic excretion. The autopsy revealed a mild hypertrophic and severely dilated cardiomyopathy with an "abnormal increase of mitochondria" concomitantly with rarefication of myofibrils, abnormal accumulation of mitochondria in heart muscle cells and glycogen increase in hepatocytes at ultrastructural level.

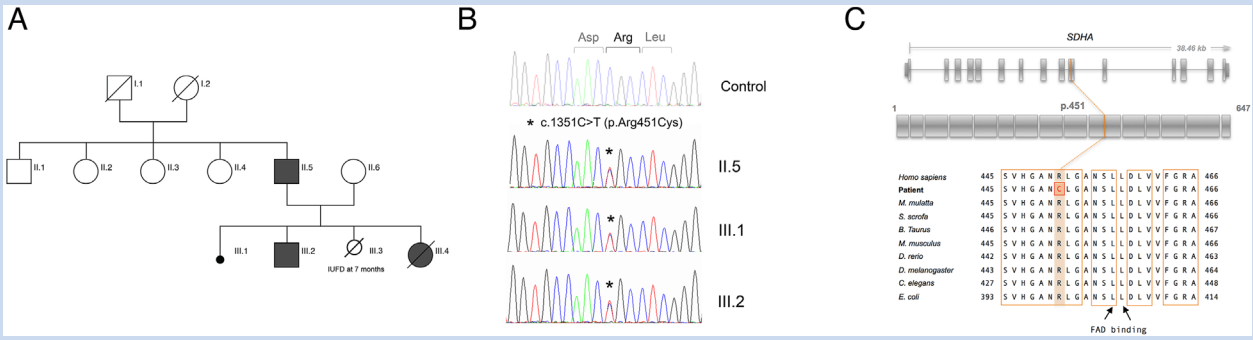


**FIG. 1. Family pedigree (A), molecular genetic analysis (B) and phylogenetic conservation of *SDHA* mutation (C). (A) II.5: index patient currently 63-year-old. III:1 miscarriage at 10th week of gestation, III.2: 30-year-old affected son, III.3: Intrauterine fetal death (IUFD) of unknown etiology at 7 months of gestation and III.4: affected daughter, deceased at 7 months of age. (B) Sequencing of *SDHA* revealed a heterozygous change in exon 10 at position c.1351C>T (p.Arg451Cys) in all affected individuals. (C) This mutation is predicted to affect substrate binding. Protein alignment over various phyla shows high conservation. [Color figure can be viewed at wileyonlinelibrary.com].**

## BIOCHEMICAL AND MOLECULAR INVESTIGATIONS

Fibroblast cultures of all patients were obtained from skin biopsies and from a heart muscle biopsy during cardiac catheterization in patient II:5. Enzymatic activities, respiratory measurements and western blotting were performed as described [Jackson et al., 2014]. Biochemical analysis of individual respiratory complexes in patient fibroblasts revealed an isolated complex II deficiency with residual activities of complex II and succinate cytochrome c oxidoreductase (complex III) coupled respiration (SCCR) of 51% and 43% (Patient II:5), 42% and 47% (Patient III:2) and 33% and 59% (Patient III:4) compared to mean of controls (Fig. 2A). Oxygen consumption in fibroblasts showed abnormal ratios of succinate-related (complex II) pyruvate (complex I) respiration (SRPR): 1.6 (II:5), 1.45 (III:2) and 1.57 (III:4), respectively (control range 0.86–1.42). Analysis of SDHA by western blotting revealed equal levels of SDHA and total mitochondrial protein demonstrating the mutant protein to be stable (Fig. 2B). Molecular genetic analysis has been performed by direct sequencing of candidate genes (*SDHx*, *SDHAF1* and *SDHAF2*) as described [Jackson et al., 2014]. Sequencing of these candidate genes in all patients, revealed a known heterozygous missense mutation c.1351C>T (p.Arg451Cys) (Fig. 1B), which is located in a highly conserved domain of *SDHA* (Fig. 1C). This variant is predicted to be deleterious in silico and was previously associated with late-onset neurodegenerative presentation with optic atrophy, ataxia and myopathy annotated as c.1375C>T (p.Arg408Cys) [Birch-Machin et al., 2000]. An additional cardiomyopathy panel encompassing 174 genes known to be associated with inherited cardiac conditions revealed only the reported heterozygous *SDHA* variant.

## STRUCTURAL ANALYSIS

To elucidate the mutational consequences, homology modeling of the mutation was performed using the resolved structure of porcine succinate-ubiquinone oxidoreductase (PDB id: 1ZOY) [Sun et al., 2005] consisting of the hydrophilic SDHA and SDHB and integral membrane subunits SDHC and SDHD. The electron transport system of complex II consists of flavin adenine dinucleotide (FAD), three iron-sulfur clusters and heme b (Fig. 3A). The native activity of the enzyme is the transfer of electrons from succinate—synthesized in the Krebs cycle—to ubiquinone. Complex II has two ubiquinone binding sites: (i) $Q_P$ located proximally, on the matrix side of the inner mitochondrial membrane and (ii) $Q_D$ located distally, facing the intermembrane space (Fig. 3A). Arg451 located in the flavoprotein subunit (SDHA) is invariant among homologues from all kingdoms of life and forms the succinate binding site together with Thr308, Glu309, and Arg340 [Sun et al., 2005]. Previous modeling of the p.Arg451Cys mutation in a bacterial homologue suggested that the enzyme is inactive due to its inability to bind FAD covalently [Birch-Machin et al., 2000]. Our modeling predicts the p.Arg451Cys mutant to bind succinate via the remaining binding determinants, but likely with a higher Km (Fig. 3C, D). Therefore, loss of the succinate dehydrogenase activity is caused by a different effect of the p.Arg451Cys mutation. Free FAD as an immediate electron acceptor from succinate has a midpoint redox potential ($E_m$) of −219 mV, while the succinate:fumarate redox pair $E_m$ is +30 mV [Blaut et al., 1989]. In order to accept electrons from succinate, the FAD midpoint potential has to be increased. Indeed protein-bound FAD in complex II has an $E_m$ of −80 mV [Yankovskaya et al., 2003], which is achieved by covalent linkage to His99 [Blaut et al., 1989] and the positively charged protonated amino acid residues surrounding the riboflavin moiety of FAD made up by His407, Arg340, and Arg451. The two latter amino acids are also directly involved in succinate binding (Fig. 3B). Removal of the positive charge of Arg451 leads to a drop in FAD $E_m$ by 60 mV resulting in approximately −140 mV. This generates an extremely high energy barrier between succinate/fumarate and FAD/FADH redox pairs in order to tunnel electrons and thereby renders the enzyme inactive.

## DISCUSSION

So far only few reports describe inherited *SDHx* gene defects as underlying cause of mitochondrial disease, involving *SDHA* (Leigh syndrome and cardiomyopathy), *SDHB*, *SDHD* or *SDHAF1* (infantile leukoencephalopathy). Mutations in these genes are known

A

Table 1. Respiratory Chain Complex Activities

| | CS[a] | Activity of Enzymatic Complex[b] | | | | |
|---|---|---|---|---|---|---|
| | | I | II | III | IV | V |
| II:5 | 188 | 0.39 | 0.17 (51%) | n.d. | 0.58 | n.d. |
| III:2 | 188 | 0.29 | 0.14 (42%) | n.d. | 0.79 | 0.19 |
| III:4 | 238 | 0.38 | 0.11 (33%) | n.d. | 0.73 | n.d. |
| Controls (n=22) | | | | | | |
| Range | 134-228 | 0.19 – 0.46 | 0.17 – 0.52 | 0.35 – 0.87 | 0.42-1.11 | 0.12– 0.38 |

[a] Values are given as mU/mg protein.[b] Values are given as mU/mU citrate synthase. For the patient the percentage of the control mean is shown in parentheses. CS – citrate synthase activity. n.d. - not determined.

B

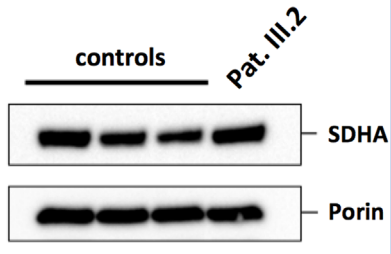


**FIG. 2. Individual respiratory complex activities in fibroblasts of all three patients (A) and western blot analysis (B). (A) All affected patients show a marked decrease of complex II activity in isolated mitochondria from fibroblasts. (B) Western blot shows comparable protein amounts for SDHA in patient III.2 relative to total mitochondrial protein mass (porin).**

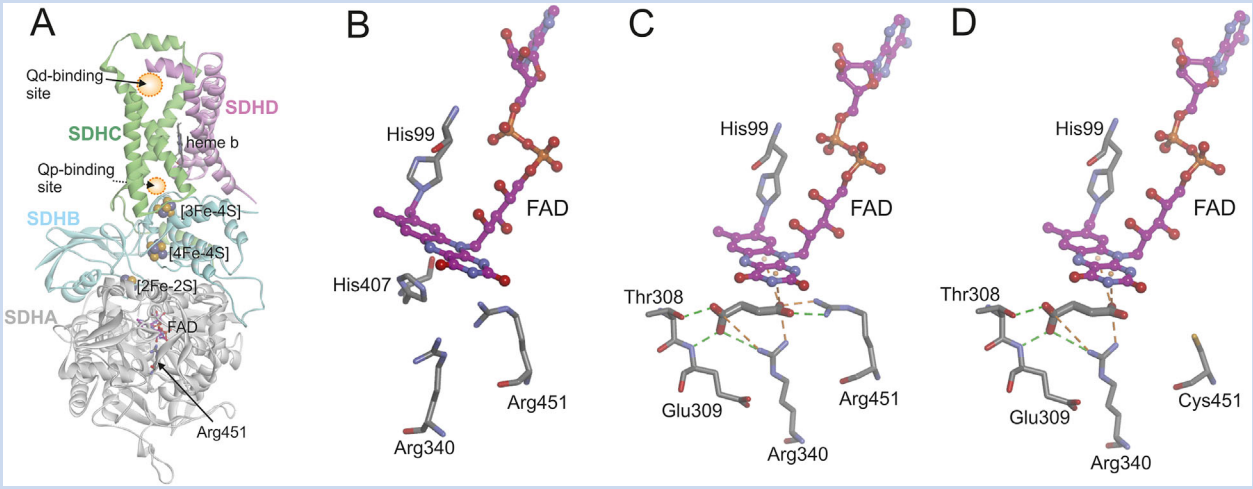


**FIG. 3. Structural analysis of Arg451Cys mutation in SDHA subunit of succinate-ubiquinone oxidoreductase (complex II). (A) Overview of modeled entire human complex II. Location of Arg451 marked by arrow. (B) Polar basic amino acid residues surrounding riboflavin moiety in a distance of 5 Å. (C) Modeled succinate binding in the vicinity of FAD. Porcine complex II used as a template for modeling was crystallized without bound substrate analogue. Therefore, orientation of side chains of amino acids coordinating succinate in human SDHA was adjusted according to positions of the corresponding residues in *E. coli* succinate dehydrogenase (PDB id 2WDQ) which is a close homologue of SDHA and which was resolved with bound succinate analogue. (D) Succinate binding by the Arg541Cys mutant. Hydrogen bonds are marked in green, electrostatic interactions are in orange. [Color figure can be viewed at wileyonlinelibrary.com].**

to follow a recessive trait. Here, we present the second report of a family with the heterozygous mutation c.1351C>T (p.Arg451Cys) in *SDHA* with apparent dominant transmission.

The spectrophotometric measurements of the respiratory chain complexes in fibroblasts revealed an isolated complex II deficiency (51% residual activity) in the index patient. The daughter shows a residual complex II activity of 33% in skin fibroblasts, the son of 42%, respectively.

Due to the isolated complex II deficiency, all coding regions with adjacent exon/intron boundaries of the *SDHx* genes and complex II assembly factors *SDHAF1* and *SDHAF2* were sequenced. All affected individuals were found to be heterozygous carriers of the mutation c.1351C>T (p.Arg451Cys). In addition, both siblings showed increased 3-methylglutaconic and 3-OH-methylglutaric acid excretion, which may have resulted in similar metabolites derived from an impaired ketone body formation due to involvement of complex II in the Krebs cycle [Wortmann et al., 2012]. The initial description of the neurodegenerative phenotype of this mutation included progressive bilateral optic atrophy with cupped optic discs (without retinopathy), myopathy, and ataxia without evidence of cardiac involvement [Taylor et al., 1996]. Interestingly, this family's phenotypic presentation was described as late-onset with clinical symptoms becoming apparent after the fourth decade of life.

Using the phylogenetic conservation of the SDH Fp subunit Birch-Machin et al. [2000] introduced the targeted mutation into an *E.coli* homolog, where the equivalent Arg398Cys mutation affects a highly conserved Flavin-binding domain rendering the enzyme inactive. The functional study showed a decrease of complex II activity of 50%, similar to what is reported in the patients.

Our structural analysis of human complex II predicts that p.Arg451Cys mutation located within 5Å from FAD affects its redox potential, which generates an impenetrable barrier precluding electron transfer from succinate. This prediction could potentially be verified by titration of redox centers in the mutant complex. The p.Arg451Cys mutation has no effect on the two quinone-binding sites. However, mutated complex II tightly binds two quinone molecules, which cannot be reduced due to the blocked electron acceptance. In heterozygous patients, therefore, the stable complex II with p.Arg451Cys mutation competes as an electron acceptor with the wild-type protein providing a possible explanation for a dominant manifestation.

In contrast to the previously reported family carrying the same heterozygous *SDHA* mutation our patients additionally present with cardiomyopathy, which is likely also caused by the complex II deficiency. The associated cardiomyopathy is only present in carriers of this variant, suggesting disease segregation. In addition, a further cardiomyopathy gene panel analysis did not reveal any additional pathogenic variants.

Although outcome of disease severity and residual enzymatic activity due to complex II mutations are not predictable, intrafamilial disease manifestation was described to be comparable [Jain-Ghai et al., 2013]. The family reported here shows high intrafamilial variability based on the early infantile to late adult onset of the clinical symptoms suggesting a possible role of hitherto not yet identified modifier genes and/or strong dependence on genetic background involved in onset, severity and expression of disease. The neurological presentation regarding the psychological status cannot be attributed purely to disease manifestation, as they are a common feature in mitochondrial disorders.

TABLE I. *SDHA* Mutations With Mitochondrial Phenotype

| Mutation | Type | Inheritance | Phenotype | References |
|---|---|---|---|---|
| c.1A>C (p.Met1Leu) | Point | Recessive | Leigh | Parfait et al. [2000] |
| c.64-2A>G | Splicing | Recessive | Not stated | Renkema et al. [2015] |
| c.117delG | Deletion | Recessive | Leigh-like | Ma et al. [2014] |
| c.221dupT | Insertion | Recessive | Leigh-like | Ma et al. [2014] |
| c.248C>T (p.Arg83Val) | Point | Recessive | Leigh | Horváth et al. [2006] |
| c.356G>A (p.Trp119*) | Point | Recessive | Leigh | Horváth et al. [2006] |
| c.565T>G (p.Cys189Gly) | Point | Recessive | Not stated | Renkema et al. [2015] |
| c.1065-3C>A | Splicing | Recessive | Not stated | Renkema et al. [2015] |
| c.1351C>T (p.Arg451Cys) | Point | Dominant | Optic atrophy, ataxia, myopathy, HCM | Birch-Machin et al. [2000], this report |
| c.1523_1526delCATCinsTATT | Insertion | Recessive | Cardiomyopathy & leukodystrophy | Alston et al. [2012] |
| c.1571C>T (p.Ala524Val) | Point | Recessive | Leigh syndrome | Parfait et al. [2000] |
| c.1660C>T (p.Arg554Trp) | Point | Recessive | Leigh syndrome | Bourgeron et al. [1995] |
| c.1664G>A (p.Gly555Glu) | Point | Recessive | Leigh-like, hypoglycaemia | van Coster et al. [2003] |

HCM, hypertrophic cardiomyopathy

Currently, there is no effective therapeutic intervention. Supplementation with riboflavin has been shown to ameliorate complex II function in patients and corresponding fibroblasts [Bugiani et al., 2006]. As phenotypic overlap of *SDHA* mutations causing a mitochondrial disease with tumor formation have been reported, we propose that patients and carriers of *SHDx* and assembly factor mutations should be monitored for possible neoplastic formation [Burnichon et al., 2010; Renkema et al., 2014].

As no tissue-specific isoforms are known (although some predicted), the tissue-specific presentation of these disorders is quite intriguing [Rustin and Rötig, 2002]. Up to date cardiomyopathies in conjunction to defective SDHA have only been reported with neonatal onset of disease and not as a dominant trait [Levitas et al., 2010b](Table I). Recessive *SDHA* mutations are the most common presentation of complex II deficiency, whereas the mutations in membrane-bound subunits might often be fatal with only two reports for *SDHD*, and none for *SDHC* [Jackson et al., 2014; Alston et al., 2015]. The rare occurrence of complex II deficiency has been attributed to possible incompatibility with life in a recent report of a complex II-related case of cardiomyopathy caused by mutations in *SDHD* with severe postnatal deterioration and death within 2 days after birth [Alston et al., 2015].

This is only the second report supporting the dominant nature of the *SDHA* c.1351C>T (p.Arg451Cys) mutation being causative for an autosomal dominantly inherited mitochondrial metabolic disorder expanding the phenotypic presentation to an earlier onset of disease with additional cardiac involvement.

## ACKNOWLEDGMENT

The authors are indebted to the family participating in this study. This work was supported by a SNF Postdoc grant to Christopher B. Jackson.

## REFERENCES

Alston CL, Davison JE, Meloni F, van der Westhuizen FH, He L, Hornig-Do HT, Peet AC, Gissen P, Goffrini P, Ferrero I, Wassmer E, McFarland R, Taylor RW. 2012. Recessive germline SDHA and SDHB mutations causing leukodystrophy and isolated mitochondrial complex II deficiency. J Med Genet 49:569–577.

Alston CL, Berti CC, Blakely EL, Oláhová M, He L, McMahon CJ, Olpin SE, Hargreaves IP, Nolli C, McFarland R, Goffrini P, O'Sullivan MJ, Taylor RW. 2015. A recessive homozygous p.Asp92Gly SDHD mutation causes prenatal cardiomyopathy and a severe mitochondrial complex II deficiency. Hum Genet 134:869–879.

Birch-Machin MA, Taylor RW, Cochran B, Ackrell BA, Turnbull DM. 2000. Late-onset optic atrophy, ataxia, and myopathy associated with a mutation of a complex II gene. Ann Neurol 48:330–335.

Blaut M, Whittaker K, Valdovinos A, Ackrell BA, Gunsalus RP, Cecchini G. 1989. Fumarate reductase mutants of Escherichia coli that lack covalently bound flavin. J Biol Chem 264:13599–13604.

Bourgeron T, Rustin P, Chrétien D, Birch-Machin M, Bourgeois M, Viegas-Péquignot E, Munnich A, Rotig A. 1995. Mutation of a nuclear succinate dehydrogenase gene results in mitochondrial respiratory chain deficiency. Nat Genet 11:144–149.

Bugiani M, Lamantea E, Invernizzi F, Moroni I, Bizzi A, Zeviani M, Uziel G. 2006. Effects of riboflavin in children with complex II deficiency. Brain Dev 28:576–581.

Burnichon N, Brière J-J, Libé R, Vescovo L, Rivière J, Tissier F, Jouanno E, Jeunemaitre X, Bénit P, Tzagoloff A, Rustin P, Bertherat J, Favier J, Gimenez-Roqueplo A-P. 2010. SDHA is a tumor suppressor gene causing paraganglioma. Hum Mol Genet 19:3011–3020.

Hoekstra AS, Bayley J-P. 2013. The role of complex II in disease. Biochim Biophys Acta 1827:543–551.

Horváth R, Abicht A, Holinski-Feder E, Laner A, Gempel K, Prokisch H, Lochmüller H, Klopstock T, Jaksch M. 2006. Leigh syndrome caused by mutations in the flavoprotein (Fp) subunit of succinate dehydrogenase (SDHA). J Neurol Neurosurg Psychiatr 77:74–76.

Jackson CB, Nuoffer JM, Hahn D, Prokisch H, Haberberger B, Gautschi M, Haberli A, Gallati S, Schaller A. 2014. Mutations in SDHD lead to autosomal recessive encephalomyopathy and isolated mitochondrial complex II deficiency. J Med Genet 51:170–175.

Jain-Ghai S, Cameron JM, Maawali AlA, Blaser S, MacKay N, Robinson B, Raiman J. 2013. Complex II deficiency-A case report and review of the literature. Am J Med Genet 161:285–294.

Levitas A, Muhammad E, Harel G, Saada A, Caspi VC, Manor E, Beck JC, Sheffield V, Parvari R. 2010. Familial neonatal isolated cardiomyopathy

caused by a mutation in the flavoprotein subunit of succinate dehydrogenase.. Eur J Hum Genet 18:1160–1165.

Levitas A, Muhammad E, Harel G, Saada A, Caspi VC, Manor E, Beck JC, Sheffield V, Parvari R. 2010. Familial neonatal isolated cardiomyopathy caused by a mutation in the flavoprotein subunit of succinate dehydrogenase. Eur J Hum Genet 18:1160–1165.

Ma Y-Y, Wu T-F, Liu Y-P, Wang Q, Li X-Y, Y Ding, Song J-Q, Shi X-Y, Zhang W-N, M Zhao, Hu L-Y, J Ju, Wang Z-L, Yang Y-L, Zou L-P. 2014. Two compound frame-shift mutations in succinate dehydrogenase gene of a Chinese boy with encephalopathy. Brain Dev 36:394–398.

Parfait B, Chrétien D, Rotig A, Marsac C, Munnich A, Rustin P. 2000. Compound heterozygous mutations in the flavoprotein gene of the respiratory chain complex II in a patient with Leigh syndrome. Hum Genet 106:236–243.

Renkema GH, Wortmann SB, Smeets RJ, Venselaar H, Antoine M, Visser G, Ben-Omran T, van den Heuvel LP, Timmers HJLM, Smeitink JA, Rodenburg RJT. 2014. SDHA mutations causing a multisystem mitochondrial disease novel mutations and genetic overlap with hereditary tumors. Eur J Hum Genet 23:202–209.

Renkema GH, Wortmann SB, Smeets RJ, Venselaar H, Antoine M, Visser G, Ben-Omran T, van den Heuvel LP, Timmers HJLM, Smeitink JA, Rodenburg RJT. 2015. SDHA mutations causing a multisystem mitochondrial disease: Novel mutations and genetic overlap with hereditary tumors. Eur J Hum Genet 23:202–209.

Rustin P, Rötig A. 2002. Inborn errors of complex II?—Unusual human mitochondrial diseases. Biochim Biophys Acta 1553:117–122.

Saraste M. 1999. Oxidative phosphorylation at the fin de siècle. Science 283:1488–1493.

Sun F, Huo X, Zhai Y, Wang A, Xu J, Su D, Bartlam M, Rao Z. 2005. Crystal structure of mitochondrial respiratory membrane protein complex II. Cell 121:1043–1057.

Taylor RW, Birch-Machin MA, Schaefer J, Taylor L, Shakir R, Ackrell BA, Cochran B, Bindoff LA, Jackson MJ, Griffiths P, Turnbull DM. 1996. Deficiency of complex II of the mitochondrial respiratory chain in late-onset optic atrophy and ataxia. Ann Neurol 39:224–232.

van Coster R, Seneca S, Smet J, Van Hecke R, Gerlo E, Devreese B, Van Beeumen J, Leroy JG, De Meirleir L, Lissens W. 2003. Homozygous Gly555Glu mutation in the nuclear-encoded 70 kDa flavoprotein gene causes instability of the respiratory chain complex II. Am J Med Genet 120A:13–18.

Wortmann SB, Kluijtmans LA, Engelke UFH, Wevers RA, Morava E. 2012. The 3-methylglutaconic acidurias: What's new? J Inherit Metab Dis 35:13–22.

Yankovskaya V, Horsefield R, Törnroth S, Luna-Chavez C, Miyoshi H, Léger C, Byrne B, Cecchini G, Iwata S. 2003. Architecture of succinate dehydrogenase and reactive oxygen species generation. Science 299: 700–704.