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Review

## Inborn errors of complex II – Unusual human mitochondrial diseases

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### Abstract

The succinate dehydrogenase consists of only four subunits, all nuclearly encoded, and is part of both the respiratory chain and the Krebs cycle. Mutations in the four genes encoding the subunits of the mitochondrial respiratory chain succinate dehydrogenase have been recently reported in human and shown to be associated with a wide spectrum of clinical presentations. Although a comparatively rare deficiency in human, molecularly defined succinate dehydrogenase deficiency has already been found to cause encephalomyopathy in childhood, optic atrophy or tumor in adulthood. Because none of the typical housekeeping genes encoding this respiratory chain complex is known to present tissue-specific isoforms, the tissue-specific involvement represents a quite intriguing question, which is mostly addressed in this review. A differential impairment of electron flow through the respiratory chain, handling of oxygen, and/or metabolic blockade possibly associated with defects in the different subunits that can be advocated to account for tissue-specific involvement is discussed. © 2002 Elsevier Science B.V. All rights reserved.

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### 1. Introduction

Complex II (CII; succinate–ubiquinone oxidoreductase (SQR); EC 1.3.5.1) is composed of four subunits, all of them being encoded by nuclear DNA [1]. Two of these subunits form the succinate dehydrogenase (SDH): the largest subunit, a flavoprotein of 70 kDa (Fp; SDH A) containing the putative active site and the covalently bound FAD moiety of the enzyme, and the iron–sulfur protein (Ip; SDH B, 30 kDa) subunit carrying three dissimilar iron-clusters,  $[2\text{Fe}-2\text{S}]^{2+,1+}$ ,  $[4\text{Fe}-4\text{S}]^{2+,1+}$ , and  $[3\text{Fe}-4\text{S}]^{1+,0}$  [2]. The SDH is anchored to the membrane by two additional

subunits, C and D (15 and 12.5 kDa respectively), which contain a single heme group and the ubiquinone (UQ) binding sites (Fig. 1). In addition to these structural subunits, a number of accessory factors unknown in human are necessary for the assembly and maintenance of the active enzyme complex.

CII is part of both the tricarboxylic acid cycle and the mitochondrial respiratory chain [1]. It catalyzes the oxidation of succinate to fumarate and transfers the electrons to the UQ pool of the respiratory chain (Fig. 1). While under most conditions electrons are subsequently directed to oxygen allowing for ATP synthesis, reverse electron flow from succinate to  $\text{NAD}^+$  might also occur in intact tissues (e.g. kidney, liver) favored by low  $p\text{O}_2$  [3], possibly playing a role in ketogenesis in liver. In keeping with this, a high CII activity compared with the activity of the respi-

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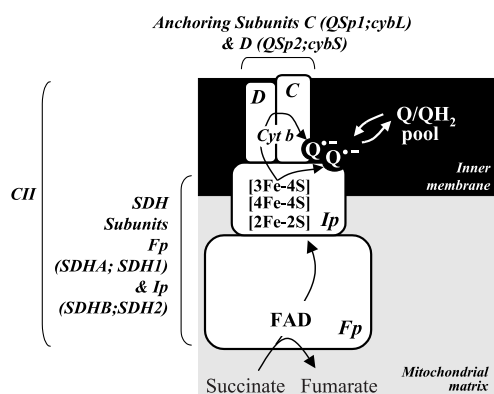


Fig. 1. A schematic view of the respiratory chain CII. Featuring the four CII subunits with their various alternative symbols, the scheme shows the SDH consisting of the Fp and Ip subunits anchored to the mitochondrial inner membrane by the subunits C and D. Stabilized semi-quinone species feed electrons to the UQ (Q) pool.

ratory chain cytochrome segment (CIII–CIV) has been observed in human liver and kidney tissues associated with comparatively high SDH mRNA levels [4].

CII defects are relatively rare compared to other respiratory chain deficiencies, yet they are associated with a wide spectrum of clinical phenotypes, ranging from encephalomyopathy in childhood to optic atrophy or tumor formation in adulthood [5]. Succinate cytochrome *c* reductase deficiency and/or SDH activity impairment has been described in a handful of patients in the last 20 years, associated with progressive encephalomyopathy with dementia, myoclonic seizure and short stature [6], Kearns–Sayre syndrome with conduction defects [7], myopathy and encephalopathy [8], isolated hypertrophic cardiomyopathy [9], hypertrophic cardiomyopathy plus skeletal muscle myopathy [10] generalized muscle weakness and easy fatigability [11]. The molecular bases of these deficiencies have not been elucidated for most cases. However, in a few cases, mutations have been identified in the genes encoding CII subunits, resulting in a wide variety of clinical presentations [12–16]. With none of these proteins being known to present isoforms and all being ubiquitously expressed, the tissue-specific involvement inferred from the various clinical presentations remains a puzzling question. In addition to isolated CII deficiency, a generalized deficiency of Fe–S cluster-containing proteins, includ-

ing the SDH, has been reported in Friedreich's ataxia due to an oxidative stress caused by a decreased fra-taxin content of the mitochondria [17]. As these centers represent a privileged target for superoxides, it can be predicted that similar damages, involving the SDH, will be progressively reported in a number of situations of oxidative stress.

This review is mostly focused on the recent progresses made in the identification of the gene mutations associated with CII defects. The strikingly varying biochemical and clinical consequences of these mutations are discussed.

## 2. Mutations in SDH A encoding the Fp subunit

Fluorescent in situ hybridization and pulse-field gel electrophoresis analysis demonstrated that SDH A is duplicated in the human genome (5p15; 3q29) [12]. Expression studies on human/hamster somatic cell hybrids showed that only the chromosome 5 gene is expressed [12]. The genomic structure of the two SDH A genes has been determined and sequence analysis of the two SDH A copies made it possible to identify a 1-bp deletion creating a frame shift in the 3q29 copy, confirming that this copy is actually a pseudogene [14]. The 5p15 gene comprises 15 exons extending over 38 kb encoding a 664-amino acid protein showing 95% homology with its bovine counterpart [18]. Two cDNA sequences with specific polymorphisms have been simultaneously described for the 5p15-encoded Fp subunit, corresponding to two different haplotypes [18,19].

The first reported nuclear gene mutation causing a respiratory chain defect was a point mutation in the gene encoding the SDH Fp subunit [12]. The mutation was identified in two sibs presenting Leigh syndrome and SDH deficiency [20]. The patients were homozygous for an Arg544Trp substitution in a conserved domain of the protein [12]. Both consanguineous parents were found heterozygous for the mutation. The deleterious effect of the Arg544Trp substitution on the catalytic activity of the enzyme was assessed in a SDH-negative mutant yeast strain transformed with wild-type or mutant Fp cDNA. In a second patient with Leigh syndrome, SDH deficiency and compound heterozygous mutations in the SDH Fp gene were afterward reported [14].

The patient, born to unrelated parents, was found heterozygous for an Ala524Val substitution inherited from the father. Transfection of the corresponding mutant cDNA into Fp-deficient cells failed to restore normal SDH activity, confirming the deleterious effect of this mutation. The second allele, inherited from the mother, carried an A-to-C substitution, changing the methionine translation initiation codon to a leucine. Instability of this mutant transcript was demonstrated by quantification (10% residual transcript).

A third case of SDH A mutation has been recently documented in two sisters with partial CII deficiency and late-onset neurodegenerative disease with progressive optic atrophy, ataxia, and myopathy [21]. The affected members of this family were shown to carry a heterozygous Arg408Cys substitution in a highly conserved region of the protein [13]. The equivalent mutation in *Escherichia coli* generated an inactive enzyme unable to bind flavin adenine dinucleotide covalently. No mutation in the other allele was identified and this was claimed to be compatible with the 50% residual CII and SDH activity found in these patients. To date, no additional mutations have been reported in the SDH A encoding the Fp subunit.

### 3. Mutations in SDH B encoding the Ip subunit

The gene encoding the human SDH Ip subunit maps to chromosome 1p36.1-p35 [22,23]. It encodes a 252-amino acid protein showing 94% homology with its bovine heart counterpart. The complete genomic sequence was described afterwards, consisting of eight exons within approximately 40 kb [24].

Very recently, gene mutations in the SDH B have been shown to cause tumor formation in familial paraganglioma (PGL) and familial pheochromocytoma [25], following the description of causative mutations in SDH C and D in patients with this condition (see below).

PGLs are vascularized, neural crest-derived tumors of the sympathetic paraganglia, being mostly located in the head and the neck, i.e. carotid bifurcation, skull base or middle ear [15]. The carotid body is the most common tumor site. This highly vascular small organ is localized at the bifurcation of the

common carotid artery and is a chemoreceptive organ that senses oxygen levels in the blood. In familial PGL, cellular hyperplasia/anaplasia is observed in the tumor in the absence of any hypoxic stimulus. Pheochromocytomas are catecholamine-producing tumors that cause excess release of the two hormones epinephrine and norepinephrine, which regulate heart rate and blood pressure. The vast majority of pheochromocytomas are observed in the adrenal medulla (about 90% of the cases). Due to their location in sympathetic paraganglia, the other 10% can be classified as PGL.

Hereditary PGL is transmitted with incomplete and age-dependent penetrance and is genetically heterogeneous. PGL has been mapped to three loci, PGL1 at 11q22.3-23.1, PGL2 at 11q13.1, and PGL3 which does not segregate with either of the loci at 11q [26–28]. PGL1 is an autosomal dominant disease with incomplete penetrance when transmitted through the father, whereas no disease occurs when maternally transmitted, suggesting genomic imprinting [15].

A loss of CII activity presumably due to damage to the SDH B-associated iron–sulfur clusters has also been reported in Friedreich's ataxia [17] and in one familial case of Ip-deficiency presented with myopathy [29].

### 4. Mutations in SDH anchoring subunits

SDH C and D are respectively the large and the small anchoring subunits of the CII (140 and 103 amino acids long). SDH C gene comprises six exons and five introns extending over 35 kb [30] and has been mapped to chromosome 1q21 [31]. SDH D gene has been mapped to chromosome 11q23 by fluorescence in situ hybridization and comprises four exons extending over more than 18 kb [31]. Noticeably, while both the Fp and the Ip subunits are highly conserved through evolution, SDH C and D show little similarity to their respective other species counterparts. It has also been stated that there are multiple SDH C genes or pseudogenes based on Southern blot analyses [32]. The identification of two of these as pseudogenes has been described afterwards [30].

Sequencing the SDH D gene made it possible to

identify missense mutations affecting conserved amino acids and nonsense mutations in several families showing significant linkage to PGL1 locus mapped to chromosome 11q22.3–23.1 in a 11.5 Mb critical interval between D11S1968 and D11S1347 containing the SDH D gene [15]. These mutations were heterozygous in both affected individuals and healthy carriers. To account for the lack of maternal disease transmission in PGL1, allele-specific expression of the SDH D gene was looked for. Biallelic expression was observed in all tissues investigated in both affected and carrier individuals, denoting that SDH D was not located in an imprinted genomic domain [15]. The imprinted inheritance pattern in PGL1 remains therefore to be explained, but the monoallelic expression of SDH D might be confined to the paraganglionic cells. On the other hand, loss of heterozygosity with exclusive loss of the normal maternal chromosome at 11q23 has been frequently observed in PGL1 tumors. In all cases studied, the mutated SDH D allele was retained in the tumor cells, while the normal allele was completely lost. The germ-line loss of function mutations of the paternal allele and the somatic loss of the maternal allele suggest that SDH D acts as a tumor suppressor gene. Mutations in SDH D were also identified in sporadic extra-adrenal pheochromocytoma [33].

The suggestion that SDH genes may act as tumor suppressor genes was strongly reinforced by the observation that mutations in a second SDH gene, SDH C, were the underlying cause of autosomal dominant PGL3 [16]. Indeed, a G-to-A transition in the exon 1 of the SDH C has been shown to destroy the start codon at nucleotide position 958 in a patient with familial PGL3 [16]. Again the mutated SDH C allele was retained in the tumor cells, while the normal allele with wild-type SDH C was lost.

Taken together, these data support the view that SDH B, C and D genes are tumor suppressor genes. Based on the phenotypic similarity between PGL tumor and normal carotid body submitted to chronic hypoxia, it may be hypothesized that these subunits are critical components of the oxygen-sensing system of the paraganglionic tissue, and that their loss actually results in oxidative stress, chronic hypoxic stimulation and cellular proliferation [15]. Interestingly enough, a mutation in the *mev-1(kn1)* gene of

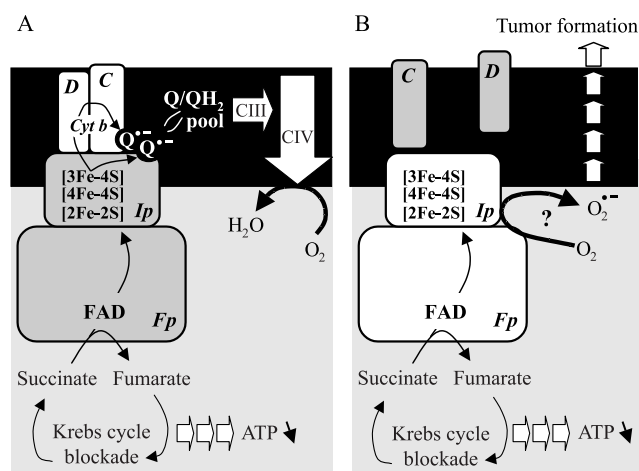


Fig. 2. Differences in the predictable consequences of mutations affecting either the SDH or the anchoring subunits C and D. A: Mutations in the SDH subunits (Fp or Ip) should tend to reduce Krebs cycle activity and consequently mitochondrial ATP synthesis. Reduced electron flow through the SDH should decrease SDH component reduction and not result in a major increase of superoxide production. Noticeably, some particular mutation could still favor an over-reduction of a given SDH component (e.g. flavin), resulting in superoxide production. B: Mutations in SDH C or D by causing reduction of the electron flow to the UQ pool will favor reduction of the several SDH components and possibly result in higher superoxide production. This latter may cause oxidative stress and favor tumor formation in patients.

*Caenorhabditis elegans*, the orthologue of the mammalian SDH C gene, causes oxidative stress and premature ageing [34]. The mutation has been shown to compromise electron transfer between the SDH, still present in the mitochondria, and the UQ pool, presumably resulting in an increased superoxide production by SDH.

As oxygen free radicals are believed to play a key role in both cell proliferation and apoptosis, and as the mitochondrial MnSOD gene has been shown to act as a tumor suppressor gene [35], it is tempting to hypothesize that superoxide overproduction resulting from SDH C or D mutations triggers tumor formation because of preserved catalytic activity of the SDH A and B (Fig. 2). However, it remains to show that the lack (or decrease) of either SDH C or D anchoring subunit is still compatible with the SDH assembly and function as proposed in the nematode. In addition, the recent report that mutations in SDH B gene also cause familial pheochromocytoma

ma and paraganglioma does not support the view that an active superoxide production triggered by a non-anchored SDH is the actual pathogenic mechanism underlying this condition. Whatever the subunit considered, the percent of residual SDH activity associated with the various types of mutation may rather be the key feature and should be urgently measured in the different cases.

### 5. Clinical presentations associated with CII defect

A mutation in the CII Fp subunit was the first mutation identified in a nuclear gene encoding a respiratory chain component [12]. Because the nuclear genes encoding the respiratory chain subunits are typical housekeeping genes, it was initially thought that a reduced clinical spectrum would be associated with mutations in these genes [36]. However, as observed, for example, in the case of the mutations affecting respiratory chain CIV assembly genes, varying clinical features may result from mutations in genes that are functionally closely related [5]. This is clearly the case for CII deficiencies, the disease causing mutations lying in the genes encoding CII subunits or in yet unknown genes. This might well be related to the several functions contributed by CII proteins in either the respiratory chain ATP synthesis and oxygen handling, or the Krebs cycle and possibly the organ-specific mitochondrial metabolism. First, as in the case of most deficiencies affecting the different respiratory chain complexes, hampering CII activity should ultimately result in reduced ATP synthesis. Accordingly, it has been shown that patients' cultured skin fibroblasts harboring a mutation in the CII Fp gene cannot survive upon glucose withdrawing from culture medium that turns down ATP-generating glycolysis [37]. In keeping with this, neuromuscular symptoms associated with CII deficiencies (myopathy and encephalopathy) are typical features associated with respiratory chain deficiencies affecting other respiratory chain complexes [5]. Similarly, a hypertrophic cardiomyopathy, as the one reported in association with few cases of CII deficiency (2 cases on more than 3000 investigations in patients at risk in our experience), is frequently observed as the result of a respiratory chain dysfunction [5]. However, it remains to be explained why a

hypertrophic cardiomyopathy is not a consistent feature in CII deficiencies. Noticeably, sequencing of the four genes encoding CII subunits in patients with isolated hypertrophic cardiomyopathy and SDH deficiency failed to reveal any mutation (unpublished data). Finally, optic atrophy, as reported in two adult patients with SDH deficiency and heterozygous mutations in SDH Fp gene, is not a rare feature in mitochondrial diseases [13]. However, its unique occurrence in these two patients with partial SDH deficiency is quite puzzling, since heterozygous parents of patients harboring other deleterious mutations in any of the four genes encoding SDH subunits do not present optic atrophy.

The causative association of respiratory chain defect and tumor formation is amazing, even if mitochondria have been long claimed to be indirectly involved in tumor processes. The handling of oxygen for the benefit of the cell is a quite dangerous task ensured by the respiratory chain and it appears that CII (SDH?) is a privileged location for superoxide production. A specific role of CII in generating/eliminating superoxides might therefore account for SDH B, C and D, acting as tumor suppressor genes.

### 6. Conclusion

The recent discovery of tumor-causing mutations in the SDH B, C and D genes has further widened the already broad spectrum of diseases primarily caused by mutations in genes encoding mitochondrial respiratory chain proteins. The particular case of CII deficiencies perfectly illustrates the complexity of these diseases that can be revealed in childhood or adulthood, that can be or not neuromuscular diseases, and that can involve quite different organs for yet unknown reasons. Beside the four structural genes involved in CII, a yet undetermined number of genes might be involved in CII assembly. Based on what has been observed for other respiratory chain complexes, mutations in assembly genes quite frequently cause respiratory chain dysfunction and it can be predicted that this will stand true as well for CII defect. Again, mutations in these genes can be at the origin of newly described diseases or of 'old' diseases of yet unknown mitochondrial origin.

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