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# The role of complex II in disease ☆

Attje S. Hoekstra, Jean-Pierre Bayley *

*Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands*

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ABSTRACT

Genetically defined mitochondrial deficiencies that result in the loss of complex II function lead to a range of clinical conditions. An array of tumor syndromes caused by complex II-associated gene mutations, in both succinate dehydrogenase and associated accessory factor genes (*SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF1*, *SDHAF2*), have been identified over the last 12 years and include hereditary paraganglioma–pheochromocytomas, a diverse group of renal cell carcinomas, and a specific subtype of gastrointestinal stromal tumors (GIST). In addition, congenital complex II deficiencies due to inherited homozygous mutations of the catalytic components of complex II (SDHA and SDHB) and the SDHAF1 assembly factor lead to childhood disease including Leigh syndrome, cardiomyopathy and infantile leukodystrophies. The role of complex II subunit gene mutations in tumorigenesis has been the subject of intensive research and these data have led to a variety of compelling hypotheses. Among the most widely researched are the stabilization of hypoxia inducible factor 1 under normoxia, and the generation of reactive oxygen species due to defective succinate:ubiquinone oxidoreductase function. Further progress in understanding the role of complex II in disease, and in the development of new therapeutic approaches, is now being hampered by the lack of relevant cell and animal models. This article is part of a Special Issue entitled: Respiratory complex II: Role in cellular physiology and disease.



## 1. Introduction

Mitochondrial function and oxidative phosphorylation via the respiratory chain are among the most fundamental processes of life. Deficiencies in mitochondrial function and in the activity of the various protein complexes of the respiratory chain have been associated with a wide variety of clinical conditions [1]. Deficiencies of complex II with a defined genetic basis have been identified in only a limited number of diseases, including the hereditary paraganglioma–pheochromocytoma tumor syndrome, a specific group of renal cell carcinomas, gastrointestinal stromal tumors (GIST) primarily associated with Carney–Stratakis syndrome, a subset of Leigh syndrome cases, and most recently, infantile leukoencephalopathy associated with mutations of *SDHAF1*.

As complex II of the respiratory chain, succinate dehydrogenase (SDH) is situated at the intersection of the tricarboxylic acid (TCA) cycle and mitochondrial oxidative phosphorylation; combining these functions places SDH at the center of the two essential energy producing processes of the cell. SDH is a heterotetramer and all four protein subunits, SDHA, -B, -C and -D, have been implicated in disease. Two recently identified SDH accessory factors, SDHAF1 and SDHAF2, are also involved in disease and although clearly essential to the normal functioning of complex II, their exact functions are still poorly defined. Despite the fact that these proteins are all associated with the same protein complex, mutations lead to clear differences in clinical phenotype. The molecular basis for this clinical divergence is as yet unknown.

Biochemical and immunohistochemical analyses have demonstrated that mutations in complex II-related genes often result in the reduction or loss of a functional enzyme complex, which may in turn lead to either the accumulation of succinate [2] or the generation of reactive oxygen species (ROS), due to frustrated electron transport [3,4]. Loss of SDH may also lead to a limited capacity to utilize oxidative phosphorylation for ATP production [5,6].

## 2. Paraganglioma–pheochromocytoma syndrome

Hereditary paraganglioma–pheochromocytoma syndrome is characterized by neuroendocrine tumors that originate from both the sympathetic and parasympathetic branches of the autonomic nervous system. Paragangliomas arise most commonly in the carotid body and the abdominal paraganglia, but may occur in a wide variety of paraganglia at other locations. In general, paragangliomas of the head and neck (HNPGL) rarely lead to malignancy or metastases, and growth is generally extremely slow [7]. In contrast, abdominal paragangliomas show relatively frequent malignancy, with a 5-year survival of 34–60% in cases of malignant tumors [8].

Pheochromocytomas (PC) originate in the sympathetic nervous system and while the term is generally reserved for tumors of the adrenal medulla, some authors include all sympathetic paragangliomas [3]. In contrast to HNPGL, pheochromocytomas show a more polygamous

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* Corresponding author. Tel.: +31 715269512; fax: +31 715268285.
*E-mail address:* j.p.l.bayley@lumc.nl (J.-P. Bayley).

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causal association and have been linked to mutations in several non-complex II genes, including VHL, RET, NF1, TMEM127 and MAX.

Adrenal pheochromocytomas are usually benign but are clinically important due to the potentially life-threatening hypertensive crises that may result from the excessive production and secretion of catecholamines.

Paraganglioma–pheochromocytoma syndrome, with some notable exceptions, shows a classic Mendelian pattern of dominant inheritance in families but as with other tumors associated with tumor suppressor genes, at the cellular level tumor occurrence resembles a recessive disease with predominantly adult onset. An inherited mutation is initially silent and carriers are phenotypically normal at birth, but at some point a chance second mutation or loss of the remaining normal allele occurs, and this so-called 'second hit' leads to the initiation and growth of a tumor (Table 1).

### 2.1. SDHD

The identification of SDHD demonstrated for the first time that mitochondrial proteins involved in intermediary metabolism could act as tumor suppressors [9]. Germline mutations in *SDHD* generally result in benign HNPGLs but are also associated with sympathetic paragangliomas and adrenal pheochromocytomas [10]. Carriers of *SDHD* mutations have a very high propensity for tumor development (penetrance), variously estimated at 87–100%, although not all carriers will develop overt clinical symptoms or even be aware of their tumor [11–13].

*SDHD* and *SDHAF2* share the same chromosomal location (Ch11) and show a very unusual pattern of inheritance-dependent expression of mutations in which carriers develop tumors only when the mutation is inherited from the father [14,15]. Although this phenomenon resembles genomic imprinting, no evidence of allelic silencing or suppression of *SDHD* or *SDHAF2* has ever been presented [9,16]. Carriers of maternally transmitted *SDHD* mutations may, very rarely, develop tumors but only following complex somatic genetic events in the tumor [17]. The *SDHA*, *-B* or *-C* genes are located on chromosomes 5 (A) and 1 (B and C) and mutation carriers show no parental effects.

Several hypotheses have been advanced to explain *SDHD*-linked parental effects [18–20] but most data support the so-called 'Hensen model' [20–22]. The loss of the entire maternal copy of chromosome 11 in tumors from paternal *SDHD* mutation carriers is a hallmark of *SDHD*-linked paragangliomas. The Hensen model proposes that maternal chromosomal loss results in the simultaneous deletion of the *SDHD* gene and an exclusively maternally expressed gene, producing a striking parent-of-origin effect [20,21,23]. The similar penetrance and clinical phenotype of *SDHAF2* and *SDHD* mutation carriers may be related to the function of the postulated maternally expressed tumor suppressor gene. An equivalent parental effect is not associated with the *SDHA*, *-B* or *-C* genes and even if the same maternally expressed gene plays a role in these tumors, the independent assortment of chromosomes prevents a parent-of-origin effect.

### 2.2. SDHB

The *SDHB* gene is the most commonly mutated of all the SDH-related genes and *SDHB* mutation carriers may develop extra-adrenal paragangliomas, adrenal pheochromocytomas or head and neck paragangliomas [24,25]. Around 20% of *SDHB* mutation carriers will develop metastatic disease [12,25]. *SDHB* mutations tend to show low penetrance and only 25–40% of all carriers will eventually develop a tumor [26–28], suggesting that many carriers go undetected. This is reflected in the apparently sporadic presentation of many patients [29], with further investigation of family members often revealing germline mutations in asymptomatic carriers, many of whom remain tumor-free to advanced age [26]. Although the underlying cause of reduced penetrance is unknown, possible genetic explanations include inhibition of cell proliferation due to secondary loss of vital genetic material in the proximity of the remaining normal allele or that additional loss of chromosome 11 is required (Hensen model) [23,30].

### 2.3. SDHC

Despite the close physical interaction of the SDHC and SDHD proteins, paragangliomas due to *SDHC* mutations are far less common than SDHD-related paragangliomas. Although the number of SDHC-related patients is still insufficient to allow firm conclusions to be drawn, the penetrance of *SDHC* mutations appears to be low, more closely reflecting that of *SDHB* than *SDHD*. The clinical expression of *SDHC* mutations appears to be comparable to that of *SDHD* however, with most patients showing primarily head and neck paragangliomas, although some sympathetic paragangliomas have been reported [31,32].

### 2.4. SDHA

The identification of mutations of complex II in hereditary paraganglioma–pheochromocytoma syndrome immediately implied a role for the major catalytic subunit, SDHA. Initial research efforts were stymied by confusion as to the extent and nature of possible additional copies of the *SDHA* gene [33], and over a decade would elapse before a

**Table 1**
Complex II associated gene mutations with clinical phenotype.

| Complex II associated gene | Phenotype/disease | Total no. pathogenic mutations | Pathogenic mutations by disease |
|---|---|---|---|
| *SDHA* | Leigh syndrome | 15 | 7 |
| | Wild-type GIST | | 6 |
| | Paraganglioma/pheochromocytoma | | 3 |
| | Cardiomyopathy (isolated) | | 1 |
| | Late-onset optic atrophy, ataxia, myopathy | | 1 |
| *SDHB* | Paraganglioma/pheochromocytoma | 186 | 180 |
| | Renal cell carcinoma | | 10 |
| | Carney–Stratakis syndrome/GIST | | 5 |
| | Infantile leukodystrophy | | 1 |
| *SDHC* | Paraganglioma/pheochromocytoma | 35 | 34 |
| | Carney–Stratakis syndrome/GIST | | 2 |
| | Renal cell carcinoma | | 1 |
| *SDHD* | Paraganglioma/Pheochromocytoma | 119 | 119 |
| | Carney–Stratakis syndrome/GIST | | 1 |
| *SDHAF1* | Infantile leukodystrophy | 1 | 1 |
| *SDHAF2* | Paraganglioma | 2 | 2 |

Data are derived from the TCA Cycle Gene Mutation Database and/or literature. See text for further details.

pathogenic mutation of *SDHA* was identified in a patient with paraganglioma [34].

An apparent theme of SDH mutations, that the number of identified mutations varies widely and is unrelated to the size of the protein, is continued with the largest protein of complex II, SDHA. Only three PGL/PC-associated mutations have been identified to date, even less than for Leigh syndrome. Nonsense *SDHA* mutations have been convincingly associated with disease through biochemical analysis, but the same mutations have been found at a relatively high frequency in healthy controls (0.5%). This suggests that *SDHA* mutations may show extremely low penetrance [35], with most mutation carriers escaping the development of clinical symptoms. SDHA is the most stable of the SDH proteins when soluble and also appears to be a component of a mitochondrial ATP-sensitive potassium channel [36]. This additional function could be an explanation for the rarity of mutations if maintenance of this complex is essential for cell viability, although this supposition is challenged by the existence of Leigh syndrome patients with homozygous *SDHA* mutations.

An alternative, genetic explanation might be that the 'second hit', usually occurring via complete or partial chromosomal loss, would result in the concomitant loss of essential genes in the proximity of *SDHA*. If these genes are essential to cell viability, only very specific and rare molecular events may be tolerated. This concept does not explain the lack of somatic mutations however.

### 2.5. SDHAF2

A recently identified, novel paraganglioma-related gene encodes a protein involved in the addition of a flavin-adenine dinucleotide (FAD) prosthetic group to form the active SDHA flavoprotein. Loss of succinate dehydrogenase complex assembly factor 2 (SDHAF2) leads to reduced complex II stability, reduced levels of all subunits and loss of function, demonstrating that correct flavination of SDHA is essential for complex II function [15]. Despite this close functional relationship with a catalytic subunit of complex II, loss of SDHAF2 results in a SDHD-like phenotype, with a highly penetrant and exclusively head and neck paraganglioma phenotype [37,38]. Another striking similarity with *SDHD* is the expression of exclusively paternal mutations; no carrier of a maternally transmitted *SDHAF2* mutation has ever developed a tumor. Although the mechanistic basis of this clinical phenotype and mode of inheritance is unknown, it accords with and is predicted by the 'Hensen model' [20].

### 2.6. Mutations

Mutations in genes associated with complex II are summarized in the TCA Cycle Gene Mutation Database (http://chromium.liacs.nl/lovd_sdh). Although we have not yet added *SDHAF1*, we plan do so in the near future.

Around 15% of all paragangliomas are attributable to mutations in genes associated with complex II (*SDHA*, *SDHB*, *SDHC*, *SDHD* and *SDHAF2*) [25,34,38]. All of these genes are thought to have a tumor suppressor function and most tumors show genetic loss of the normal allele in conjunction with a germline mutation, resulting in the loss of the specific protein subunit that in turn destabilizes complex II and reduces or abolishes enzymatic activity [39–41].

For unknown reasons, there is wide variation in the number of unique mutations reported for each subunit gene. *SDHB* predominates, with 186 pathogenic mutations listed in the TCA Cycle Gene Mutation Database, followed by *SDHD* ($n=119$), *SDHC* ($n=35$), *SDHA* ($n=15$, and mainly associated with LS) and *SDHAF2* ($n=2$) [42]. In addition to the widely differing frequency of mutations, the phenotypes resulting from SDH mutations show differences in tissue-specificity, behavior and penetrance. Current understanding of the biology of complex II offers no ready explanation for these differences.

## 3. Gastrointestinal stromal tumors

Gastrointestinal stromal tumors (GISTs) are rare mesenchymal tumors of the gastrointestinal tract, and while generally diagnosed around the age of 60, tumors may occur in children or young adults in association with familial syndromes including Carney triad (GIST, paraganglioma, pulmonary chondroma) and Carney–Stratakis syndrome (GIST, paraganglioma). Around two-thirds of GISTs occur in the stomach, with the remaining cases occurring in the esophagus and throughout the gastrointestinal tract [43]. Originally considered to be of smooth muscle origin, GISTs are now thought to originate from the pacemaker cells regulating digestive tract peristalsis, the so-called 'interstitial cells of Cajal' (ICCs) [44]. Most GISTs carry somatic gain-of-function mutations in the KIT gene and non-KIT tumors often harbor similar mutations in the platelet derived growth factor receptor-a (PDGFRA) gene [45–47] or may occur in neurofibromatosis type 1 patients [48].

### 3.1. Pediatric wild type GISTs

GISTs in children and young adults appear to be a distinct clinical entity and are referred to as 'pediatric wild type GISTs' [49]. Together with an absence of KIT/PDGFRA mutations, these tumors show several other characteristic features including occurrence exclusively in the stomach, a multinodular growth pattern and a propensity to metastasize [50,51]. Pediatric wild type GISTs show similar clinical features and morphology to GISTs that occur together with paragangliomas. The Carney triad is a rare and sporadic tumor syndrome of unknown etiology, chiefly affecting young adult females, and showing a co-occurrence of GISTs, pulmonary chondromas and/or extra-adrenal paragangliomas [52]. The closely related Carney–Stratakis syndrome is a very rare condition showing a co-occurrence of KIT-negative GIST and familial paraganglioma. The first report of this syndrome described 12 young patients (average age of 23 years) from five unrelated families. These patients showed multifocal and frequently metastatic GISTs, with multicentric paragangliomas [53]. A genetic basis for Carney–Stratakis syndrome was subsequently elucidated with the discovery of germline mutations in the *SDHB*, *SDHC* and *SDHD* genes in 8 of the 11 cases tested [54].

More recently, Pantaleo et al. reported the discovery of *SDHA* mutations in two young adults with wild type GISTs [55] and subsequently reported a further two patients [56]. Interestingly, all cases showed germline or somatic point mutations affecting both alleles, rather than the gross chromosomal losses more typical of tumor suppressor genes.

### 3.2. SDHB immunohistochemistry

While germline SDH mutations have been found in only a subset of GISTs, loss of SDHB protein expression is relatively common in WT GISTs. In 2010, Gill et al. reported that both Carney triad associated GISTs and a pediatric GIST showed negative immunohistochemical staining for SDHB, while 97% of sporadic GISTs were positive [49].

Other studies confirmed and extended this finding, showing that 'SDH deficient GISTs' are also associated with Carney–Stratakis and a fraction of apparently sporadic adult wild type GISTs. Janeway et al. evaluated 30 WT GIST tumors without associated SDH mutations and found loss of SDHB protein expression in all 18 pediatric WT GISTs tested, and weak or absent expression in all 12 adult WT GISTs. For comparison, only 1 of 23 KIT or NF-1-associated GISTs showed loss of SDHB expression. Loss of SDHB expression suggests that complex II enzyme activity will also be affected and these authors showed that two WT GISTs without an associated SDH mutation also lacked enzyme activity. Janeway and colleagues investigated several possible mechanisms of SDHB loss including decreased mRNA expression of *SDHB*, *SDHC*, or *SDHD* due to epigenetic modifications and loss of function mutations in the *SDHA* or *SDHAF2* genes, but found no evidence for either

mechanism [57]. A study by Miettinen et al. identified 66 SDH-deficient GISTs among 756 gastric GISTs (7.5%) and noted that most gastric GISTs in patients <20 years and a substantial percentage in patients <40 years were SDH deficient. Very few SDHB-negative tumors were seen in older cases, and all non-gastric GISTs ($n = 378$) were SDHB positive [58].

Negative SDHB immunohistochemistry is an established and reliable means of identifying pheochromocytomas and paragangliomas caused by SDH mutations, and now appears to be relevant to a specific group of GISTs. Gaal et al. explored the relationship between SDHB immunohistochemistry and SDH mutations in four GISTs from Carney–Stratakis syndrome patients and six from Carney triad patients, together with 42 apparently sporadic GISTs. Negative SDHB immunohistochemistry was followed by mutational analysis of *SDHB*, *SDHC*, and *SDHD*. All Carney–Stratakis and Carney triad syndrome GISTs stained negative for SDHB, but a germline mutation (*SDHB*) was found in only one patient. Of the 42 sporadic tumors, only one was SDHB-negative and no mutation was detected in this tumor [59].

The loss of SDHB expression and lack of complex II activity suggests that defects in complex II or cellular respiration may play an important oncogenic role in a particular subset of GISTs. The SDH mutations reported in some of these tumors are clearly pathogenic and thus can be assumed to be causative, but the absence of mutations in most cases suggests that mechanisms of complex II deactivation of a more generic nature must also be at play. SDH-deficient GISTs now appear to represent a distinctive tumor type, with a specific morphology and set of associated clinical features including an exclusive localization to the stomach, a multilobulated growth pattern, frequent but indolent metastases, and occurrence in children and young adults [60].

## 4. Renal cell carcinoma

The most common type of renal cell carcinoma (RCC) is clear cell RCC (ccRCC), a tumor of the renal cortex characterized by tumor cells with a clear cytoplasm and a well-vascularized, nested growth pattern. Other non-clear cell types include the papillary, type II papillary, collecting duct and chromophobic tumors [61]. Only 3–4% of all cases of RCC are hereditary and these are attributable to mutations in a heterogeneous collection of genes including VHL, MET, TSC1, TSC2, FH and FLCN. A wide variety of genotype–phenotype correlations have been described in familial RCC, and the most prominent include the association of VHL mutations with ccRCCs, MET with type 1 papillary RCC, and FH with type 2 papillary RCC [62,63]. VHL is the most common cause of inherited ccRCC and accounts for around 30% of all cases [61].

### 4.1. SDHB

More recently, mutations in subunit genes of complex II have been recognized to confer risk for RCC, indicating that RCC should be now be included, together with GIST, in the spectrum of tumors associated with pheochromocytoma-paraganglioma (PGL) syndrome [49,64–66]. The association of RCC with SDH mutations was not entirely unexpected, as both VHL and FH have strong mechanistic links to SDH. The SDH-related development of RCC currently appears to be dominated by germline DNA mutations of *SDHB*.

The association of germline *SDHB* mutations with renal tumors was first described by Vanharanta et al. (2004), who reported two families with early-onset RCC and germline *SDHB* mutations. The first family included two members with both early-onset RCC and paraganglioma. The second family comprised a patient diagnosed with ccRCC and his mother was diagnosed with malignant paraganglioma, both of whom carried a germline *SDHB* mutation [66]. That hereditary RCC can occur without any personal or family history of pheochromocytoma or paraganglioma was shown when Ricketts et al. [65] described germline *SDHB* mutations in RCC-only cases. These studies suggest that genetic screening for germline *SDHB* mutations should be considered in patients with familial RCC.

### 4.2. Histopathologic subtypes

There are no clear correlations of histopathologic subtypes of RCC with germline *SDHB* mutations and the most frequently reported SDHB-associated renal tumors are also the most common, the ccRCCs. A variety of other types of renal tumors have been described in patients with *SDHB* mutations, including chromophobic RCC, type II papillary RCC, and oncocytoma [65–68]. Housley et al. [69] reported a morphologically unique renal tumor associated with a germline *SDHB* mutation and described distinctive intracytoplasmic inclusions, demonstrating that these inclusions represent giant mitochondria. This study was followed by Gill et al. [64] who described the morphology and clinical features of renal tumors associated with germline *SDHB* mutations. These tumors displayed eosinophilic cytoplasm with intracytoplasmic inclusions and indistinct cell borders, reminiscent of the morphological features described by Housley et al., suggesting that giant mitochondria may be a key morphologic feature of SDHB-associated renal tumors. However, these morphological features have not been described for all SDHB-associated renal tumors, although this might be due to lack of detailed analysis. Ultrastructural analysis of paragangliomas has shown that giant mitochondria are also a feature of this tumor, but this characteristic is also often overlooked [40].

A recent report suggests that *SDHC* mutations may also be associated with RCC. Malinoc et al. [70] investigated known carriers of germline *SDHC* mutations for renal tumors, and found one patient with both a PGL and two RCCs. Molecular investigation of the tumors, metachronous bilateral ccRCCs and a papillary RCC, revealed no somatic *SDHC* mutations but did show LOH of intragenic and flanking markers at the *SDHC* locus. SDHB immunostaining showed that the two ccRCCs were negative but the papillary RCC stained positive for SDHB, perhaps suggesting that this tumor was coincidental [70]. Further studies are needed to confirm these findings.

## 5. Leigh syndrome and cardiomyopathy

Leigh syndrome (LS) is a progressive neurodegenerative disorder of infancy or childhood and is characterized by diverse neuropathologic features including focal, necrotizing lesions of the basal ganglia, diencephalon, cerebellum, or brainstem. The clinical hallmarks of LS vary but may include psychomotor delay or regression, muscular hypotonia, ataxia, respiratory insufficiency, and tremor due to lactate academia [71]. There is currently no effective treatment for LS and many patients die within months of being diagnosed. LS is a genetically heterogeneous disease and mutations in complexes I–V of the respiratory chain, coenzyme Q, and the pyruvate dehydrogenase complex all lead to a deterioration in mitochondrial function. Deficiencies of complex II are particularly rare and account for around 2% of all respiratory chain deficiencies [72].

### 5.1. SDHA

Bourgeron et al. (1995) described the first mutation in a nuclear encoded subunit of complex II. Two siblings with LS and a deficiency of complex II both carried a homozygous missense mutation in the *SDHA* gene. The parents were first cousins and both were heterozygous carriers of the mutation [71]. Both patients presented with neurological signs after the age of 10 months and showed developmental regression over the following months. Increased levels of serum lactate were detected and magnetic resonance imaging (MRI) showed abnormalities characteristic for LS in the cerebral white matter and basal ganglia.

The second patient with a mutation in complex II and a complex II deficiency was described by Parfait et al. (2000). In this case the patient was born to unrelated parents and developed normally until

9 months of age, when psychomotor delay was first noticed. Developmental abnormalities were apparent by 16 months of age, and mildly elevated lactate was also noted. MRI revealed necrotic lesions in the basal ganglia, confirming the diagnosis of LS. Enzyme analyses identified a complex II defect and showed complex activity of a third of normal levels. The patient was found to carry compound heterozygous mutations in the *SDHA* gene, including a nonsense mutation affecting the translation initiation codon and a missense mutation [72].

A report by Van Coster et al. (2003) described a patient, born to first cousins, with low weight gain at 5 months. A hospital admission due to respiratory difficulties revealed poor muscle tone, and an enlarged liver and spleen. An additional enlargement of the heart and serious cardiac dysrythmia resulted in the death of the patient during the first day of hospitalization. Although the patient was too young to allow assessment of LS symptoms, genetic analysis identified a homozygous missense mutation of *SDHA* [73]. The same homozygous missense mutation was reported in a patient with a relatively mild LS and complex II deficiency phenotype [74]. This patient, also born to first cousin parents, initially showed normal developmental but by 22 months, myopathy, poor walking and frequent falls were apparent. Further examination revealed elevated blood lactate and abnormalities on brain MRI. However, the patient then showed a gradual improvement and by the age of 10 could occasionally walk up to 200 m. He also showed a lack of cognitive impairment and repeat MRI demonstrated no new lesions and improvements in previous abnormalities [74].

### 5.2. Cardiomyopathy

Dilated cardiomyopathy is characterized by impaired systolic function due to dilatation of the left or both ventricles and is a major cause of morbidity and death, due to sudden cardiac arrest. Levitas et al. [75] described 15 Bedouin patients from a single tribe who all presented with dilated cardiomyopathy. Despite a mild increase in lactate, all patients had normal growth, normal muscle bulk and strength, and normal reflexes and gait. The psychomotor development of all patients was also normal and none had seizures. LS was ruled out as a diagnosis by brain MRI. Eight infants were diagnosed with left ventricular non-compaction (LVNC), a condition characterized by an incomplete development of the heart. Assessment of respiratory chain enzymes in skeletal and cardiac muscle biopsies showed a 50–60% reduction in complex II activity in skeletal muscle but an up to 85% reduction in cardiac muscle. A homozygous missense mutation in the *SDHA* gene, p.Gly555Glu, was identified in all patients. While this mutation caused only cardiomyopathy in these patients, the same mutation has also been reported in connection with myopathy, cardiomyopathy and mild Leigh-like symptoms [73,74].

Horvath et al. [76] described a case of a child of unrelated parents showing developmental delay and muscle weakness at 5 months of age, but with a normal heart and abdominal organs. Electroencephalography (EEG) showed some irregularities, lactate levels were mildly increased, and the child developed spasticity and other abnormalities. By the age of 9 the patient showed psychomotor arrest, blindness, seizures, and severe muscle contractures. Biochemical analysis of muscle biopsy specimens showed a residual complex II activity of only 23%. Sequencing of *SDHA* identified compound heterozygote mutations, including both a nonsense and a missense mutation.

More recently, Alston and colleagues described a patient with compound heterozygous mutations in the *SDHA* gene (p.Thr508Ile; p.Ser509Leu). At 3 months of age he presented with increasing shortness of breath, sweating, and feeding difficulty. Cardiomegaly and ischemia were evident, with a dilated and enlarged left ventricle, and he was diagnosed with dilated cardiomyopathy. In addition, the patient developed motor delay, knee and hip contractures, and generalized muscle weakness. Cranial and spinal MRI at 2.5 years age also demonstrated extensive abnormalities. A muscle biopsy revealed severely reduced SDH activity and BN/SDS-PAGE analysis showed loss of fully assembled complex II and SDHA protein [77].

### 5.3. Late-onset disease

A remarkable exception to the early onset seen in most cases of mitochondrial deficiency was reported by Birch-Machin et al. These authors described an autosomal dominant condition in a family with several affected cases, all of whom carried a heterozygous missense mutation of *SDHA* [78]. Patients showed an approximately 50% decrease in both complex II and SDH activity, and an unusual phenotype of late-onset neurodegenerative disease characterized by progressive optic atrophy, ataxia, and myopathy. While these findings suggest that a 50% reduction in *SDHA* RNA and protein content (haploinsufficiency) can result in a clinically relevant complex II deficiency and late-onset neurodegenerative disease, it is worth noting that the parents of the juvenile cases described above also carry heterozygous mutations but have not been reported to develop clinical symptoms.

### 5.4. Clinical and metabolic diversity

These findings illustrate the diversity of *SDHA*-related phenotypes that can result from similar or even identical mutations, with homozygous mutations resulting in phenotypes ranging from devastating developmental abnormalities to a purely cardiovascular phenotype – free of neurological involvement – and heterozygous mutations leading to late-onset neurodegenerative disease. While the basis of this clinical diversity is unknown, a clue may have been provided by Levitas et al. who described a healthy carrier of homozygous *SDHA* mutations [75]. This individual was the father of one of the patients but in contrast to his son, he exhibited normal LV function and dimension and was normal on clinical assessment, despite having lost three young siblings to cardiovascular failure. He was also capable of heavy physical work, while still showing complex II activity levels similar to those of the clinical cases. This apparent metabolic adaptation might have a genetic basis, but the child described by Pagnamenta et al. suggests that some physiological adaptation may also take place [74].

While the heterozygous patients described by Birch-Machin et al. developed late-onset disease, the lack of clinical symptoms in parents of homozygous patients could be due to mutation-specific effects, although the structure of SDHA and the range of known mutations suggest otherwise. It is worth noting that all patients show residual complex II activity and mutations are predominantly missense, with nonsense mutations only occurring when accompanied by a missense mutation [76], suggesting that complete loss of SDHA protein function may be incompatible with life. The molecular defects resulting from complex II deficiencies are still rather poorly understood, but both oxidative phosphorylation and the TCA cycle show dysfunction, and hypoglycemia and lacticacidemia are commonly seen in patients [71,76].

The heterozygous parents of homozygous *SDHA* mutation carriers are also theoretically prone to paraganglioma development, as mutations of *SDHA* have been shown to be a (rare) cause of paraganglioma. However, the extreme rarity of SDHA-related tumors may be due to the relatively low frequency of the loss of the chromosomal region containing the *SDHA* locus (5p15), compared with the 1p36 (*SDHB*) and 11q23 (*SDHD*) loci that often show loss in tumor tissues [34]. On the other hand, the neurodegenerative disorders and cardiomyopathies associated with SDHA have not (yet) been linked to mutations in the *SDHC* or *SDHD* genes. This may be due to the structural role of the SDHC and SDHD proteins, with pathogenic mutations resulting in complete rather than partial loss of function. Such mutations may be poorly tolerated and incompatible with life in a homozygous state, while missense mutations with relatively mild affects on the catalytic functions of SDHA or SDHB can occur in a homozygous state. The role of haploinsufficiency for individual components of complex II may

also play a role, as a 50% reduction in genetic content for the *SDHB*, -C, or -D genes results in no noticeable physiological phenotype, while heterozygous mutations of *SDHA*, accompanied by reduced enzymatic activity, can give rise to late-onset neurodegenerative disease [78].

## 6. Infantile leukodystrophies

### 6.1. SDHAF1

A specific infantile leukoencephalopathy was recently identified in several cases in two large, unrelated consanguineous families [79]. Clinical features in both families were very similar and further investigation revealed elevated blood lactate and pyruvate levels. Further characteristics included an initial 6–11 month disease-free interval followed by a rapidly progressive psychomotor regression, weakness of all four limbs, loss of postural control, and involuntary muscle contractions. MRI of the brain showed severe changes to the brain white matter, and accumulation in the brain of lactate and succinate was also detected. Patient muscle and fibroblasts showed only 20–30% residual complex II activities. The clinical condition of the patients stabilized and while showing consistently impaired growth, several patients survived for over a decade.

Genetic mapping using linkage analysis identified a common homozygous region on chromosome 19 and further analysis identified homozygous mutations in a previously unknown gene, now called succinate dehydrogenase complex assembly factor 1 (*SDHAF1*) [79]. The encoded protein is ubiquitously expressed in mitochondria, and although novel, it carries a tripeptide LYR motif found in several proteins involved in Fe–S metabolism. SDHAF1 may therefore be associated with SDHB and involved in the insertion or retention of the complex II Fe–S center. Other Fe–S-dependent activities tested in SDHAF1-defective organisms were normal, implying that SDHAF1 may be specific for complex II.

The association of mutations of *SDHAF1* with an infantile leukoencephalopathy reminiscent of Leigh syndrome was somewhat unexpected – as was the association of mutations of *SDHAF2* with paraganglioma – because these co-factors interact with complex II subunits that generally give very different phenotypes when mutated. If SDHAF1 is a cofactor of SDHB, heterozygous mutations might be expected to result in typical SDHB-associated tumors including paraganglioma, pheochromocytoma, GIST and renal cell carcinoma, while the SDHA cofactor SDHAF2 would be predicted to give rise to extra-adrenal paraganglioma, GIST and Leigh syndrome (when homozygously mutated). Although such clinical phenotypes may yet come to light, at the moment it appears that we still have much to learn regarding the cofactors of complex II.

### 6.2. SDHB

More recently, Alston and colleagues described a female child, born to consanguineous parents, who showed apparently normal developmental to 1 year of age, but then regressed rapidly over a 6-week period [77]. In addition to unsteadiness, repeated falls, and eventual loss of walking ability, the child showed loss of muscle tone with poor head control and difficult feeding. Following this period of deterioration, the child showed slow developmental progress but remained wheelchair-dependent at the age of 4 years. MRI demonstrated leukodystrophy of deep cerebral white matter at both the initial presentation and at 4.5 years, with dystrophic white matter showing increased lactate and succinate.

A diagnostic muscle biopsy showed severely reduced SDH and complex II activity, and genetic analysis identified a novel homozygous mutation in the *SDHB* gene (c.143A>T, p.Asp48Val). Further investigations showed an almost complete absence of the SDHB subunit by SDS-PAGE analysis, and studies of the equivalent mutation in yeast showed a 50% reduction in SDH activity.

As well as being the first association of an SDHB defect with a neurological phenotype, this finding represents the first description of a homozygous *SDHB* mutation in any patient (or in any multicellular organism, for that matter). Although no systematic functional study of the effect of putative mutations of SDH subunits has yet been described, several studies have focused on specific subunits and certain variants [80–82]. An earlier study may provide a clue as to why the p.Asp48Val mutation can exist in a homozygous state [83]. Goffrini and colleagues have previously studied a p.Cys191Tyr mutation, found in a patient with head and neck paraganglioma, and could show that the ability of the yeast strain, BY4741Δ*sdh2*, to grow on 2% ethanol could not be complemented by the mutation ($sdh2^{C184Y}$), whereas the growth of the p.Asp48Val ($sdh2^{N42V}$) strain was almost equivalent to complementation with a wild type construct. Equally, the $sdh2^{C184Y}$ strain showed a profound defect in respiration, while the $sdh2^{N42V}$ strain showed no change. The 50% reduction in SDH activity seen in the $sdh2^{N42V}$ strain also contrasted with the approximately 95% reduction seen in the $sdh2^{C184Y}$ strain.

These differences, although only broadly concordant with the declines in complex II activity seen in muscle biopsies, may suggest that the p.Asp48Val variant induces relatively minor changes in the SDHB protein compared to the paraganglioma-associated p.Cys191Tyr mutation. Only time will tell if further *SDHB* variants will emerge with an 'intermediate' genetic phenotype and an association with neurological disorders.

## 7. Conclusions

Defects in complex II lead to a diverse array of clinical symptoms, ranging from serious developmental delay to tumors as varied as renal cell carcinoma and paraganglioma. Is there a common theme that unites these clinical syndromes? One broad theme that is perhaps fundamental to all these diseases, and also a 'new frontier' for the understanding of disease in general, is that of cell specificity. Cell types show profound differences that often survive cancerous transformation, and during development and throughout life cells differ in their requirements for ATP, redox agents and the metabolic intermediates produced by the TCA cycle. These differences may make certain cells particularly vulnerable to complex II-mediated transformation and neoplasia. Cardiac cells and cells of the ascending and descending tubules of the kidney have a heavy energy dependency and show a high level of expression of complex II; tumorigenic cells of paragangliomas, pheochromocytomas and GISTs all have a neuronal function. These similarities may be coincidental but might hint at some underlying commonality.

### 7.1. Biochemical studies

Despite considerable progress in understanding the biochemistry resulting from loss of complex II [84,85], no direct link to tumorigenesis has yet been established. We still do not know if the primary link between loss of complex II and tumorigenesis is due to the activation of HIF1 through the accumulation of succinate [86,87], due to the generation of ROS [3] or to other mechanisms such as inhibition of histone demethylases [88] or inhibition of EglN3-mediated apoptosis [89]. While one or more of these mechanisms may be important, studies have been contradictory. Loss of SDH has been shown to lead to oxidative stress and genomic instability by inducing a pseudo-hypoxic response [3,4,80,90,91]. Conversely, other studies reported no signs of ROS production or oxidative stress resulting from SDH mutations, but rather an accumulation of succinate and HIF1a activation [5,92].

### 7.2. Animal and cell models

Elucidating the primary link between loss of complex II and disease is an important clinical aim that could open the way to new

treatments. Although various mechanisms and pathways have been implicated in disease, no direct causal link to disease has yet been established. A major barrier to further progress is the current lack of relevant animal and cell models. Although several mouse lines carrying knockouts of SDH genes have been described, heterozygous animals do not develop disease and homozygous knockout is lethal [93,94]. Conditional and tissue-specific models also fail to develop disease [95] (and unpublished results). To date, no cell line from a paraganglioma or other complex II-related tumor has been described. Until these models are established the resolution of currently conflicting data and further progress towards potential therapies will remain beyond our reach.

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