Hereditary Paraganglioma/ Pheochromocytoma and Inherited Succinate Dehydrogenase Deficiency

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Key Words

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Abstract

Mitochondrial complex II, or succinate dehydrogenase, is a key enzymatic complex involved in both the tricarboxylic acid (TCA) cycle and oxidative phosphorylation as part of the mitochondrial respiratory chain. Germline succinate dehydrogenase subunit A (*SDHA*) mutations have been reported in a few patients with a classical mitochondrial neurodegenerative disease. Mutations in the genes encoding the three other succinate dehydrogenase subunits (*SDHB*, *SDHC* and *SDHD*) have been identified in patients affected by familial or 'apparently sporadic' paraganglioma and/or pheochromocytoma, an autosomal inherited cancer-susceptibility syndrome. These discoveries have dramatically changed the work-up and genetic counseling of patients and families with

paragangliomas and/or pheochromocytomas. The subsequent identification of germline mutations in the gene encoding fumarate hydratase – another TCA cycle enzyme – in a new hereditary form of susceptibility to renal, uterine and cutaneous tumors has highlighted the potential role of the TCA cycle and, more generally, of the mitochondria in cancer.

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Introduction

Mitochondrial diseases result in a number of clinical presentations, ranging from organ-specific involvement to multisystemic disorders, often with prominent neurological features [1]. The recent discovery of germline mutations in the *SDH* genes encoding succinate dehydrogenase in patients with familial paraganglioma (PGL) or pheochromocytoma (PHEO) has revealed an unexpected and primary role of mitochondrial deficiency in carcinogenesis [2]. *SDH* gene mutations are also frequently encountered in patients with an apparently sporadic form of PGL or PHEO. Further evidence that mitochondria play a role in carcinogenesis has been provided by the

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identification of mutations affecting the fumarate hydratase (*FH*) gene [3]. This review focuses on the recently established link between mitochondrial dysfunction and tumorigenesis. The consequences of these new findings for the diagnosis, treatment and follow-up of patients with PHEO and PGL will be discussed in detail.

Mitochondrial Complex II or Succinate Dehydrogenase

Description

Most of the energy used by the cell is generated by mitochondrial oxidative phosphorylation, via the respiratory chain, which produces ATP from the energy released by substrate oxidation. The metabolic pathways responsible for substrate oxidation depend on the tissues and cells concerned, but occur in the mitochondrial matrix. The tricarboxylic acid (TCA) cycle, β -oxidation of fatty acids, and many other enzymatic reactions integrate mitochondrial matrix metabolism into the general metabolic network of the cell. Indeed, as many as a thousand nuclear genes are thought to encode the components of the mitochondrial metabolic machinery. The oxidation of organic acids via the TCA cycle produces the reduced equivalents – NADH and FADH₂ – required for the production of most of the cell's ATP via the respiratory chain. Oxidative phosphorylation couples ATP synthesis to the flow of electrons from NADH or FADH₂ to O₂, thanks to the proton gradient setup by electron transfer through the respiratory chain complexes of the inner mitochondrial membrane. With only four subunits, mitochondrial complex II (succinate:ubiquinone oxidoreductase, SQR, EC 1.3.5.1) is the smallest complex in the respiratory chain. It is located at the crossroads between the respiratory chain and the TCA cycle. Together with a number of inner membrane-bound dehydrogenases, it feeds electrons to the quinone pool, reducing ubiquinone (Q) to ubiquinol (QH₂). The specific redox properties of this complex suggest that it is a key enzyme in control of the redox status of the Q pool, and therefore in the regulation of oxidative stress [4]. In the TCA cycle, it catalyzes the oxidation of succinate to fumarate (fig. 1). The complex comprises four subunits: SDHA, SDHB, SDHC and SDHD. The hydrophilic, catalytic part of the complex consists of a 70-kDa flavoprotein (Fp, SDHA) and a 30-kDa iron-sulfur protein (Ip, SDHB), which form the succinate dehydrogenase (SDH) enzyme. The hydrophobic part of the complex consists of the 15-kDa (SDHC) and 12.5-kDa (SDHD) subunits, which anchor the com-

plex in the inner mitochondrial membrane and are necessary for electron transfer to the Q pool.

The succinate binding site of the enzyme is located on the Fp subunit, to which a flavin adenine dinucleotide (FAD) binds covalently. Electrons derived from the oxidation of succinate are transferred to the flavin moiety (FADH₂) and then on to the three iron-sulfur clusters – [2Fe-2S], [4Fe-4S], [3Fe-4S] – of the Ip subunit. They are then transferred to the Q pool via the anchorage domain of complex II, which contains a b-type cytochrome and Q binding sites [5]. The three-dimensional structure of *Escherichia coli* SDH was recently elucidated and shows that SQR plays a role in preventing the formation of reactive oxygen species (ROS) [6].

The SDH Genes

The four-subunit complex II is the only respiratory chain complex exclusively encoded by nuclear genes – *SDHA* (15 exons, 664 amino acids) [7], *SDHB* (8 exons, 281 amino acids) [8, 9], *SDHC* (6 exons, 170 amino acids) [10], and *SDHD* (4 exons, 160 amino acids) [10] – located on four different chromosome regions: 5p15, 1p35–36.1, 1q21 and 11q23, respectively. Two forms of *SDHA* have been described in humans, and are expressed differentially according to the cell type [11]. A point mutation in the *SDHC* gene of *Caenorhabditis elegans*, giving rise to the *mev-1* mutant, causes premature aging and oxidative stress by increasing ROS production [12]. In humans, mutations in *SDH* genes have been shown to cause two very different types of disease. Mutations in *SDHA* cause a neurological disease, with an early onset and brain lesions typical of Leigh syndrome. Mutations in *SDHB*, *SDHC* and *SDHD* predispose the individual to a particular type of cancer: hereditary PGL/PHEO.

SDHA Mutations Cause Leigh Syndrome

Only five different germline mutations have been described in *SDHA*, which is located on chromosome 5 (table 1). The first one (R554W) was identified in one consanguineous family. The patients, homozygous for the mutation, presented early-onset neurodegenerative disease with Leigh syndrome (MIM 256000), due to a severe complex II deficiency. The expression of this mutation in yeast resulted in a 50% decrease in SDH activity [13]. A similar phenotype was described in a compound heterozygous patient with a paternally inherited A524V mutation and a maternally inherited M1L mutation [14]. Another homozygous mutation (G555D) was identified in a

Fig. 1. The key role of mitochondrial complex II. Complex II connects the respiratory chain in the inner mitochondrial membrane with the tricarboxylic acid (TCA) cycle in the matrix. In the respiratory chain, complexes I and II reduce ubiquinone (Q) to ubiquinol (QH₂). In the TCA cycle, succinate is oxidized by succinate dehydrogenase to generate fumarate. The enzymes involved in this process are: (1) fumarase, (2) malate dehydrogenase, (3) aspartate aminotransferase, (4) α-ketoglutarate dehydrogenase, (5) succinyl CoA synthetase, (6) pyruvate dehydrogenase, (7) citrate synthetase, (8) aconitase, and (9) isocitrate dehydrogenase.

Table 1. Mutations in *SDHA* gene

Inheritance	Protein change	Phenotype	Ref.
Recessive	p. R554W	Early-onset Leigh syndrome	13
Recessive	p. A524V/M1L	Early-onset Leigh syndrome	14
Recessive	p. G555D	Early-onset Leigh syndrome	15
Dominant	p. R451C	Late-onset optic atrophy, ataxia, myopathy	16

child with complex II deficiency, who died at 5.5 months of age following a respiratory infection [15]. Finally, a heterozygous base change (R408C) was reported in 2 sisters with a partial complex II deficiency (residual activity >50%) and caused a milder phenotype associating optic atrophy, ataxia and myopathy [16]. Leigh syndrome and

the various neuromuscular symptoms observed in this small number of patients with mutations in the *SDHA* gene are features frequently observed in patients with respiratory chain deficiency [17].

Hereditary Paraganglioma and Pheochromocytoma: *SDHD*, *SDHB*, *SDHC* Deficiencies

Clinical Presentation of Paraganglioma and Pheochromocytoma

PGLs are neuroendocrine tumors that may secrete catecholamines. They occur most frequently in the head (glomus tympanicum and jugulare), neck (carotid body and glomus vagale), adrenal medulla and extra-adrenal sympathetic ganglia. The paraganglia and other elements of the autonomic nervous system arise from neural crest

Fig. 2. Neural crest cell migration. Schematic representation of the migration of neural crest cells in the fifth week of development, in a transverse section of a human embryo. The parasympathetic ganglia in the head and neck, the sympathetic thoracic and abdominal ganglia, the organ of Zuckerkandl at the aortic bifurcation and the adrenal medulla develop from these cells.

cells, as the thyroid C-cells, and are distributed from the base of the skull to the pelvic floor. During embryogenesis, neural crest cells migrate from the neural tube to form the parasympathetic system in the head and neck, the sympathetic nervous system in the thoracic and abdominal region and the adrenal medulla (fig. 2). PGLs may occur at any location in this migration process. PGLs arising from the parasympathetic system (carotid body, glomus vagal, glomus jugulare and tympanicum) usually have no endocrine activity. However, PGLs arising from the adrenomedulla or sympathetic ganglia (organ of Zuckerkandl or periaortic region, perirenal region, bladder, mediastinum, etc.) may be functional and secrete catecholamines. According to current nomenclature, the term 'pheochromocytoma' corresponds to a secreting PGL evolving from the adrenal medulla, whereas a 'functional PGL' refers to an extra-adrenal secreting PGL, which was previously known as an 'ectopic PHEO'. The tumors now described as 'carotid body PGL' were formerly known as 'chemodectoma' due to the chemoreceptor function of the carotid body.

PGLs are generally benign, highly vascularized tumors occurring close to the major blood vessels and cranial nerves. About 10% of these tumors are malignant. PGLs

are usually diagnosed on the basis of the presence of a mass in the neck, pulsatile tinnitus or hearing loss. A head and neck MRI or CT scan can be used to characterize the tumor in terms of vascularization, as well as location and extent of the tumor [18]. The only curative therapy is early surgical resection of the tumor. Before commencing surgery, other sites of PGL or PHEO should be sought by determining 24-hour urinary or plasma metanephrine concentration and radiological methods such as ^{123}I -metaiodobenzylguanidine (mIBG) scintigraphy and/or octreotide scintigraphy and/or ^{18}F -DOPA or ^{18}F -fluorodopamine whole-body positron emission tomography (PET). Finally, angiography is worthwhile to assess the extent of the tumor and for presurgical embolization [19]. In cases of PHEO, surgery must be accompanied by preoperative drug treatment and the assistance of a specialized team of anesthetists [20]. Radiation therapy may also be proposed for large, rapidly growing tumors.

Hereditary Paraganglioma

The existence of familial forms of PGL has been suspected since the 1940s, with reports of several cases of head and neck PGL in the same family. PGLs seem to be inherited in about 30% of cases. The hereditary form of PGL is usually characterized by an early onset and a more severe presentation than the sporadic form. These tumors often display bilateral and multiple locations and may be recurrent or malignant. The presence of one or several secreting tumors is not rare and contributes to the severity of inherited PGL [21]. Such PGLs constitute a genetically heterogeneous group of diseases as four different loci have been implicated: *PGL1* on 11q23, *PGL2* on 11q13, *PGL3* on 1q21 and *PGL4* on 1p36. This condition is subject to autosomal dominant inheritance, with genomic imprinting of the maternal allele for the *PGL1* locus [22]. The disease has incomplete penetrance and variable expressivity.

In 2000, linkage analysis and positional cloning allowed Baysal et al. [23] to report the first deleterious mutations in the *SDHD* gene, corresponding to the *PGL1* locus. The use of a candidate gene approach has led to the identification of mutations in *SDHC* (*PGL3*) [24] and *SDHB* (*PGL4*) [25]. Several mutations in these three genes were subsequently reported in patients with hereditary PGL, and in apparently sporadic cases [26].

Genetics of Hereditary Pheochromocytomas

Prior to 2000, three different familial and syndromic diseases were known to result in adrenomedulla tumors or PHEOs: multiple endocrine neoplasia type II (MEN2;

Table 2. Genotyping of pheochromocytoma-susceptibility genes in two different populations

	271 patients with non-syndromic pheochromocytoma ¹	84 patients with apparently sporadic pheochromocytoma ²
Hereditary pheochromocytoma	24% (66/271)	12% (10/84)
<i>RET</i>	5% (13/271)	0% (0/84)
<i>VHL</i>	11% (30/271)	2% (2/84)
<i>SDHB</i>	4% (11/271)	10% (8/84)
<i>SDHD</i>	4% (12/271)	0% (0/84)
	'Nonsyndromic' but during the follow-up: (a) 12 medullary thyroid carcinomas and 6 family history among the 13 patients with <i>RET</i> mutation; (b) 15 other features and 12 family history among the 30 patients with <i>VHL</i> mutation, and (c) 4 glomus tumors among the 23 patients with <i>SDH</i> mutation	'Sporadic' because: (a) normal calcitonin level before and after pentagastrin infusion; (b) no other feature detected (fundoscopic examination/CT scan); (c) patients with personal or family history of MEN, <i>VHL</i> , <i>NF1</i> and PGL were excluded, and (d) systematic follow-up (8.8 ± 5.7 years)

¹ For the Freiburg-Warsaw-Columbus pheochromocytoma group [31].
² For the COMETE Network [32].

MIM 171400), induced by germline-activating mutations in the *RET* proto-oncogene [27], von Hippel-Lindau disease (*VHL*; MIM 193300), due to mutations in the tumor suppressor gene *VHL* [28], and neurofibromatosis type 1 (MIM 162200), caused by mutations in the *NF1* gene [29]. In 2000/2001, the identification of mutations in the *SDH* (*SDHD*, *SDHB*, *SDHC*) genes in hereditary PGLs (MIM 168000) and in PHEOs led to changes in the genetic counseling and work-up for affected patients [30]. In 2002, Neumann et al. [31] demonstrated the importance of screening for mutations in the genes conferring susceptibility to PHEO in a large, apparently non-syndromic population (271 patients). They reported 24% inherited disease, with 66 patients having a germline mutation in the *VHL*, *RET*, *SDHD* or *SDHB* gene. Some of these patients had a family history or multifocal disease, identified after genetic testing. In 2003, a retrospective study was performed on 84 patients with apparently sporadic PHEO, from which patients with a family history or syndromic disease were excluded. In this French series, 12% of the patients harbored mutations, mostly in the *VHL* and *SDHB* genes [32] (table 2). In this population, followed in a single center for a mean of 9 years, the identification of a mutation in the *SDHB* gene was associated with a high risk of extra-adrenal location and, particularly, of malignant disease. A preliminary survey of 227 patients with PHEOs from the French cohort showed that one third of these unselected patients had a germline mutation in one of the known susceptibility genes. In two

thirds of these cases, the molecular diagnosis was oriented by clinical symptoms or a family history, especially for diseases resulting from inherited mutations in *NF1*, *RET*, *VHL* and *SDHD*. However, 90% of cases with apparently sporadic presentation are due to mutations in *SDHB* or *VHL* [33].

These recent genetic advances have rapidly led to changes in the management of patients with PHEO. The biological and radiological diagnosis of PHEO in patients of any age should now lead to a search for an inherited disease and the family pedigree should be analyzed. The work-up should comprise (1) a physical examination, including a search for neurofibromas, coffee-colored pigmented spots, and the determination of plasma thyrocalcitonin concentration; (2) a fundoscopic examination looking for retinal hemangioblastomas, and (3) head and neck and abdominal CT or MRI scans to search for cervical PGL, and renal or pancreatic tumors. Targeted genetic testing should be offered to patients with phenotypic features typical of MEN2, *VHL* disease or hereditary PGL. In patients with a regular, apparently sporadic presentation, the *VHL* and *SDHB* genes should be analyzed as a matter of priority.

Genetic Counseling

Genetic testing is indicated for all patients with a PGL and/or a PHEO, whatever the location of the tumor and the age of the subject, but, as discussed above, such testing may also be indicated on the basis of clinical and fa-

Fig. 3. A family with an *SDHD* mutation. **a** Angiography showing bilateral cervical PGL (subject I.1). **b** The family pedigree, suggesting autosomal dominant transmission with maternal imprinting: subject III.1 had a glomus tumor whereas subject II.1 is asymptomatic. **c** Analysis of the *SDHD* gene in the leukocyte DNA of subject I.1 showed a mutation in the *SDHD* gene affecting its splicing (IVS II-1 G>T).

miliar features (fig. 3). Phenotype-genotype correlations are useful for genetic testing, which should initially target the *SDHD* gene in cases of head and neck PGL, or the *SDHB* gene in cases of extra-adrenal PGL or PHEO. *SDHD* gene mutations often result in multiple tumors, primarily in the head and neck. In carriers of *SDHB* mutations, the resulting tumor is often a functional extra-adrenal PGL or PHEO, which may be malignant [34]. Nonsense and missense mutations, insertions and deletions are frequently encountered in the *SDHD* and *SDHB* genes, but only 4 familial cases have so far been linked to the *SDHC* gene [35]. However, this small number of cases may be accounted for by intragenic deletions of the *SDHC* gene not detected by direct sequencing [36].

The family pedigree may also direct genetic testing. The disease displays autosomal dominant inheritance for the *SDHB* and *SDHC* genes, with maternal imprinting for the *SDHD* gene. Consequently, in families with *SDHD* mutations, the disease is transmitted exclusively via the father. A heterozygous mother has a 50% risk of transmitting the mutation to her child, but children carrying the maternal mutant allele do not go on to develop the disease. In contrast, the *SDHB* and *SDHC* mutations are both maternally and paternally transmitted.

The identification of a causative mutation in an affected patient should lead to presymptomatic familial genetic testing because the early detection of small tumors in individuals at risk can reduce the morbidity of the disease. Presymptomatic genetic testing should be offered to all first-degree relatives if an *SDH* mutation is detected in the index case. Such testing should make it possible to detect presymptomatic tumors (size <1 cm) in positive carriers, which can be treated surgically with less risk of complication. Several clinical research networks throughout the world focus on hereditary PGL and PHEO. In

France, the PGL.NET Network recommends clinical testing including head and neck MRI and metanephrine determination in patients with an *SDH* mutation. If metanephrine levels are normal, octreotide scintigraphy should be performed to search for non-functional PGLs. If metanephrine levels are high, suggesting a possible functional PGL, ^{123}I -mIBG scintigraphy should be performed to detect the tumor. In Germany and the USA, MRI of the neck, thorax and abdomen-pelvis and/or ^{18}F -DOPA or ^{18}F -dopamine PET are recommended as screening tests [37, 38]. Such tests should be carried out annually for patients with *SDHB* mutations and every 2 years for patients with *SDHD* mutations. It is important to note that all these guidelines are empiric and require validation by prospective studies in families with *SDH* mutations.

***SDH* Genes and Tumorigenesis**

SDH genes (*SDHD*, *SDHC*, *SDHB*) have been shown to be tumor suppressor genes. Consistent with Knudson's two-hit hypothesis for tumorigenesis involving a tumor suppressor gene, a heterozygous germline mutation in an *SDH* gene is usually associated with somatic loss of the normal allele (loss of heterozygosity), leading to inactivation of the *SDH* gene. We have shown that this inactivation results in a total lack of electron transfer from succinate in the tumor [39, 40], for all subunits except SDHA, for which mutations never result in the complete abolition of SDH enzymatic activity (25–50% decrease). The production of fumarate by SDH is immediately followed by the conversion of fumarate into malate by fumarate hydratase (see fig. 1). Mutation of the *FH* gene, which encodes fumarate hydratase (also known as fumarase),

Fig. 4. Strong expression of genes encoding angiogenic factors in hereditary PGL. (a) EPAS1, (b) VEGF and (c) VEGFR-1 mRNA profiles on in situ hybridization (signal visible as white dots) in a mutated *SDHD* carotid-body PGL.

has been found to cause another autosomal disorder characterized by tumors of the skin and uterus and/or renal cancer [3]. These recent discoveries suggest that impairment of the TCA cycle may be involved in cancer [41].

The molecular and cellular mechanisms linking *SDH* mutations and tumorigenesis remain unknown and experimental models for the study of these disorders have not yet been developed. Several non-mutually exclusive hypotheses have been put forward [2, 42]. They involve a mitochondrial dysfunction leading to resistance to apoptosis, accumulation of oxygen free radicals and oxidative stress, or pseudo-hypoxia acting as a protumoral factor [2]. Hence, in renal cell carcinoma occurring in patients with VHL disease – a known genetic cause of PHEO – it is established that the abnormal stabilization of the hypoxia-inducible factor EPAS1/HIF2 α is directly responsible for tumorigenesis [43]. It has therefore been suggested that activation of the hypoxic pathway is also directly involved in the tumorigenic process of *SDH*-mutated PGLs and PHEOs. Indeed, *SDH* gene mutations are associated with the induction of hypoxia response genes such as that encoding vascular endothelial growth factor (VEGF), and this induction is thought to be mediated by the abnormal activation of HIF1 α and EPAS1/HIF2 α (fig. 4). The subsequent stimulation of angiogenesis probably accounts for the high level of vascularization of these tumors [39, 40], but hypoxia-inducible transcription factors (HIFs) may also be responsible for carcinogenesis [for review, see 44]. This hypothesis is supported by the high incidence of head and neck PGLs in populations living at elevated altitude [45]. Several mechanisms accounting for the putative link between *SDH* mutations and HIF

stabilization have been put forward. One is the accumulation of succinate, which would lead to inactivation of the prolyl-4-hydroxylases required for HIF degradation in normoxia [46]; another involves the ROS pathway. The validity of these mechanisms remains to be demonstrated in appropriate experimental models providing an understanding of the molecular and cellular mechanisms involved in the induction of tumorigenesis by *SDH* mutations.

Conclusion

The recent discovery of mutations in the *SDHD*, *SDHC* and *SDHB* genes has shed new light on the primary disorders responsible for PHEOs and PGLs in particular, and more generally, in the elucidation of novel, unexpected oncogenic mechanisms involving mitochondria.

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