Succinate dehydrogenase (SDHx) mutations in pituitary tumors: could this be a new role for mitochondrial complex II and/or Krebs cycle defects?

Paraskevi Xekouki and Constantine A Stratakis

Abstract

Succinate dehydrogenase (SDH) or mitochondrial complex II is a multimeric enzyme that is bound to the inner membrane of mitochondria and has a dual role as it serves both as a critical step of the tricarboxylic acid or Krebs cycle and as a member of the respiratory chain that transfers electrons directly to the ubiquinone pool. Mutations in SDH subunits have been implicated in the formation of familial paragangliomas (PGLs) and/or pheochromocytomas (PHEOs) and in Carney–Stratakis syndrome. More recently, SDH defects were associated with predisposition to a Cowden disease phenotype, renal, and thyroid cancer. We recently described a kindred with the coexistence of familial PGLs and an aggressive GH-secreting pituitary adenoma, harboring an SDHD mutation. The pituitary tumor showed loss of heterozygosity at the SDHD locus, indicating the possibility that SDHD’s loss was causatively linked to the development of the neoplasm. In total, 29 cases of pituitary adenomas presenting in association with PHEOs and/or extra-adrenal PGLs have been reported in the literature since 1952. Although a number of other genetic defects are possible in these cases, we speculate that the association of PHEOs and/or PGLs with pituitary tumors is a new syndromic association and a novel phenotype for SDH defects.

Introduction

Succinate dehydrogenase (SDH) or succinate-coenzyme Q reductase is a multimeric enzyme that is bound to the inner membrane of mitochondria (Oyedotun & Lemire 2004). It has a dual role as it serves both as a critical step of the tricarboxylic acid (TCA) or Krebs cycle and as a member of oxidative phosphorylation, the respiratory chain that transfers electrons directly to the ubiquinone pool (Kantorovich & Pacak 2010). It is a highly conserved protein complex that consists of four subunits: two hydrophilic, a flavoprotein (SDHA) and an iron–sulfur protein (SDHB) that together form the catalytic core of the enzyme (SDHA serves as the substrate binding site for succinate), and two hydrophobic subunits, SDHC and SDHD, that anchor the holotetramer to the membrane and serve as the ubiquinone site (Oyedotun & Lemire 2004, Kantorovich & Pacak 2010).

Syndromes related to SDHx mutations

The discovery that mutations in genes coding for the subunits SDHB, SDHC, and SDHD were responsible for the formation of multiple and possibly coexisting parasympathetic and sympathetic paragangliomas (PGLs) and/or pheochromocytomas (PHEOs) (Baysal et al. 2000, Astuti et al. 2001) made obsolete (Dluhy 2002) at least one part of the axiom that had been proposed by Bravo & Gifford (1984); the so-called ‘10 rule’ had stated that 10% of PHEOs were bilateral, 10% malignant, 10% normotensive, 10% extra-adrenal, and 10% genetic origin. Today, we know that as many as 40% of PHEOs/PGLs may be due to a genetic defect (Raygada et al. 2011); in children and young adults, this may be true in as many as three out of four patients.

In 2007, Stratakis et al. described germline mutations of the SDHB, SDHC, and SDHD genes in...
### Table 1: Reported cases of coexistence of PHEO/PGL and pituitary adenoma

<table>
<thead>
<tr>
<th>Report</th>
<th>Age&lt;sup&gt;a&lt;/sup&gt;/sex</th>
<th>Case</th>
<th>Genetic screening</th>
<th>Family history for endocrine tumors</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iversen (1952)</td>
<td>44/M</td>
<td>Acromegaly/PHEO</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Kahn &amp; Mullon (1964)</td>
<td>40/M</td>
<td>Acromegaly/PHEO</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>O’Higgins &lt;i&gt;et al.&lt;/i&gt; (1967)</td>
<td>21/F</td>
<td>Acromegaly/PHEO</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Steiner &lt;i&gt;et al.&lt;/i&gt; (1968)</td>
<td>41/M</td>
<td>Cushing’s disease/bilateral PHEOs/medullary thyroid cancer</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Positive for MEN for VI generations</td>
<td></td>
</tr>
<tr>
<td>Wolf &lt;i&gt;et al.&lt;/i&gt; (1972)</td>
<td>43/F</td>
<td>Pituitary adenoma (probably nonfunctioning), PHEO/PHEO, medullary thyroid cancer</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Farhi &lt;i&gt;et al.&lt;/i&gt; (1976)</td>
<td>19/F</td>
<td>Acromegaly/PGLs/, parathyroid hyperplasia, pigmentary abnormalities</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Kadowaki &lt;i&gt;et al.&lt;/i&gt; (1976)</td>
<td>44/M</td>
<td>Acromegaly/PHEO</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Osamura &lt;i&gt;et al.&lt;/i&gt; (1977)</td>
<td>58/M</td>
<td>Acromegaly/PHEO/renal carcinoma</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Manger &lt;i&gt;et al.&lt;/i&gt; (1977)</td>
<td>15/F</td>
<td>Acromegaly/PHEO</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Melicow (1977)</td>
<td>52/F</td>
<td>Chromophobe adenoma of pituitary/papillary carcinoma of thyroid/PHEO diagnosed postmortem</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Positive for PHEOs/islet cell tumor/renal adenoma</td>
<td></td>
</tr>
<tr>
<td>Janson &lt;i&gt;et al.&lt;/i&gt; (1978)</td>
<td>28/F</td>
<td>Pituitary adenoma (probably nonfunctioning) /bilateral PHEO</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Positive for PHEOs/islet cell tumor/renal adenoma</td>
<td></td>
</tr>
<tr>
<td>Alberts &lt;i&gt;et al.&lt;/i&gt; (1980)</td>
<td>36/F</td>
<td>Pituitary adenoma (?) /PHEO/islet cell tumor (gastrinoma), Cushing’s syndrome (adrenal cortical adenoma), parathyroid hyperplasia</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Myers &amp; Eversman (1981)</td>
<td>53/F</td>
<td>Acromegaly/PHEO/PHP</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Anderson &lt;i&gt;et al.&lt;/i&gt; (1981)</td>
<td>53/F</td>
<td>Acromegaly/PHEO/parathyroid hyperplasia (diagnosed post-mortem)</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Negative</td>
<td>Hypertension in one sibling (PHEO?)</td>
</tr>
<tr>
<td>Anderson &lt;i&gt;et al.&lt;/i&gt; (1981)</td>
<td>58/F</td>
<td>Acromegaly/PHEO</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Meyers (1982)</td>
<td>35/F</td>
<td>Prolactinoma/PHEO</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
<td>Ectopic GHRH secretion from PHEO</td>
</tr>
<tr>
<td>Roth &lt;i&gt;et al.&lt;/i&gt; (1986)</td>
<td>43/M</td>
<td>Acromegaly (nodular somatotroph hyperplasia)/PHEO</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Bertrand &lt;i&gt;et al.&lt;/i&gt; (1987)</td>
<td>26/M</td>
<td>Prolactinoma/PHEO/bilateral medullary thyroid carcinoma (MTC), parathyroid adenoma</td>
<td>_&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Father: metastatic MTC/probably PHEO</td>
<td></td>
</tr>
<tr>
<td>Teh &lt;i&gt;et al.&lt;/i&gt; (1996)</td>
<td>41/M</td>
<td>Acromegaly/PHEO/abdominal PGL</td>
<td>RET: (−)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Baughan &lt;i&gt;et al.&lt;/i&gt; (2001)</td>
<td>43/M</td>
<td>Acromegaly/PHEO/hemangioma/lipoma/parotid adenoma</td>
<td>RET: (−)</td>
<td>Negative</td>
<td>Maybe ectopic GHRH secretion (not measured)</td>
</tr>
<tr>
<td>Dünser &lt;i&gt;et al.&lt;/i&gt; (2002)</td>
<td>56/M</td>
<td>Pituitary adenoma (probably not secreting) /PHEO</td>
<td>Not performed</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Sleilati &lt;i&gt;et al.&lt;/i&gt; (2002)</td>
<td>57/F</td>
<td>Acromegaly/PHEO</td>
<td>RET: negative</td>
<td>Negative</td>
<td>Negative for GHRH ectopic secretion</td>
</tr>
</tbody>
</table>
patients with PGLs and gastrointestinal stromal tumors (GISTs), negative for mutations in PDGFRA or KIT genes (McWhinney et al. 2007). GISTs from these patients showed allelic losses of the SDHB and SDHC chromosomal loci pointing to a tumor-suppressor function of SDH subunits (SDHx) in these neoplasms. This was the first time that a germline mitochondrial oxidation defect was linked to predisposition for development of a sarcoma. More recently, SDHx mutations (or functional variants) were associated with predisposition to a Cowden disease-like phenotype that consisted of breast, endometrial, thyroid, kidney, colorectal cancers, dermatological features such as oral and skin papillomas, and neurological manifestations such as autism and Lhermitte-Duclos disease (Ni et al. 2008), as well as with renal and thyroid cancer (Neumann et al. 2004, Vanharanta et al. 2004).

### Pituitary adenomas and PHEOs/PGLs as part of multiple endocrine neoplasia syndromes

Pituitary adenomas represent one of the components of multiple endocrine neoplasia type 1 (MEN1) due to mutations in MEN1 gene, the other components being primary hyperparathyroidism and pancreatic tumors. Adenomas and adenomatous hyperplasia of the thyroid and adrenal glands may also occur in patients with MEN1 (Thakker 2010).

PHEOs/PGLs, primary hyperparathyroidism, and medullary thyroid cancer are the main tumors that occur in multiple endocrine neoplasia type 2 (MEN2), with particular marfanoid habitus in MEN2B subtype. The genetic causes are gain-of-function mutations in RET proto-oncogene (Wohllk et al. 2010).

There have been some reports in the literature of MEN1 where PHEOs (unilaterally or bilaterally) were identified in patients with proven MEN1 mutations. However, the prevalence of PHEOs in MEN1 appears to be <0.1% (Gatta-Cherifi et al. 2012). We just described a kindred with the coexistence of familial PGLs and an aggressive GH-secreting pituitary adenoma, harboring a SDHD mutation (Xekouki et al. 2012). The pituitary tumor showed loss of heterozygosity (LOH) at the SDHD locus, indicating the possibility that this gene’s loss was causatively linked to the development of the neoplasm (Xekouki et al. 2012). Until this report, coexistence of a pituitary adenoma and PHEOs/PGL was not recognized as a distinct entity. However, since 1952, we have identified 29 cases in the literature of pituitary adenomas copresenting with PHEO and/or extra-adrenal PGLs (Table 1); in most reports, the coexistence of these two tumors was described as a unexpected ‘coincidence’. Unfortunately, no genetic

<table>
<thead>
<tr>
<th>Report</th>
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<th>Case</th>
<th>Genetic screening</th>
<th>Family history for endocrine tumors</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breckenridge et al. (2003)</td>
<td>59/M</td>
<td>Pituitary adenoma (non-secreting)/PHEO</td>
<td>Not performed</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>López-Jiménez et al. (2008)</td>
<td>60/M</td>
<td>Prolactinoma/nonsecreting PGL</td>
<td>SDHC (+)</td>
<td>2/4 children are carriers of the same mutation. Parents' history: negative</td>
<td></td>
</tr>
<tr>
<td>Saito et al. (2010)</td>
<td>40/M</td>
<td>Acromegaly/MTC</td>
<td>RET (+)</td>
<td>Mother PHEO/MTC RET (+)</td>
<td></td>
</tr>
<tr>
<td>Zhang et al. (2011)</td>
<td>45/M</td>
<td>Acromegaly/PGLs</td>
<td>Not performed</td>
<td>Father and sister neck PGLs</td>
<td></td>
</tr>
<tr>
<td>Heinlen et al. (2011)</td>
<td>60/M</td>
<td>PHEO/nonsecreting pituitary adenoma/MTC</td>
<td>RET (+)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Sisson et al. (2011)</td>
<td>29/M</td>
<td>Bilateral PHEOs/ acromegaly/PTC</td>
<td>Not performed</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Xekouki et al. (2012)</td>
<td>37/M</td>
<td>Acromegaly/bilateral PHEOS/PGLs</td>
<td>SDHD (+) LOH of SDHD locus in pituitary adenoma</td>
<td>Sister, paternal uncle: neck PGLs (same mutation)</td>
<td></td>
</tr>
</tbody>
</table>

NA, not available; PHEO, pheochromocytoma; PGL, paraganglioma; PTC, papillary thyroid cancer; MTC, medullary thyroid cancer; PHP, primary hyperparathyroidism; MEN, multiple endocrine neoplasia; M, male; F, female.

²The age at first visit.

²DNA testing was not available at that time.
testing was available until the 1990’s, so we can only hypothesize that some of the early cases presented in Table 1 could represent cases of MEN syndromes or may be due to SDHx mutations. The most recent cases described by Sisson et al. (2011) and Zhang et al. (2011) have a lot of similarities with our case and most probably are cases of familial PGLs.

The only other reported patient with a pituitary tumor and neck PGLs due to an SDHC splice site mutation is the one reported by López-Jiménez et al. (2008). However, the prolactinoma could not be tested for LOH, so we can only speculate that this particular mutation may have contributed to the patient’s pituitary tumor formation.

**Proposed mechanisms**

The above data and the number of cases in Table 1 indicate that the association of certain pituitary tumors and SDHx mutations may be a real one, adding this neoplasm to the ever increasing list of lesions associated with SDH deficiency. How could this be

![Figure 1](image)

**Figure 1** (a) Mechanisms of pseudohypoxia in inherited PHEO and/or PGLs: inactivation of SDH leads to the abnormal stabilization of HIFs in normoxia that escape degradation and translocate to the nucleus, where they dimerize with HIF1β and promote transcription of genes that enhance tumorigenesis, e.g. VEGF, PDGF-β (2-OG, α-ketoglutarate; HIFs, hypoxia-inducible factors; PHD, prolyl hydroxylases; SDH, succinate dehydrogenase; VHL, Von Hippel–Lindau). (b) Oxidation of succinate to fumarate transfers the electrons through a sequence of steps from the flavin moiety in SdhA to a set of three iron–sulfur clusters in SdhB, to the ubiquinone binding site in SdhC and SdhD. When complex II is disrupted due to mutations in SdhB, SdhC, or SdhD, the electron transfer is impaired promoting superoxide generation through the autoxidation of the reduced flavin group by O₂ in the matrix (FM, flavin moiety; Fe-S, iron–sulfur clusters; e⁻, electron transfer; QH, ubiquinone; QH₂, ubiquinol).
molecularly plausible? Several mechanisms have been proposed to explain how the dysfunction of SDHx can lead to the formation of PHEOs/PGLs. The first model is ‘pseudohypoxia’ and accumulation of reactive oxygen species (ROS). In normoxic conditions, a family of oxygen-dependent enzymes known as prolyl hydroxylases (PHD) 1, 2, and 3 (also known as Egln2, Egln1, and Egln3) hydroxylate the three α subunits of hypoxia inducible factor α (HIF1α, HIF2α, and HIF3α). The hydroxylated HIFαs are then targeted by von Hippel–Lindau (VHL) protein, an E3 ubiquitin ligase, polyubiquitinated and degraded in the proteasome. Only hydroxylated HIFαs can be targeted by VHL for degradation. However, if PHDs are inhibited by the accumulated succinate (such as when SDHx are mutated), HIFαs are not hydroxylated, escape degradation, and translocate to the nucleus, where they dimerise with HIF1β and bind to specific promoter elements of target genes including critical angiogenic factors such as vascular endothelial growth factor (VEGF), enzymes involved in glucose metabolism, and cell survival, and possibly a number of others (Raimundo et al. 2011; Fig. 1a). Activation of the HIF pathway and the resulting angiogenic and glycolytic response in SDHx-mutated tumors was first reported by Selak et al. (2005) and has been replicated in many studies. Certainly, the ‘pseudohypoxia’ hypothesis is not new: Otto Warburg in the 1920s described a striking rate of glycolysis and lactate production in tumor cells, in the presence of normal oxygen concentrations (Warburg 1956). Warburg proposed that this phenomenon might be related to a defect in mitochondrial respiration, or some other mechanism that allows the tumor cell to function as hypoxic under normoxic conditions. It took over 80 years for the ‘Warburg effect’ to be confirmed, and today, it is the basis for the use of functional imaging strategies such as the [18F]deoxyglucose-positron emission tomography ([18F-FDG PET) for the diagnosis of PHEO/PGLs (Bayley & Devilee 2010). The generation of ROS due to the SDH/complex II deficiency in the presence of SDHx mutations has also been implicated in tumor formation, although ROS are usually a ‘by-product’ of other elements of the electron transport chain, particularly complex I (NADH-ubiquinone oxidoreductase) and III (ubiquinone-cytochrome c oxidoreductase). ROS might promote tumor formation in SDH-deficient cells by inhibiting PHD activity similar to succinate accumulation (Bardella et al. 2011; Fig. 1b). The accumulation of succinate in SDH-deficient tumors may also inhibit other components of α-ketoglutarate-dependent enzymes besides PHDs. It was recently demonstrated that loss of SDHB subunit in a yeast model led to succinate accumulation, which could cause the inhibition of two different α-ketoglutarate-dependent dioxygenases: the Jlp1, involved in sulfur metabolism, and the histone demethylases Jhd1, which belongs to the JmjC-domain-containing histone demethylase (JHDM) enzymes. It was also demonstrated that JmjD2D, the corresponding human JHDM, was inhibited by succinate accumulation (Smith et al. 2007). Inhibition of the histone demethylases could certainly lead to tumor formation by a variety of epigenetic changes (Bardella et al. 2011). Indeed, increased methylation of histone H3 that can be reversed by overexpression of the JmjD3, indicating that histone demethylases may be involved in the formation of paragangliomas (and related tumors).

**Figure 2** Methylation of histones like trimethylated lysine 27 on histone H3 (H3K27me3) controls transcription by allowing chromosomal regions to alternate between ‘on’ and ‘off’. Jumonji domain-containing 3 (JmjC) domain to catalyze demethylation on lysines. In cells with inactivated  SDHB, increased methylation of histone H3 can be reversed by overexpression of the JjmD3, indicating that histone demethylases may be involved in the formation of paragangliomas (and related tumors).

**Conclusions**

Could all of this be happening in the pituitary as well? The mechanism by which SDHx germline mutations might contribute to pituitary tumor formation is still
In our studies, we showed increased expression of HIF1α in the SDHD-mutant tumor cells compared with normal pituitary and GH-secreting adenoma cells without SDH defects (Xekouki et al. 2012). Clearly, further research is needed to prove SDHx mutation involvement in predisposition to pituitary tumors; however, the clinical cases and the preliminary laboratory data make this enzyme a likely candidate for yet another molecular mechanism through which pituitary tumors may form.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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