Non-pheochromocytoma (PCC)/paraganglioma (PGL) tumors in patients with succinate dehydrogenase-related PCC–PGL syndromes: a clinicopathological and molecular analysis

Thomas G Papathomas¹, Jose Gaal¹, Eleonora P M Corssmit², Lindsey Oudijk³, Esther Korpershoek¹, Ketil Heimdal³, Jean-Pierre Bayley⁴, Hans Morreau⁵, Marieke van Dooren⁶, Konstantinos Papaspyrou⁷, Thomas Schreiner⁸, Torsten Hansen⁹, Per Arne Andreassen¹⁰, David F Restuccia¹, Ingrid van Kessel⁶, Wolf Mann⁷, Francisc H van Nederveen¹², Ozgur Mete¹³,¹⁴, Sylvia L Asa¹³,¹⁴, Ronald R de Krijger¹,¹⁵ and Winand N M Dinjens¹

¹Department of Pathology, Josephine Nefkens Institute, Erasmus MC, University Medical Center, PO Box 2040, 3000 CA Rotterdam, The Netherlands, ²Department of Endocrinology, Leiden University Medical Center, Leiden, The Netherlands, ³Section for Clinical Genetics, Department of Medical Genetics, Oslo University Hospital, Oslo, Norway, ⁴Department of Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands, ⁵Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands, ⁶Department of Clinical Genetics, Erasmus MC, University Medical Center, Rotterdam, The Netherlands, ⁷Department of Otorhinolaryngology, Head and Neck Surgery, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany, ⁸Section for Specialized Endocrinology, Department of Endocrinology, Oslo University Hospital Rikshospitalet, Oslo, Norway, ⁹Institute of Pathology, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany, ¹⁰Department of Pathology, Oslo University Hospital, Oslo, Norway, ¹¹Division of Endocrinology, Department of Internal Medicine, Erasmus MC, University Medical Center, Rotterdam, The Netherlands, ¹²Laboratory for Pathology, PAL Dordrecht, Dordrecht, The Netherlands, ¹³Department of Pathology, University Health Network, Toronto, Ontario, Canada, ¹⁴Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada and ¹⁵Department of Pathology, Reinier de Graaf Hospital, Delft, The Netherlands

Abstract

Objective: Although the succinate dehydrogenase (SDH)-related tumor spectrum has been recently expanded, there are only rare reports of non-pheochromocytoma/paraganglioma tumors in SDHx-mutated patients. Therefore, questions still remain unresolved concerning the aforementioned tumors with regard to their pathogenesis, clinicopathological phenotype, and even causal relatedness to SDHx mutations. Absence of SDHB expression in tumors derived from tissues susceptible to SDH deficiency is not fully elucidated.

Design and methods: Three unrelated SDHD patients, two with pituitary adenoma (PA) and one with papillary thyroid carcinoma (PTC), and three SDHB patients affected by renal cell carcinomas (RCCs) were identified from four European centers. SDHAA/SDHB immunohistochemistry (IHC), SDHx mutation analysis, and loss of heterozygosity analysis of the involved SDHx gene were performed on all tumors. A cohort of 348 tumors of unknown SDHx mutational status, including renal tumors, PTCs, PAs, neuroblastic tumors, seminomas, and adenomatoid tumors, was investigated by SDHB IHC.

Results: Of the six index patients, all RCCs and one PA displayed SDHB immunonegativity in contrast to the other PA and PTC. All immunonegative tumors demonstrated loss of the WT allele, indicating bi-allelic inactivation of the germline mutated gene. Of 348 tumors, one clear cell RCC exhibited partial loss of SDHB expression.
Conclusions: These findings strengthen the etiological association of *SDHx* genes with pituitary neoplasia and provide evidence against a link between PTC and *SDHx* mutations. Somatic deletions seem to constitute the second hit in *SDHB*-related renal neoplasia, while *SDHx* alterations do not appear to be primary drivers in sporadic tumorigenesis from tissues affected by SDH deficiency.

Introduction

Familial paraganglioma (PGL) syndromes, caused by *SDHx* (A, B, C, D, and -AF2) mutations, are rare syndromes inherited as autosomal dominant traits with *SDHD* and *SDHAF2* mutations being associated with a striking parent of origin phenotypic expression (1). The *SDHA/B/C/D* genes encode for the four subunits of succinate dehydrogenase (SDH) or mitochondrial complex II, while the *SDHAF2* gene encodes SDH complex assembly factor 2 (SDHAF2) that ensures flavination of SDHA, which is essential for the functional and structural integrity of the SDH complex (2). Mitochondrial complex II, bound to the inner mitochondrial membrane, is the only enzyme participating both in the tricarboxylic acid/Krebs cycle and oxidative phosphorylation and thereby links deregulation of these cellular functions to tumorigenesis (2).

Although familial PGL syndromes were initially thought to predispose only for pheochromocytoma (PCC) and PGL, other tumor types such as gastrointestinal stromal tumors (GISTs) (2, 3), renal cell carcinomas (RCCs) (4), and pituitary adenomas (PAs) (5, 6) have expanded the *SDHx*-associated tumor spectrum. Several other neoplasms have been reported in *SDHx* mutation carriers including papillary thyroid carcinoma (PTC), medullary thyroid carcinoma, pancreatic neuroendocrine tumor, adrenal cortical adenoma, neuroblastoma (NBL), ganglioneuroma (GN), adenomatoïd tumor of the adrenal gland, melanoma, lung cancer, breast carcinoma, oesophageal cancer, rectal and ovarian carcinomas, uterine adenocarcinoma, uterine leiomyoma, testicular seminoma, bladder cancer, meningioma, oligodendroglioma, cecal polyps, and hematolymphoid malignancies (7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19).

However, whether these tumors coincidentally occurred in these patients or are causally related to the *SDHx* germline mutation is largely unknown. Biallelic inactivation of *SDHx* genes has only been reported in six RCC cases (8, 16, 20, 21), two PAs (5, 6), one NBL (14), and one testicular seminoma (15) but was not identified in a PA (7), PTC (16), or small-cell lung carcinoma (8). Despite the fact that *SDH* inactivation may contribute to these particular phenotypes, the significance of this contribution remains unclear given the relative lack of studies displaying an increased lifetime risk for these tumors arising in the context of an SDH-deficient state (22).

With regard to *SDHB*-related RCCs, two independent groups have described specific morphological features (11, 23), suggesting a genotype–phenotype association similar to what has been described for other hereditary forms of VHL-, MET-, FLCN-, FH-, MITF-, TSC1/2-, and *PTEN*-related renal neoplasia (24). By contrast, previous reports of a diverse histopathological spectrum, encompassing oncocytoma (14, 25), clear cell RCC (26, 27), eosinophilic chromophobe RCC (27), papillary RCC (type II) (28, 29, 30), poorly differentiated tumor with a papillary architecture and sarcomatoid areas (11, 31), and angiomylipoma (32), indicated morphological heterogeneity in *SDHB*-related neoplasia.

In an effort i) to determine whether the occurrence of two PAs was related to *SDHD* mutations, ii) to elucidate PTC as a component of the *SDH*-related tumor spectrum, and iii) to search for potential genotype–phenotype correlations and clarify the nature of the second hit in *SDHB*-associated renal neoplasia, seven tumors from six *SDHB*- or *SDHD*-mutated patients were meticulously investigated. Moreover, we explored loss of the SDH complex by SDHB immunohistochemistry (IHC) in a large series of 348 tumors of unknown *SDHx* mutational status, including renal tumors, PAs, PTCs, neuroblastic tumors, seminomas, and adenomatoid tumors.

Subjects and methods

Tissue samples

For the case series, archival specimens of tumor and normal formalin-fixed paraffin-embedded (FFPE) tissues...
were provided by four hospitals from The Netherlands (Erasmus MC, University Medical Center (EMC) and Leiden University Medical Center (LUMC)), Norway (Oslo University Hospital), and Germany (University Medical Center of the Johannes Gutenberg University Mainz). All available cases (n = 6) were ascertained from the histopathology archives at each center. Clinical and genetic characteristics of these patients are detailed in Table 1. Informed consent was obtained for genetic analysis and access to the clinical data in accordance with institutional guidelines.

The second series included 348 tumors of unknown SDHx mutational status diagnosed at Erasmus MC (130 renal tumors (80 clear cell RCCs, 19 papillary RCCs, 15 chromophobe RCCs, and 16 oncocytomas), 60 PTCs, 47 peripheral neuroblastic tumors (38 primary tumors and nine metastases), and four composite PCC/GN, 50 seminomas, ten adenomatoid tumors, and 41 PAs (27 GH-, eight PRL-, one FSH-, and one ACTH-secreting and/or positive on IHC as well as four non-functional ones)) and at the Department of Pathology, University Health Network (six PAs (acidophil stem cell adenomas)). These were assessed anonymously according to the Proper Secondary Use of Human Tissue code established by the Dutch Federation of Medical Scientific Societies (http://www.federa.org). The Medical Ethical Committee of the Erasmus MC approved the study.

### SDHA/SDHB IHC

All non-PCC/PGL tumors from three SDHB- and three SDHD-mutated patients were analyzed with SDHA and SDHB IHC. Stainings were performed on 4–5 μm sections of FFPE blocks as described previously (9, 33). The following primary antibodies against SDHA and SDHB were used: mouse monoclonal 2E3GC12FB2AE2 (Mitosciences, Abcam; 1:500) and rabbit polyclonal HPA002868 (Sigma–Aldrich Corp.; 1:400) respectively.

All 348 tumors from the second series were initially analyzed with SDHB IHC (and SDHA IHC in the eventuality of SDHB immunonegativity). If the internal control (granular staining in endothelial cells) was negative for SDHB, the tumor was considered SDHB immunonegative and SDHA immunopositive.

#### Table 1: Clinical and molecular characteristics of non-PCC/PGL tumors in SDHB/SDHD-mutated patients

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Germline SDHx mutation</th>
<th>Tumors observed (age at detection)</th>
<th>SDHB IHC</th>
<th>SDHA IHC</th>
<th>Second hitb (LOH)</th>
<th>Status at last follow-up/age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60/M</td>
<td></td>
<td>SDHD c.274G&gt;T (p.Asp92Tyr)</td>
<td>HN PGLs (60 years) PA (60 years) PCC (62 years)</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
<td>AWED/68</td>
</tr>
<tr>
<td>2</td>
<td>56/F</td>
<td></td>
<td>SDHD c.274G&gt;T (p.Asp92Tyr)</td>
<td>HN PGLs (56 years) PA (56 years) CBT (17 years) GJT (17 years) CBT (25 years) PTC (25 years) PCCs (26 years) ea PGL (26 years) ea PGL (25 years) RCC1 (L) (25 years) RCC2 (L) (25 years) RCC (R) (31 years) RCC (23 years)</td>
<td>Pos</td>
<td>Pos</td>
<td>Negc</td>
<td>AWED/68</td>
</tr>
<tr>
<td>3</td>
<td>25/F</td>
<td></td>
<td>SDHD c.14G&gt;A (p.Trp5X)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NA/27</td>
</tr>
<tr>
<td>4d</td>
<td>25/M</td>
<td></td>
<td>SDHB c.3G&gt;A (p.Met1Ile)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AWED/31</td>
</tr>
<tr>
<td>5</td>
<td>23/M</td>
<td></td>
<td>SDHB c.3G&gt;A (p.Met1Ile)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AWED/28</td>
</tr>
<tr>
<td>6</td>
<td>36/M</td>
<td></td>
<td>SDHB Exon 3 del</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AWED/47</td>
</tr>
</tbody>
</table>

AWD, alive with disease; AWED, alive without evidence of disease; CBT, carotid body tumor; ea PGL, extra-adrenal paraganglioma; GJT, glomus jugulare tumor; HN PGL, head and neck paraganglioma; IHC, immunohistochemistry; L, left; NA, not available; PA, pituitary adenoma; PCC, pheochromocytoma; PTC, papillary thyroid carcinoma; R, right; RCC, renal cell carcinoma.

dNone of the evaluated tumors displayed a somatic mutation as a second hit.

1iSequencing chromatograms of PTC DNA displayed i) both the mutated and the WT allele at a ratio 50:50, ii) no somatic mutation as a second hit, and iii) retention for a microsatellite marker telomeric to SDHD with LOH analysis. It should be noted that the patient was homozygous (not informative) for two markers centromeric to SDHD (LOH electropherograms not shown).

2Non-PCC/PGL tumors of this sibling were tested in Norway.
positive, slides were considered suitable. From SDHB immunonegative/SDHA immunopositive tumors, i) the entire SDHB, SDHC, SDHD, and SDHAF2 coding sequences were assessed for mutations and large intragenic deletions at the germline and somatic level by direct sequencing and multiplex ligation-dependent probe amplification (MLPA) assay with a commercially available kit (SALSA MLPA P226-B2; MRC Holland, Amsterdam, The Netherlands) and ii) loss of heterozygosity (LOH) analysis was performed for polymorphic microsatellite markers flanking the SDHB, SDHC, SDHD, and SDHAF2 genes.

**Mutation screening**

DNA isolation from tumors was carried out using standard procedures following manual microdissection (to assess somatic mutations as a potential second hit). All tumor samples were estimated to contain at least 80% neoplastic cells. The full coding sequence, including intron–exon boundaries, of the SDHD and SDHB genes was screened by direct sequencing in forward and reverse orientation (experimental details available on request).

**LOH analysis**

LOH analysis was performed for polymorphic microsatellite markers flanking either the SDHB or the SDHD gene. For this, PCR was performed with fluorescence-labeled primers (Invitrogen; primer sequences are available on request) for 35 cycles with an annealing temperature of 60 °C and amplified products analyzed, along with LIZ 500 size standard (Applied Biosystems), using capillary electrophoresis on an ABI 3130-XL genetic analyzer (Applied Biosystems). Data were analyzed using GeneMarker Software (Soft-Genetics LLC, State College, PA, USA).

**Results**

**Case series**

**Case 1** A 60-year-old male with a history of multiple head and neck PGLs (HN PGLs) and a previously diagnosed germline c.274G>T (p.Asp92Tyr) SDHD gene mutation presented with a PRL-producing macroadenoma causing bitemporal hemianopia and was treated with dopamine agonists, subsequently leading to PA shrinkage (10). Two years later, the patient developed meningitis and liquor-rhoea, for which he was treated with antibiotics and underwent a transsphenoidal partial resection of the macroadenoma along with restoration of the sella turcica.

The same year, a right-sided PCC was laparoscopically resected. Postoperatively, urinary catecholamine excretion normalized. On the preoperative magnetic resonance imaging (MRI), an additional iodine-123-metaiodobenzylguanidine (MIBG)-negative 7 mm nodule was visualized in the left adrenal gland, suspicious of an adenoma, for which a wait and scan strategy has been followed. Endocrine tests revealed no hormone production by this nodule. Owing to increasing prolactin levels postoperatively and residual tumor on the pituitary MRI, cabergoline therapy was reintroduced leading eventually to normal prolactin levels. Family history was positive for HN PGL, but negative for pituitary tumors. The patient is alive 6 years after the resection of the macroadenoma and PCC and is receiving hormone replacement for hypopituitarism with levothyroxine, hydrocortisone, androgel, and mimrin.

**Case 2** A 56-year-old female with a history of type 2 diabetes mellitus, hypertension, and a nontoxic nodular goiter was referred due to a right-sided vocal cord paralysis on the basis of a vagal PGL (10). On further evaluation, bilateral carotid body tumors were revealed. Screening MRI along with endocrine tests demonstrated a GH-producing pituitary macroadenoma, which was partially removed by transphenoidal resection followed by treatment with somatostatin analogs. The diagnosis was confirmed on histopathological grounds (Fig. 1). The patient was subsequently shown to harbor a heterozygous germline mutation c.274G>T (p.Asp92Tyr) in the SDHD gene. Family history was positive for HN PGL (father, two sisters) and gastric GIST (sister), while negative for pituitary tumors. The patient is alive with intact pituitary function 12 years after surgery.

**Case 3** A previously reported 25-year-old female with a history of HN PGLs was operated on for a left carotid body tumor and PTC (T1N0 unicentric tumor in the right lobe; Fig. 2) (34, 35). One year later, she presented with bilateral PCCs and an intra-abdominal extra-adrenal (ea) PGL and was subsequently shown to harbor a heterozygous germline SDHD mutation c.14G>A (p.Trp5X). Although there was suspicion based on imaging studies for metastatic foci affecting the lungs and a peri-pancreatic lymph node, no biopsy was performed in order to confirm and specify the origin of metastases. The patient was lost to follow-up 12 months postoperatively. No other family members were evaluated for SDHD mutations or screened for relevant tumors.
Cases 4 and 5  A 25-year-old male (case no. 4) presented with periods of headache, throbbing chest pain, sweating, dizziness, and palpitations associated with episodic hypertension. On laboratory investigation, the noradrenaline levels in blood and urine appeared almost 20 times higher than the upper normal limit. Computed tomography (CT) scan of the abdomen revealed a para-aortic mass as well as two large solid tumors in the left kidney with relative homogeneous uptake. MRI scanning of the brain, spine, neck, and thorax showed no abnormalities. The patient underwent a left-sided nephrectomy and resection of the retroperitoneal mass and was subsequently diagnosed with two RCCs measuring 9 cm (RCC1) and 2.8 cm (RCC2) in maximum diameter and an intra-abdominal ea PGL. Histopathologically, the RCCs were originally classified as combined oncocytoma/chromophobe RCC and unclassified RCC respectively. Twenty-four-hour urinary excretion of catecholamines and total metanephrines were postoperatively normalized. SDHB sequence analysis revealed a heterozygous germline start codon mutation c.3G>A (p.Met1Ile). An MIBG scan and CT scan performed 6 months postoperatively showed no evidence of recurrent or metastatic disease. However, 6 years postoperatively, a 1.3 cm tumor in the right kidney close to the hilus was treated by open radiofrequency ablation. Identical mutations were identified in both his father and two brothers. One of the latter underwent biochemical and radiological screening for catecholamine-secreting PGLs. Although these investigations were negative for PGLs, the younger sibling (case no. 5) suffered from a 2.5 cm renal tumor requiring a right-sided kidney resection at age 23 years. On histopathological grounds, the renal tumor was originally classified as clear cell RCC (Fuhrman grade 2).

Case 6  A 47-year-old male was originally diagnosed with a left jugulotympanic PGL at the age of 30 years after a period of unilateral hearing loss and tinnitus. The tumor was surgically resected without major complications. Four years later, a carotid body PGL was detected and treated.

Figure 1
(A) H&E staining and SDHB/SDHA IHC in two pituitary adenomas arising in two unrelated patients carrying the SDHD c.274G>T (p.Asp92Tyr) mutation: case no. 1 showed loss of SDHB expression in the neoplastic cells with normal (endothelial) cells serving as positive internal controls, while case no. 2 retained SDHB labeling; on reticulin stain, the latter revealed breakdown of the reticulin fiber network, thereby excluding the possibility of a hyperplastic SDHBpos lesion (image not shown). Both tumors showed strong positive staining for SDHA. (B) Sequencing chromatograms of tumor DNA and healthy germline tissue in case nos 1–2: mutational analysis revealed the germline SDHD c.274G>T (p.Asp92Tyr) mutation in both the pituitary adenomas and the corresponding normal tissues; only case no. 1 displayed relative loss of the WT SDHD allele. (C) Loss of heterozygosity (LOH) electropherograms of case nos 1–2: LOH in the pituitary adenoma (case no. 1) was confirmed using polymorphic microsatellite markers flanking the SDHD locus. The red arrows indicate the allele with relative loss. LOH analysis of the other pituitary adenoma (case no. 2) exhibited retention of heterozygosity.
with radiotherapy. At the age of 36 years, the patient was diagnosed with a renal tumor measuring 13 cm in maximum diameter for which he underwent a left-sided nephrectomy. Histopathologically, the renal tumor was initially diagnosed as eosinophilic chromophobe RCC, while on revision as eosinophilic clear cell RCC (Fuhrman grade 3). Several times hereafter, he suffered from intra-abdominal RCC recurrences and splenic metastasis necessitating surgical resection. Nine years postoperatively, liver metastases were detected on imaging; hence, the patient was treated with radiofrequency ablation and sunitinib. Although being unresponsive to the latter, he is currently alive with disease 11 years postoperatively. On genetic analysis, the patient was subsequently shown to harbor a heterozygous germline SDHB mutation (exon 3 deletion). Family history was negative for HN PGL. Results of the SDHA/SDHB IHC, mutation screening, and LOH analysis of non-PCC/PGL tumors (case nos 1–6) are detailed in Table 1.

### Histopathology of the SDHB-associated RCCs

The RCCs from case nos 4–6 shared similar morphological features, as illustrated in Fig. 3 and Supplementary Figs 1 and 2, see section on supplementary data given at the end of this article. All RCCs displayed characteristic eosinophilic appearance and pale eosinophilic and/or bubbly intracytoplasmic inclusions, as described previously (11, 23). In particular, the neoplastic cells were polygonal with eosinophilic granular cytoplasm and centrally placed nuclei, which were either relatively uniform with inconspicuous nucleoli (RCC1 case nos 4 and 5) or pleomorphic with prominent nucleoli (RCC2 case nos 4 and 6). Tumor cells were arranged mainly in nested and solid growth patterns with occasional tubular formations. In addition, case no. 6 displayed a focal papillary growth pattern (Supplementary Fig. 2). All tumors were well-circumscribed exhibiting areas of cystic change, which contained eosinophilic material (Fig. 3). RCC1 case nos 4 and 6 showed necrotic areas. All RCCs demonstrated SDHB immunonegativity and SDHA immunoreactivity, as highlighted in Fig. 3 and Supplementary Figs 1 and 2.

### SDHx alterations in unselected tumors of unknown SDHx mutational status

Of the 348 genetically uncharacterized tumors, one clear cell RCC was partly immunonegative for SDHB (1/130 renal tumors, 0.8%; Table 2). Histopathologically, the tumor measuring 9.6 cm in maximum diameter comprised low-grade (Fuhrman grade 1) and high-grade areas (up to Fuhrman grade 4) along with sarcomatoid differentiation and extensive areas of necrosis. Although distinctive cytoplasmic inclusions could not be detected, focal vacuolation was present only in high-grade areas. All RCCs demonstrated SDHB immunonegativity and SDHA immunoreactivity, as highlighted in Fig. 3 and Supplementary Figs 1 and 2.
with the latter, LOH analysis revealed LOH both at the SDHAF2 locus (Fig. 4) and for a microsatellite marker centromeric to SDHD, while the patient was homozygous (not informative) for three markers on the telomeric side (data not shown). No germline SDHx mutations or large deletions were detected by direct sequencing or MLPA respectively.

**Discussion**

Familial PGL syndromes are multiple neoplasia syndromes primarily characterized by the development of PCCs/PGLs. Consistent with the wide expression and the functional role of the SDHx genes, it has been suggested that associations with neoplasms other than PCCs/PGLs may exist (36). In this study, we further strengthen the link between pituitary neoplasia and germline SDHx mutations and suggest that the occurrence of PTC in SDHx mutation carriers might rather be coincidental.

In accordance with the classical ‘two-hit hypothesis’ of Alfred Knudson (the combination of an inactivating germline mutation as a first hit and somatic loss of function of the WT allele as a second hit being essential for the tumor development) (37), one of the PAs of an SDHD patient displayed somatic allelic deletion at the SDHD locus resulting in SDHB immunonegativity (Fig. 1). This is consistent with Xekouki et al. (6) who observed SDHD LOH accompanied by decreased SDH enzymatic activity in one GH-secreting PA and preliminary data concerning another relevant case in the SDH-deficient setting (5) (Supplementary Table 1, see section on supplementary data given at the end of this article). By contrast, the other PA demonstrated retention of SDHD heterozygosity, which is in accordance with the positive

**Table 2** Immunohistochemical SDHB losses in 348 neoplasms of unknown SDHx mutational status.

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>SDHB immunonegativity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal tumors</td>
<td>1/130</td>
</tr>
<tr>
<td>Clear cell renal cell carcinoma</td>
<td>1/80</td>
</tr>
<tr>
<td>Papillary renal cell carcinoma</td>
<td>0/19</td>
</tr>
<tr>
<td>Chromophobe renal cell carcinoma</td>
<td>0/15</td>
</tr>
<tr>
<td>Oncocytoma</td>
<td>0/16</td>
</tr>
<tr>
<td>Papillary thyroid carcinoma</td>
<td>0/60</td>
</tr>
<tr>
<td>Pituitary adenoma</td>
<td>0/47</td>
</tr>
<tr>
<td>Neuroblastic tumors</td>
<td>0/47</td>
</tr>
<tr>
<td>Composite PCC/GN</td>
<td>0/4</td>
</tr>
<tr>
<td>Testicular seminoma</td>
<td>0/50</td>
</tr>
<tr>
<td>Adenomatoid tumor</td>
<td>0/10</td>
</tr>
</tbody>
</table>

GN, ganglioneuroma; PCC, pheochromocytoma.
SDHB expression (Fig. 1). These findings indicate that this tumor was coincidental and most likely not caused by involvement of the germline SDHD mutation. However, SDHx gene haploinsufficiency contributing to the tumor formation cannot be ruled out from our analyses. Haploinsufficient effects for the SDHx genes have been suggested in: i) bilateral adrenal medullary hyperplasia associated with a germline SDHB mutation showing retention of heterozygosity (38); ii) PCCs without loss of the WT SDHD allele arising in SDHD-mutated patients (39); and iii) somatic SDHD inactivation being accompanied by consistent reduction of transcript levels in various tumors (40, 41). Albeit, in mouse models, no indications were obtained for an SDHB/SDHD haploinsufficient contribution to tumorigenesis (42, 43) (J Favier 2013, personal communication).

An extensive literature search revealed ten reported cases of PAs arising in SDHD/R/C/A-mutated patients (Supplementary Table 1), with a further 31 PAs co-occurring with PCCs and/or PGLs pointing to a causative association with SDHx, MEN1, or other yet unidentified predisposing genes (44, 45, 46). SDHx-mutated patients appear to have a characteristic clinical phenotype with PRL-secreting macroadenomas, and to a lesser extent GH-secreting macroadenomas, usually accompanied with a personal history of PCC/PGL, implying that SDH deficiency might promote tumor growth with lactotrophs being more susceptible to this particular deficient state.

Although PTC has been previously suggested as a component of familial PGL syndromes and the risk of thyroid tumor development in SDHB mutation carriers has been estimated at 2.5% up to age 70 years (22), a causal role of SDH deficiency in thyroid tumorigenesis has not yet been established. Arguing against such a causal role, our case displayed SDHB immunoreactivity with retention of the WT allele (Fig. 2) being in accordance with a previously reported PTC (16), a rather small number of reported PTC-affected individuals carrying germline SDHx mutations and a lack of family PTC history in four of five cases in total (Supplementary Table 2, see section on supplementary data given at the end of this article).

According to prior studies from three large multinational and/or multicenter cohorts (22, 35, 47), examining 110, 116, and 358 SDHB/SDHD mutation carriers respectively, only six thyroid tumors were identified: two PTCs (35), one thyroid PGL (47, 48), as well as three thyroid tumors of unknown histopathology (22). Given the high PTC incidence rates (incidence of PTC estimated at 7.3/100 000) (49, 50), it is no surprise that thyroid tumors are relatively frequently encountered as incidental findings in patients who are under active surveillance. Taken together, the occurrence of PTCs in the SDH-deficient state may be either coincidental, reflecting the highly prevalent nature of this particular malignancy, or may be less likely attributable to cross talk of SDH- and PTEN-related signaling pathways leading to tissue-specific Cowden syndrome (CS)/CS-like tumorigenesis (51, 52).
With regard to SDHB-related renal neoplasia, all RCC cases (case nos 4–6) confirm previous histopathological and SDHB immunohistochemical observations (11, 23), highlighting the fact that distinct morphological traits along with SDHB immunonegativity could aid in screening for SDHB mutations. In addition, LOH seems to constitute the main mechanism of inactivation of the WT allele in these tumors as observed in SDHx-related tumorigenesis (40, 53). Taking into consideration: i) the rarity of SDHB-related RCC cases (8, 11, 14, 16, 21, 22, 23, 25, 26, 27, 28, 29, 30, 31, 32, 35, 54); ii) the broad distribution of various germline mutations (missense, nonsense, frameshift, and splice site) and large deletions throughout the SDHB gene (Supplementary Fig. 3, see section on supplementary data given at the end of this article); and iii) the fact that mutations within the same codon can generate variable phenotypes (current study, SDHB c.3G > A p.Met11Le; PCC/PGL and/or RCC), previous challenges in establishing genotype–phenotype correlations become conceivable (22).

Interestingly, one clear cell RCC with sarcomatoid dedifferentiation was found to be partly SDHB immunonegative (1/130, 0.8%; Fig. 4) being in accordance with other SDHB mutational or immunohistochemical studies (31, 55, 56). Given that the low-grade tumor component retained SDHB expression, this raises the question as to whether this finding is simply a serendipitous secondary event resulting from genomic instability or has an active role in RCC progression. Mechanisms other than germline SDHx mutations, including mutations in as yet unidentified susceptibility gene(s) affecting SDH complex assembly/function, abnormal functional interactions with mitochondrial proteins, and/or epigenetic alterations, could be responsible for loss of protein expression (57, 58). Another plausible explanation is that HIF1α accumulation due to impaired hydroxylation in hypoxic areas (59) might have resulted in downregulated SDHB expression either in an auto-regulatory loop (60) or through a putative signaling axis involving HIF1α/microRNA-210/iron-sulfur cluster scaffold protein (ISCU) (61).

By analyzing various tumors from 348 patients of unknown SDHx mutational status, all except one were found to be SDHB immunopositive. This is consistent with the notion that mutations of hereditary cancer genes may not be directly involved in tumorigenesis of their sporadic counterparts as reflected in the role of fumarate hydratase (FH) in sporadic vs hereditary leiomyomatosis and RCC (62). Although a high percentage of apparently sporadic PCC/PGL cases is causatively linked to germline mutations (1), this is not the case for other tumor types. In particular, only 5–8% of RCCs, 5% of follicular cell-derived well-differentiated thyroid cancers, <5% of PAs, 1–2% of testicular germ cell tumors, and NBLs respectively are familial when compared with 30–35% of PCC/PGLs (1, 24, 63, 64, 65, 66). With regard to the adenomatoid tumors, there is not only lack of any association with syndromic or genetic conditions but also no example of a typical two-hit mechanisms of TSG inactivation (12).

In conclusion, the current study further strengthens the etiological association of SDHx genes with pituitary neoplasia, while it does not support a causative link between PTC- and SDH-deficient state. Additionally, we elucidate clinicopathological and genetic aspects of SDHB-related renal neoplasia and report a 0.8% frequency of SDHB immunonegativity in renal tumors, reinforcing the notion that hereditary cancer genes may not always be involved in sporadic tumorigenesis.

**Supplementary data**
This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-13-0623.

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

**Funding**
This work was supported by the Seventh Framework Programme (FP7/2007-2013) under grant agreement number 259735 (ENS@T-Cancer).

**Acknowledgements**
The assistance of Frank van der Panne in preparing the images is gratefully acknowledged.

**References**

www.eje-online.org
European Journal of Endocrinology

Non-PCC/PGL tumors in the SDH deficiency

170: Non-PCC/PGL tumors in the SDH deficiency

46 Xekouki P & Stratakis CA. Succinate dehydrogenase (SDHx) mutations in pituitary tumours: could this be a new role for mitochondrial complex II and/or Krebs cycle defects? Endocrine-Related Cancer 2012 19 C33–C40. (doi:10.1530/ERC-12-0118)

www.eje-online.org

