Germline SDHB and SDHD mutations in pheochromocytoma and paraganglioma patients

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Abstract

Pheochromocytoma and paragangliomas (PCC/PGL) are neuroendocrine tumors that arise from chromaffin cells of the adrenal medulla and sympathetic/parasympathetic ganglia, respectively. Of clinical relevance regarding diagnosis is the highly variable presentation of symptoms in PCC/PGL patients. To date, the clear-cut correlations between the genotypes and phenotypes of PCC/PGL have not been entirely established. In this study, we reviewed the medical records of PCC/PGL patients with pertinent clinical, laboratory and genetic information. Next-generation sequencing (NGS) performed on patient samples revealed specific germline mutations in the SDHB (succinate dehydrogenase complex iron-sulfur subunit B) and SDHD (succinate dehydrogenase complex subunit D) genes and these mutations were validated by Sanger sequencing. Of the 119 patients, two were identified with SDHB mutation and one with SDHD mutation. Immunohistochemical (IHC) staining was used to analyze the expression of these mutated genes. The germline mutations identified in the SDH genes were c.343C>T and c.541-542A>G in the SDHB gene and c.334-337delACTG in the SDHD gene. IHC staining of tumors from the c.343C>T and c.541-2A>G carriers showed positive expression of SDHB. Tumors from the c.334-337delACTG carrier showed no expression of SDHD and a weak diffused staining pattern for SDHB. We strongly recommend genetic testing for suspected PCC/PGL patients with a positive family history, early onset of age, erratic hypertension, recurrence or multiple tumor sites and loss of SDHB and/or SDHD expression. Tailored personal management should be conducted once a patient is confirmed as an SDHB and/or SDHD mutation carrier or diagnosed with PCC/PGL.

Key Words
- PCC/PGL
- SDHB
- SDHD
- genotype–phenotype correlation

Introduction

Pheochromocytomas/paragangliomas (PCC/PGLs) are tumors, arose from neural crest-derived chromaffin cells, produce and secrete catecholamines (1, 2, 3). PCCs are tumors of the adrenal medulla and PGLs originate from sympathetic (e.g. organ of Zuckerkandl) or parasympathetic (e.g. carotid body) paraganglia. The incidence of PCC/PGL is up to 8 per 100,000 with its peak onset around the 4th decade of lives (4, 5, 6). Most PCC/PGLs are benign but with high morbidity and mortality due to hypersecretion of catecholamines and metanephrines, which induce hypertension and cardiovascular diseases. It is estimated that ~30% PCC/PGLs are genetically inherited disease, and this percentage may rise as new PCC/PGL-causing mutations are being identified.
Succinate dehydrogenase (SDH) is a protein complex involving in both citric acid cycle and respiratory electron transfer chain reactions (7). The SDH complex comprises two anchoring subunits SDHC (succinate dehydrogenase subunit C) and SDHD and two catalytic subunits SDHA (succinate dehydrogenase complex flavoprotein subunit A) and SDHB. SDHB, an eight-exon gene localized on chromosome 1p36.13 and part of the mitochondrial electron transport complex II, is the most commonly mutated subunit in hereditary forms of PCC/PGLs. SDHD, the four-exon gene positioned on chromosome 11q23, is another member of the SDH complex (8). If any component of the SDH complex is lost, SDHB IHC becomes negative (9). Loss of SDHB by immunohistochemistry (IHC) in PCC/PGL is strongly correlated with SDH subunit gene mutation. So far, SDH deficiency has been observed in PCC/PGLs, gastrointestinal stromal tumors, pancreatic neuroendocrine tumor, renal carcinoma, pituitary adenoma and pulmonary chondroma (9, 10).

The Cancer Genome Atlas (TCGA) molecular taxonomy divides PCC/PGL into four main clusters: pseudohypoxia, Wnt-signaling, kinase-signaling and cortical mixture (11). The pseudohypoxia group can be divided into at least two subgroups. The tricarboxylic acid (TCA) cycle-related subgroup contains germline mutations in succinate dehydrogenase subunits SDHA, SDHB, SDHC, SDHD as well as SDHAF2 (succinate dehydrogenase complex assembly factor 2), FH (fumarate hydratase), MDH2 (malate dehydrogenase 2) and GOT2 (glutamic-oxaloacetic transaminase 2) (12, 13). The VHL/HIF2A-related subgroup shows both somatic and germline mutations (13). Germline mutations in SDH gene are responsible for 6–9% of sporadic PCC/PGLs, 29% of pediatric cases, 38% of malignant tumors and more than 80% of familial aggregations of PGL and PCC (14). Germline mutations in the SDHB gene are associated with hereditary paraganglioma syndrome type 4 (PGL4), while germline mutations of SDHD are present in hereditary paraganglioma syndrome type 1 (PGL1). The penetrance in SDHB and SDHD mutation-positive non-probands by age 60 years was only 21.8 and 43.2%, respectively (15). Furthermore, maternal transmission and genomic imprinting in SDHD could mask the hereditary nature of paraganglioma in rare cases (16). The difficulty of making a precise diagnosis delays appropriate treatment. Thus, hereditary PCC/PGL poses a significant challenge to clinicians.

Although the genetic basis of PCC/PGL is well characterized, the cancer-driving mutations for all PCC/PGL remain unknown. Here, we report the identification of a nonsense mutation and a splice site mutation in the SDHB gene and an SDHD frameshift mutation by genetic screening and immunohistochemistry.

Materials and methods

Patients and genetic testing

The Institutional Review Board of Daping Hospital of the Third Military Medical University approved this study. Written informed consents were obtained from the patients for use of their medical records and related images. A total of 119 PCC/PGL patients were diagnosed and underwent resection of their tumors in our institute between 2011 and 2018. The diagnoses were confirmed by three licensed pathologists based on H&E-stained tumor specimens (Fig. 1) and tumor-specific expression of CgA (chromogranin A), Syn (synaptophysin), CD56 (neural cell adhesion molecule 1), S-100 (S100 calcium-binding protein B), CK (choline kinase beta), MelanA (protein melan-A), HMB45 (human melanoma black), CD34 (CD34 molecule), SMA (survival of motor neuron 1, telomeric) and Ki-67 (proliferation marker protein Ki-67) (data not shown). For the genetic testing study, inclusion criteria consisted of the early age of onset, extra renal lesions, bilateral adrenal gland lesions, positive family history, recurrent or multifocal disease. To conduct Target Capture-Based Deep Sequencing (BGI Health, Shenzhen, Guangdong, China), total DNA isolated from peripheral blood cells of the patients was used to screen for potential mutations in the following genes: SDHAF2, SDHB, SDHC, SDHD, MAX (MYC associated factor X), NF1 (neurofibromin 1), RET (Ret proto-oncogene), VHL (von Hippel–Lindau) and TMEM127 (transmembrane protein 127). Upon identification of the mutations, Sanger sequencing was conducted on DNA of the probands’ family members to identify the specific mutation. Of these patients, 3 with SDHB or SDHD mutations; 21 in 5 families with VHL mutations; 10 in 4 families with RET mutations and 1 with somatic HIF2A, which has been described in our previous study (17, 18, 19).

Immunohistochemistry

Immunohistochemical (IHC) staining was performed as described previously (9, 17, 20, 21). In brief, the tumor specimens were retrieved from the Department of Pathology of Daping hospital and IHC staining was performed on formalin-fixed paraffin-embedded tissues. The sections were deparaffinized and heat antigen
retrieved using a citric acid buffer. The antibodies against SDHB (1:200, Proteintech; catalog number: 10620-1-AP) and SDHD (1:200, Bioss, Beijing, China; catalog number: ab08187596; immunogen range: 81–159 amino acid residue) were used. The HRP-labeled secondary goat anti-rabbit antibody was purchased from EnVisio Detection Systems (Dako). A peroxidase-labeled polymer was conjugated to immunoglobulins (DAKO) with 3,3-diaminobenzidine as a chromogen. The GIST (gastrointestinal stromal tumor) tissues were stained and served as an external positive control (9).

Results

Clinical characteristics

Of the 119 cases, 90 (75.6%) developed unilateral neoplasia, 10 (8.4%) developed bilateral tumors, 10 (8.4%) located in bladder, two in carotid body, two in duodenum and one in cerebellum, ear, mediastinum, pleura, rectum, respectively. Of note, four patients (3.4%) presented with malignant PCC/PGL. Among all the patients, three were identified with SDHx mutations.

Proband 1 was a 14-year-old boy. With blurred vision, intermittent headache and high blood pressure (208/156 mmHg), he was diagnosed as hypertensive retinopathic in November 2011. His VMA level was approximately two times of the normal level (72 µmol/24 h urine; normal level <35 µmol/24 h urine) (Table 1). Although craniocerebral MRI revealed no abnormalities, ultrasonography results suggest he had a paraganglioma. His blood pressure became normal 3 days after tumor resection. Enhanced CT scanning of the thorax, abdomen and pelvic cavities showed no recurrence or metastasis. His blood pressure became normal in all the follow-ups and the last one was in August 2017. Briefly, in proband 1’s family, his father died of a stroke at age of 32 years. His mother was tested with Sanger sequencing, but no mutation was identified. His only uncle has hypertension. Therefore, we speculated that the mutation of the proband was inherited from his father. Other family members showed no evidence of PCC/PGL.

Proband 2 was a 32-year-old male admitted to our hospital with a history of hypertension for 3 years. His blood pressure was 160/100 mmHg at diagnosis. Physical examination found no abnormalities. Laboratory test showed an elevated urine norepinephrine 1890 µg/L (normal range: 10–70 µg/L). MRI scans showed a 3 × 2 cm para-aortic mass in the middle of his abdomen (Fig. 2B and E). Laparoscopic surgery was attempted initially, but ultimately open surgery was required to remove the mass in December 2017. Pathological examination of the mass revealed a parangangioma. His blood pressure became normal ten days after the surgery. In proband 2’s family, the father has hypertension for many years, and the mother did not have any abnormality. MRI or CT scan showed no evidence of PCC/PGL. The other family members refused referrals for further medical examination.

Proband 3 was a 45-year-old female with intermittent dizziness, palpitation and nausea for 1 year. History showed that a PGL located in the region of the right jugular foramen was diagnosed 5 years ago and resected at the West China Hospital (Sichuan Province, China). Hyperthyroidism was diagnosed 2 years ago. Enhanced CT scans revealed a 2.7 × 2.9 cm mass located at the bifurcation of the abdominal aorta (Fig. 2C and F). Laboratory tests revealed no abnormalities. She underwent laparoscopic
tumor resection on December 12, 2014. Pathological examination revealed a paraganglioma. After surgery, her blood pressure returned to normal without medication. CT scans from the neck to pubic regions on her last follow-up in August of 2016 revealed no lesion. Proband 3’s parents and her two children showed no sign of PGLs and refused to be tested with Sanger sequencing.

Identification of mutations in the SDHB and SDHD genes

We identified two heterozygous germline mutations in the SDHB gene: c.343C>T in proband 1 (Fig. 3A) and c.541-2A>G in proband 2 (Fig. 3B). In addition, a frame-shift variant (c.334_337delACTG, p.Asp113Metfs*21) in exon 4 of the SDHD gene was detected in proband 3 (Fig. 3C). In addition, we identified a somatic point mutation in the SRD5A2 gene (c.578A>G) in proband 2. Of note, all the mutations were further confirmed by Sanger sequencing. There was no mutation in the remaining susceptibility gene panel.

Expression of the mutated SDHB and SDHD

Since multiple lines of evidence indicate that IHC staining of SDHB is a robust and reliable surrogate marker for SDH gene mutations (9, 20, 21, 22, 23, 24), we conducted IHC of SDHB on all the tumor tissues. Positive expression of SDHB

Table 1  Characteristics of patients carrying SDH gene mutations.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Patient one</th>
<th>Patient two</th>
<th>Patient three</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender/age</td>
<td>SDHB</td>
<td>SDHB</td>
<td>SDHD</td>
</tr>
<tr>
<td>Site</td>
<td>Male/14</td>
<td>Male/32</td>
<td>Female/45</td>
</tr>
<tr>
<td>Size (cm)</td>
<td>Post-caval</td>
<td>Para-aortic</td>
<td>Bifurcation of abdominal aorta</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>PGL</td>
<td>PGL</td>
<td>Hereditary PGL</td>
</tr>
<tr>
<td>Headache</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Palpitation</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Diaphoresis</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dizziness</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Nausea</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Hypertension (mmHg)</td>
<td>208/156</td>
<td>160/100</td>
<td>154/75</td>
</tr>
<tr>
<td>Nucleotide change</td>
<td>c.343C&gt;T</td>
<td>c.541-2A&gt;G</td>
<td>c.334_337delACTG</td>
</tr>
<tr>
<td>Mutation</td>
<td>P.Arg115Ter</td>
<td>IVS5-2A&gt;G</td>
<td>p.Asp113Metfs*21</td>
</tr>
<tr>
<td>Mutation type</td>
<td>Detrimental mutation</td>
<td>Pathogenic</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>Het</td>
<td>Het</td>
<td>Het</td>
</tr>
<tr>
<td>Aldosterone (erect position/decubitus; ng/mL)</td>
<td>0.254/0.257</td>
<td>NA</td>
<td>0.13/0.14</td>
</tr>
<tr>
<td>Cortisol (8/16/24 h; ng/mL)</td>
<td>NA</td>
<td>NA</td>
<td>215/261/102</td>
</tr>
<tr>
<td>Increased E</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Increased NE</td>
<td>NA</td>
<td>1.89 ng/mL</td>
<td>NA</td>
</tr>
<tr>
<td>VMA</td>
<td>72.0 µmol/24 h</td>
<td>178.36 µmol/24 h</td>
<td>–</td>
</tr>
<tr>
<td>MN</td>
<td>5.1 µmol/24 h</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PTH</td>
<td>4.04 pg/mL</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>17-OH</td>
<td>13.5 µmol/24 h</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>17-KS</td>
<td>30.2 µmol/24 h</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Renin</td>
<td>NA</td>
<td>35.71 µUI/mL; 50.38 µUI/mL (2h after motivated)</td>
<td>1.08 ng/mL</td>
</tr>
<tr>
<td>PRA (erect position/decubitus; ng/mL)</td>
<td>NA</td>
<td>NA</td>
<td>0.75 ng/mL</td>
</tr>
<tr>
<td>Angiotensin II (erect position/decubitus; pg/mL)</td>
<td>NA</td>
<td>NA</td>
<td>1.08/0.75</td>
</tr>
<tr>
<td>ARR (erect position/decubitus)</td>
<td>NA</td>
<td>NA</td>
<td>39/35</td>
</tr>
<tr>
<td>ACTH (8/16/24 h; pg/mL)</td>
<td>NA</td>
<td>NA</td>
<td>12/18</td>
</tr>
<tr>
<td>Other disease</td>
<td>Thyroid nodule</td>
<td>Atrial flutter</td>
<td>HNPGL; renal cyst</td>
</tr>
<tr>
<td>Metastasis</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Follow-up (months)</td>
<td>76</td>
<td>5</td>
<td>41</td>
</tr>
<tr>
<td>Outcome</td>
<td>NED</td>
<td>NED</td>
<td>NED</td>
</tr>
</tbody>
</table>

*+* represented existence of this phenotype, ‘–’ nonexistence; 17-KS, 17-ketosteroid; 17-OH, 17-OH-corticosteroid; ACTH, adrenocorticotropic hormone; ARR, aldosterone renin ratio; E, epinephrine; MN, noradrenaline; NA, not available; NE, norepinephrine; NED, no evidence of disease; PRA, plasma renin activity; PTH, parathyroid hormone; VMA, vanillylmandelic acid.

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was observed using IHC staining in proband 1-derived tumor tissues that harbor the c.343C>T SDHB gene mutation (Fig. 4B). Expression of the c.541_2A>G SDHB mutant allele (proband 2) in PGL cells and surrounding endothelial and inflammatory cells revealed a distinct cytoplasmic granular staining pattern (Fig. 4C). Tissue samples of proband 3 (c.334_337delACTG mutation) were negative for SDHD (Fig. 4H) and showed weak diffused SDHB staining (Fig. 4D).

Discussion

The literature search identified a total of eight reports with 13 c.343C>T SDHB mutation carriers in eight families (1, 4, 25, 26, 27, 28, 29, 30). Of which, Ivana Jochmanova reported the c.343C>T as a function affected mutation; van Hulsteijn et al. reported the c.343C>T as a pathologic mutation, which leads to malignant PGL with bone metastasis. In this study, we found this mutation caused an early onset of disease with a broad profile of clinical manifestations. Although the c.343C>T mutation results in the replacement of an arginine by a termination codon (p.Arg115Ter), IHC staining showed positive SDHB in the tumor from the 14-year-old boy (Fig. 4B). This is consistent with previous studies showing that this nonsense mutation produces a truncated protein of less than half the full-length protein of 280 amino acids (1, 25).

A recent nationwide study of 194 SDHB mutation carriers found the prevalence of c.343C>T mutation is about 1.5% (3/194; 1 with PCC and 2 with PGLs) (30), suggesting that this mutation is likely to be underestimated.

Since Timmers et al. first reported the c.541-2A>G mutation in 2007 (26), five additional reports have documented the same mutation. Four probands showed a positive family history of PCC/PGLs, and three had affected relatives while one presented with metastases (1, 26, 31, 32, 33). Noticeably, an infant carrier was diagnosed with leukoencephalopathy without PCC/PGL (33); a 19-year-old female carrier was diagnosed with hereditary oncocytic renal cancer (31) and an 11-year-old boy was diagnosed with polycythemia and abdominal PGL (32).
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In 2017, our team reported a case with a HIF2A somatic mutation-induced polycythemia and PCC and a case of HIF2A germline-mutation-induced polycythemia in a patient with VHL-associated renal cell carcinoma (17, 34). It is likely for this reason that the pseudohypoxia-related PCC/PGL is fundamentally a metabolic disease. In our study, the expression of SDHB was similar to the external positive control (Fig. 4C). This is most likely due to the fact that the primary antibody targets only the amino acids present on the truncated protein. Therefore, antibodies specifically against the full-length, the N-terminal or C-terminal portions should be used in future studies.

It seems that the c.541-2A>G carriers had a higher penetrance, early onset, more severe and complicated phenotypes, which warrants further investigation.

Though more than 130 unique SDHD gene mutations have been reported in hereditary PGL1 (35), only two studies listed the c.334_337delACTG variant as we report here (4, 36). Amar et al. reported the c.334_337delACTG mutant in a sporadic carrier and a syndromic or familial carrier (36), while Benn et al. reported two carriers of this mutant in a family with PCC, an abdominal PGL and HNPGL (4). Since none of these groups investigated the expression of this mutated gene, we are the first to study the expression of SDHD and SDHB in the c.334-337delATCG carrier. The results showed a weak and diffused SDHB staining pattern and with negative staining for SDHD (Fig. 4D and H). A previous study suggests that a weak-diffused pattern of SDHB may have a stronger correlation with mutations in SDHD rather than SDHB (37). Based on the findings in our study, c.334_337delATCG in the SDHD gene appeared to affect SDHB expression and thus linked to a more grievous phenotype (simultaneous PCC and PGL lesions). In addition, the adjacent mutation (c.337_340delIGACT) has the same amino acid change (p.Asp113Metfs*21) with our case, which may indicate it is a hotspot mutation region.

PCC/PGL present as solitary lesions in 90–95% of cases (38). SDHB mutations mainly predispose to extra-adrenal PGLs and to a lesser extent to adrenal PCCs and HNPGLs, while SDHD mutations are typically associated with multifocal HNPGLs and less frequently with adrenal PCCs and extra-adrenal PGLs (39). PGLs are more frequently located in the head and neck region at the carotid bifurcation (carotid body tumor), along with the vagal nerve, in the jugular foramen and the middle ear space. Less common sites are close to the larynx, thyroid, urinary bladder and the upper mediastinum (14). The three probands identified in this study presented with retroperitoneal or pelvic PGLs. Notably, the c.334_337delACTG carrier in this study showed HNPGL in the right jugular foramen five years before entry into our study. In addition, we previously reported on multiple PGL patients with three tumors around the aorta abdominal and the inferior vena cava (17).

Malignant PCC/PGLs are defined by distant metastases commonly found in the liver, lung, bone, and lymph nodes. The term ‘metastatic PCC/PGL’ has been used to replace ‘malignant PCC/PGL’ in the latest WHO endocrine tumors classification (40). Only a minority of PCC/PGL patients harbor malignant tumors. Reported proportions of malignant PGL vary considerably between most genotype-phenotype studies, ranging from 31 to 71.4% in SDHB-mutation carriers to 0 to 22.7% in SDHD-mutation carriers (41). Although death can occur within a year of diagnosis, metastatic disease can be stable for more than 40 years. Detection of metastatic tumors can occur prior to the detection of primary tumors, but metastatic lesions also could be discovered more than 50 years after the primary diagnosis (42). Metastasis is more commonly associated with primary tumors located in the mediastinum (69%) and the infradiaphragmatic para-aortic area, including the organ of Zuckerkandl.
In our cohort, 3.4% (4/119) presented with malignant tumors at diagnosis. The two SDHB germline mutation carriers did not present with metastases, but a literature review suggests that patients with such mutations may present with metastases in the neck, lung, mediastinum, abdomen and pelvic region. Rare cases of metastatic HNPGLs have been described within SDHD mutation carriers and their estimated prevalence is 0–10% (39). So far, metastatic lesions have not been recorded in c.334_337delACTG carriers.

SDH-deficient renal carcinoma defined by loss of SDHB expression represents a distinct and rare renal neoplasm subtype (9), showing a strong correlation with germline SDH mutations (44). Though it is likely that not all SDHB IHC-negative tumors will carry SDH mutations, IHC remains a phenotypic test as well as an indirect genotypic test. Though our patients presented with no signs of renal cancer, it is important to note the elevated life-long risk of PGL and renal cancer co-occurrence in such patients. At the same time, it is worthwhile to exclude the possibility of other tumors like GIST, pancreatic neuroendocrine tumor, pituitary adenoma and pulmonary chondroma.

In conclusion, we presented three gene-specific germline mutations in SDH genes and their relevant phenotypes. Findings of our study suggest that the incidence of c.343C>T mutations is likely underestimated in PCC/PGL patients. Patients with the SDHB mutation, c.541-2A>G, had severe and complicated phenotypes. The c.334_337delATCG SDHD mutation appears to influence SDHB expression and associates with a more aggressive phenotype. These specific cases add to our knowledge of PCC/PGLs and may help with the genetic counseling of patients. Genotype-tailored treatment options, follow-up and preventive care are warranted.

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.

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