A registry-based study of thyroid paraganglioma: histological and genetic characteristics

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†M Brauckhoff died on September 10, 2014. We dedicate this manuscript to his memory.

Abstract

The precise diagnosis of thyroid neoplasias will guide surgical management. Primary thyroid paraganglioma has been rarely reported. Data on prevalence, immunohistochemistry (IHC), and molecular genetics in a systematic series of such patients are pending. We performed a multinational population-based study on thyroid paraganglioma and analyzed prevalence,

Key Words
- neuroendocrinology
- thyroid
- molecular genetics

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IHC, and molecular genetics. Patients with thyroid paraganglioma were recruited from the European-American-Head-and-Neck-Paraganglioma-Registry. Demographic and clinical data were registered. Histopathology and IHC were re-investigated. All patients with thyroid paraganglioma underwent molecular genetic analyses of the SDHA, SDHB, SDHC, SDHD, SDHAF2, VHL, RET, TMEM127, and MAX genes. Analyses included Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) for detection of large rearrangements. Of 947 registrants, eight candidates were initially identified. After immunohistochemical analyses of these eight subjects, 5 (0.5%) were confirmed to have thyroid paraganglioma. IHC was positive for chromogranin, synaptophysin, and S-100 and negative for calcitonin in all five thyroid paragangliomas, whereas the three excluded candidate tumors stained positive for pan-cytokeratin, a marker excluding endocrine tumors. Germline variants, probably representing mutations, were found in four of the five confirmed thyroid paraganglioma cases, two each in SDHA and SDHB, whereas the excluded cases had no mutations in the tested genes. Thyroid paraganglioma is a finite entity, which must be differentiated from medullary thyroid carcinoma, because medical, surgical, and genetic management for each is different. Notably, approximately 80% of thyroid paragangliomas are associated with germline variants, with implications for additional tumors and a potential risk for the family. As opposed to sporadic tumors, surgical management and extent of resection are different for heritable tumors, each guided by the precise gene involved.

**Introduction**

Neuroendocrine tumors of the thyroid gland are rare, and virtually all are medullary thyroid carcinoma (Ferri et al. 2009). Paragangliomas of the head and neck are neuroendocrine neoplasms developing from parasympathetic paraganglia, which occur at skull base and cervical sites, mostly the carotid body and the tympanic, vagal, and jugular paraganglia. The inferior laryngeal paraganglia can be located within the thyroid capsule explaining why this tumor might occur in the thyroid gland.

Head and neck paragangliomas can occur as sporadic tumors or as a manifestation of familial paraganglioma syndromes type 1–5 (PGL 1 to PGL 5). PGL 1 to PGL 5 are associated with germline mutations of the genes encoding succinate dehydrogenase subunit D, SDHD (PGL 1), succinate dehydrogenase complex assembly factor 2, SDHAF2 (PGL 2), succinate dehydrogenase subunit C, SDHC (PGL 3), succinate dehydrogenase subunit B, SDHB (PGL 4), and succinate dehydrogenase subunit A, SDHA (PGL 5), the SDHx genes (Zantour et al. 2004, Boedeker et al. 2009). Heritable non-SDHx paraganglioma can also occur in tumor syndromes mainly associated with pheochromocytomas such as von Hippel–Lindau disease (associated with mutations of the VHL gene), multiple endocrine neoplasia type 2 (RET gene), and neurofibromatosis type 1 (NF1 gene) (Boedeker et al. 2009, Neumann et al. 2009, Burnichon et al. 2010, Castro-Vega et al. 2014, Yang et al. 2015). In patients carrying germline mutations of the new susceptibility genes FH, PHD1 (EGLN2), and PHD2 (EGLN1), head and neck paragangliomas have not been reported (Castro-Vega et al. 2014, Yang et al. 2015). Somatic mutations of the HIF2A (EPAS1) gene have been reported in abdominal paraganglial tumors but not in head and neck paragangliomas (Toledo et al. 2013). We therefore sought to determine the frequency and characteristics of thyroid paragangliomas in our population-based European-American-Head-and-Neck-Paraganglioma-Registry based in Freiburg, Germany.

**Methods**

**Patients and data collection**

We utilized the population-based European-American-Head-and-Neck-Paraganglioma-Registry (European-American-HNPGL-Registry), which is based in Freiburg/Germany and currently has 947 registrants. Registration policy has been described in detail previously (Neumann et al. 2004, 2009, Schiavi et al. 2005). Of these 947 registrants with head and neck paragangliomas, only eight carried the initial (working) diagnosis of thyroid paraganglioma. For these eight, we re-reviewed registered
data for their putative thyroid paraganglioma including demographic information such as age, sex, symptoms, location and size of the tumor, radiological imaging, and results from fine-needle aspiration cytology and histopathology of the surgical specimens. Tumors were diagnosed as malignant, only if patients showed metastases (Lloyd et al. 2004).

**Histology and immunohistochemistry**

Tumor materials from eight patients who had a working (initial) diagnosis of thyroid paraganglioma were collected and new slides from the paraffin blocks were centrally sectioned and re-analyzed by our internal reference center in the Department of Pathology of the University of Helsinki Central Hospital using conventional H&E histology and immunohistochemistry (IHC). The panel of IHC included staining against chromogranin A, synaptophysin, S-100, pan-cytokeratin (CK-PAN), calcitonin, thyroid transcription factor 1 (TTF1), MIB1, and p53. Slides were processed through deparaffinization in xylene followed by rehydration with graded alcohol series. Endogenous peroxidase was blocked with 0.3% Dako REAL Peroxidase-Blocking Solution. Detection was performed using a Dako REAL EnVision/HRP detection system in Autostainer (Labvision Autostainer 480S). Slides were counterstained with Meyer’s hematoxylin and mounted in mounting medium (Fluka, Eukitt Quick-hardening mounting medium). In each immunohistochemical staining run, a positive and a negative control were used. Each of the different IHC procedures was performed on the same day for all eight cases and blindly scored by J A and H L with 100% concordance.

These results were compared with those primarily obtained in the participating centers. The diagnosis paraganglioma was newly established according to the criteria of the WHO (DeLellis 2004). All patients provided written informed consent.

**Genetic mutation analyses**

All patients were offered molecular testing for germline mutations of the genes, which have been reported to cause head and neck paragangliomas: SDHA, SDHAF2, SDHB, SDHC, SDHD, VHL, RET, MAX, and TMEM127 (Neumann et al. 2004, Schiavi et al. 2005, Burnichon et al. 2010). In these genes we looked for mutations in all exons except for RET, for which exons 8, 10, 11, and 13–16 only were analyzed. Mutation screening was not performed for the new candidate genes FH, EGLN2, EGLN1, and EPAS1.
because such patients seem to have only extremely rare head and neck paragangliomas: in addition, EPAS1 mutations are typically associated with polycythemia, which was not seen in any of our registrants. Genomic DNA was obtained from EDTA-anticoagulated whole blood. We performed bidirectional Sanger sequencing of the coding regions and splice sites of all genes. In order to find a deletion or duplication of the listed genes, we performed multiplex ligation-dependent probe amplification (MLPA) analyses for VHL, SDHB, SDHC, SDHD, SDHAF2, MAX, and SDHA and semi-quantitative multiplex PCR for TMEM127. We did not scan RET for large rearrangements. The sequences of the primers used for these analyses and PCR conditions are available upon request. To elucidate the extent of large deletions, breakpoints were located through quantitative real-time PCR gene-dosage determination, and then characterized by long-range PCR and nucleotide sequencing. The results were subjected to in silico analyses using the programs SIFT, MutationTaster, and PolyPhen-2 in order to predict pathogenicity of the DNA variants. They are considered to be probably pathogenic if at least two of the three software packages predicted them to be damaging/pathogenic.

Figure 1
Immunohistochemistry of case 1: H&E, chromogranin, synaptophysin, S-100, CK-PAN, calcitonin, TTF1, and internal control for CK-PAN. Note that there is positive staining of the tumor for chromogranin and synaptophysin, and of sustentacular cells for S-100. The internal control for CK-PAN shows positive staining of epithelial cells.

Figure 2
Immunohistochemistry of case 2: H&E, chromogranin, synaptophysin, S-100, CK-PAN, calcitonin, and TTF1. Note that there is positive staining of the tumor for chromogranin and synaptophysin, and of sustentacular cells for S-100. Negative staining is shown for CK-PAN, calcitonin, and TTF1.
Results

Prevalence of thyroid paraganglioma

As of May 1, 2014, the European-American-HNPGL-Registry based in Freiburg, Germany, comprises 947 registrants. Among these 947 patients, 939 carried the diagnosis of head and neck paragangliomas (HNPs): 55% had tympanojugular HNPs, 45% had carotid body tumors, 5% had vagal HNPs, and 2% had HNPs with other locations; bilateral HNPs and HNPs at several locations were present. Of the 947 patients, eight carried the putative diagnosis of thyroid paraganglioma. After comprehensive re-analysis (described below), three tumors did not meet the WHO criteria of thyroid paraganglioma (see below); thus, in the European-American-HNPGL-Registry, the prevalence of documented thyroid paraganglioma was estimated to be 0.5% (5/944).

Patients

The eight patients with the initial working diagnosis of thyroid paraganglioma comprised five females and three males with ages at diagnosis of 27–71 (median 40) years.

Figure 3
Immunohistochemistry of case 3: H&E, chromogranin, synaptophysin, S-100, CK-PAN, calcitonin, and TTF1. Note that there is positive staining of the tumor for chromogranin and synaptophysin, and of sustentacular cells for S-100. Negative staining is shown for CK-PAN, calcitonin, and TTF1.

Figure 4
Immunohistochemistry of case 4: H&E, chromogranin, synaptophysin, S-100, CK-PAN, calcitonin, and TTF1. Note that there is positive staining of the tumor for chromogranin and synaptophysin, and of sustentacular cells for S-100. Negative staining is shown for CK-PAN, calcitonin, and TTF1.
All patients had focal enlargement of the thyroid gland. One had dysphagia. Thyroid ultrasonography revealed tumors with a largest diameter of 22–60 (median 44) mm. None of the patients had either additional neuroendocrine tumors or metastases. Clinical findings are given in Table 1. Family history for paraganglial tumors was positive only in one patient (case 1).

**Fine-needle aspiration cytology**

Fine-needle aspiration cytology was performed in five of the eight patients. Cytology revealed findings suggesting a ‘tumor’ in one, an adenoma in one, and a follicular neoplasia in three patients (Table 1).

**Table 2** Results of immunohistochemistry performed in this study

<table>
<thead>
<tr>
<th>Case</th>
<th>Chromogranin A</th>
<th>Synaptophysin</th>
<th>S-100</th>
<th>Calcitonin</th>
<th>TTF1</th>
<th>CytokeratinPAN</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Thyroid paraganglioma</td>
</tr>
<tr>
<td>2</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Thyroid paraganglioma</td>
</tr>
<tr>
<td>3</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Thyroid paraganglioma</td>
</tr>
<tr>
<td>4</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Thyroid paraganglioma</td>
</tr>
<tr>
<td>5</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Thyroid paraganglioma</td>
</tr>
<tr>
<td>A</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>No paraganglioma</td>
</tr>
<tr>
<td>B</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>A few positive cells</td>
<td>Negative</td>
<td>Positive</td>
<td>No paraganglioma</td>
</tr>
<tr>
<td>C</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative (a few positive cells)</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>No paraganglioma</td>
</tr>
</tbody>
</table>

**Treatment**

All patients underwent surgery for removal of the thyroid tumor. This was performed in two patients by hemithyroidectomy and in six patients by total thyroidectomy.

**Histology and IHC**

Histology was studied using hematoxylin and eosin (H&E) stains and IHC was systematically performed and re-investigated at one of the participating centers (Helsinki). Histology together with IHC revealed that five of the eight tumors had the characteristics of paragangliomas (Figs 1, 2, 3, 4, and 5). On the basis of conventional histology,
### Table 3: Results of mutation screening. Four patients had blood DNA variants in the SDHA or SDHB genes

<table>
<thead>
<tr>
<th>Case</th>
<th>Gene RefSeq</th>
<th>Nucleotide variation and predicted effect on protein</th>
<th>Protein domain</th>
<th>Public databases</th>
<th>Pathogenicity clues (in silico analysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>SDHB NM_003000.2</td>
<td>c.530G &gt; A p.Arg177His 4Fe–4S ferredoxin-type</td>
<td></td>
<td>dbSNP</td>
<td>rs150437793 (MAF: T = 0.000/0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>EUR. Am.: T = 0.00%</td>
<td>Highly conserved nucleotide (phyloP: 0.84 (− 5.2; 1.1))</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AFR. Am.: T = 0.02%</td>
<td>Highly conserved amino acid, up to Baker's yeast (considering 14 species)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Small physicochemical difference between Arg and His (Grantham dist.: 29 (0–215))</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Align GVGD: C25 (GV: 0.00–GD: 28.82)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SIFT: deleterious (score: 0, median: 4.07)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MutationTaster: disease causing (P value: 1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Polyphen-2 HumDiv: possibly damaging – score 0.611 (sensitivity: 0.87; specificity: 0.91)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Polyphen-2 HumVar: benign – score 0.380 (sensitivity: 0.85; specificity: 0.79)</td>
</tr>
<tr>
<td>Case 2</td>
<td>SDHB NM_003000.2</td>
<td>c.201-1339_239delInsA-InYb8 p.?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 3</td>
<td>SDHA NM_004168.2</td>
<td>c.394T &gt; C p.Trp132Arg FAD-binding domain</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Endocrine-Related Cancer

E von Dobschuetz et al. A registry-based study of thyroid paraganglioma

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there were cells with diffuse sheet-like patterns of growth or nests (the classic Zellballen). These cells had mostly a clear or basophilic abundant cytoplasm according to H&E staining and some variations in the nuclei. These cells represented chief cells. The mitotic rate was low. Around the sheets or nests of chief cells, there were cells that represented sustentacular cells. Between tumor cells, there were highly vascularized fibrous septa. 

IHC of all these five tumors revealed chief cells positive for the neuroendocrine markers chromogranin A and synaptophysin. Sustentacular cells were positive for S-100. CK-PAN, TTF1, and calcitonin were also investigated and were negative in all primary thyroid paragangliomas. Both the histology and immunohistochemical profile were consistent with the WHO classification of tumors (DeLellis 2004).

The levels of the proliferation marker MIB1 were low, between 3 and 5% in all five thyroid paragangliomas, and p53 was negative, which did not indicate a mutation in this tumor-suppressor gene.

Three cases (cases A, B, and C in Tables 1, 2, and 3) that were initially included in the study as thyroid paragangliomas were excluded because of their immunohistochemical profiles (Figs 6, 7, and 8). All these cases stained positive with CK-PAN, a marker which would exclude paraganglioma. The tumor of patient A is only positive for CK-PAN, which resulted in an immunohistochemical diagnosis of an epithelial neoplasia. The tumor of patient B is a medullary thyroid carcinoma or a metastasis of a neuroendocrine tumor. The tumor of patient C is a metastasis of a neuroendocrine tumor or a calcitonin-negative medullary thyroid carcinoma. A summary of the IHC results is given in Table 2.

**Molecular genetics and family history**

All eight patients were analyzed for nine known paraganglioma predisposition genes as part of this study. The three cases that were excluded as thyroid paragangliomas were found not to carry any germline mutations in the nine genes. Of the five patients with confirmed thyroid paragangliomas, four were found to have germline DNA variants, which probably represent mutations (Table 3).

In the first confirmed thyroid paraganglioma case, the youngest of our series who was 27 years old at the time of diagnosis, the mutation was known from a relative carrying the germline DNA variant SDHB c.664G>A p.Arg177His. This family consisted of ten members and one unrelated wife (1st generation). Of the ten relatives, there were eight mutation carriers and two members in whom the mutation is not present (Fig. 9). Six mutation
carriers had paragangliomas or pheochromocytomas. Among these six patients is the 27-year-old patient with thyroid paraganglioma (III.1), the first symptomatic patient of this family (II.1) who presented with metachronous bilateral pheochromocytoma. One 80-year-old mutation carrier underwent computerized tomography of the neck, chest, and abdomen with normal results. One 14-year-old mutation carrier was not investigated thus far. The two relatives without mutations had not undergone surgery for any tumor. In silico analyses of the variant associated with disease indicated this variant to be deleterious according to the SIFT program, to be disease causing according to the MutationTaster program, and to be possibly damaging according to the PolyPhen-2 program. Together, the results of the in silico analyses and the segregation of mutation and phenotype in the pedigree make the germline variant most probably pathogenic. The phenotypic data are in accordance with the well-known reduced penetrance of tumors in subjects carrying mutations of the SDHB gene.

The second case (case 2) was a 32-year-old male who was found to have a complex rearrangement, consisting of a partial deletion of intron 2 and exon 3 of the SDHB gene and an Alu insertion (Alu family Yb8) (Fig. 10). The latter

Figure 6
Immunohistochemistry of case A: H&E, chromogranin, synaptophysin, S-100, CK-PAN, calcitonin, and TTF1. Note that there is positive staining for CK-PAN and negative staining for chromogranin, synaptophysin, S-100, calcitonin, and TTF1.

Figure 7
Immunohistochemistry of case B: H&E, chromogranin, synaptophysin, S-100, CK-PAN, calcitonin, and TTF1. Note that there is positive staining not only for CK-PAN, but also for chromogranin, synaptophysin, and TTF1. Staining for S-100 is negative, and staining for calcitonin shows only very few positive cells.
mutation has not been reported thus far. There is no additional patient in the family of this case with known paraganglial tumors.

Two more patients, 36 and 37 years old at diagnosis, had germline DNA variants of the SDHA gene. In silico analyses results indicated both variants to be deleterious according to SIFT, disease causing according to Mutation-Taster, and probably damaging according to PolyPhen-2. Thus, these two DNA variants are most probably mutations rather than polymorphisms (Table 3). Family history was negative for paraganglial tumors in these two patients.

The blood DNA variants detected in cases 1, 3, and 4 are shown in Fig. 11. The four patients who probably are carriers of germline mutations were the four youngest of this series.

Postoperative and long-term follow-up

Plasma and 24-h urine catecholamines or metanephrines were measured in all five patients with confirmed thyroid paraganglioma, preoperatively in one and postoperatively in four, and all measurements revealed normal results. In addition, results obtained from whole-body nuclear medicinal investigation using 131I-metaiodobenzylguanidine scintigraphy (in three cases), 111In somatostatin receptor scintigraphy (in two cases), 18F-fluoro-dihydroxyphenylalanine positron emission tomography (PET) (in one case), and 68Ga-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-octreotate (DOTATATE) PET/CT (in one case) were postoperatively normal. After a median 5 years of follow-up of the patients with confirmed thyroid paraganglioma, none had been shown to have developed metastases. It is noteworthy that case 4 is a 37-year-old female patient with SDHA c.1799G>A p.Arg600Gln who was diagnosed with a recurrent/relapsed tumor in the thyroid bed 12 years after thyroidectomy for thyroid paraganglioma. The recurrent tumor was histologically proven to be a paraganglioma again.

Figure 8

Immunohistochemistry of case C: H&E, chromogranin, synaptophysin, S-100, CK-PAN, calcitonin, and TTF1. Note that there is positive staining not only for CK-PAN, but also for chromogranin and synaptophysin. Staining for S-100, calcitonin, and TTF1 are negative.

Figure 9

Pedigree of the family with the mutation SDHB c.530G>A (p.Arg177His) (family of case 1): family members highlighted in blue are mutation carriers, whereas those in white have been demonstrated not to carry the mutation. Patient III.1 is the patient with thyroid paraganglioma. Patient II.1 was diagnosed with bilateral pheochromocytoma at the age of 18 and 35 years. Patients II.2 and II.3 had bilateral carotid body tumors at ages of 45 and 42 respectively. Patient II.4 had thoracic paraganglioma at the age of 40. Patient II.5 had an extra-abdominal paraganglioma at the age of 33. Patient I.1 had CT of the neck, thorax, and abdomen at the age of 80 with normal results. Patient III.3 is a 14-year-old boy awaiting clinical investigations.
Figure 10

Genomic sequence and electropherogram of the SDHB genomic rearrangement of case 2: the genomic sequences of intron 2 and exon 3 (in bold) are shown in the figure, and the intronic repeat markers are shown in italics (AluSZ and AluJb). The deleted region is highlighted in red and the inserted segment (AluYb8 sequence) with its polyA tail in green.

Long-range PCR was used to amplify the deleted region and produced an approximately 1.1 kb mutant product compared with the approximately 2.5 kb WT genomic sequence, predicting a deletion of approximately 1.4 kb; bidirectional sequencing of the mutant PCR product confirmed the partial deletion of intron 2 and exon 3 and showed the insertion of approximately 150 bp corresponding to an AluYb8 sequence (shown in the figure).
Discussion

In the European-American-HNPGL-Registry, the prevalence of confirmed thyroid paragangliomas was 0.5%, of which all but one are very likely to carry germline mutations in SDHx genes. Despite the expertise and experience of our registry and the associated investigators, only five of the eight initially identified putative thyroid paragangliomas turned out to be confirmed thyroid paragangliomas, based on immunohistochemical re-analyses and re-review.

The diagnosis of a paraganglioma in the thyroid gland is a challenge. In our series, preoperative reports of results of fine-needle cytology, which was performed in three cases, were not suspicious in any case for paraganglioma, but showed atypical cells as observed in follicular neoplasias or adenomas (Table 1). Other thyroid neoplasias easily mistaken for thyroid paraganglioma include follicular and papillary carcinoma as well as non-paraganglial neuroendocrine neoplasias, chief of which is medullary thyroid carcinoma (Skiaias et al. 2001, Zantour et al. 2004, Yano et al. 2007, Yu et al. 2013). As illustrated by our study, the most important clinical technique for differentiating thyroid paraganglioma from other histologies is IHC. We reviewed the literature finding 48 available case reports (Basu & Viswanathan 2011, Phitayakorn et al. 2011, Armstrong et al. 2012, Capel 2012, Castelblanco et al. 2012, Costinean et al. 2012, Evankovich et al. 2012, Kieu et al. 2012, Mohyuddin et al. 2013, Yu et al. 2013).

Similar to our cases, true thyroid paragangliomas are characterized by immunohistochemical positive staining for chromogranin A (30 of 30 investigated tumors/case), synaptophysin (21/21), S-100 (27/28), and neuron-specific enolase (18/18). Of equal importance, thyroid paragangliomas stain negative for calcitonin (38/38), carcinoembryonic antigen (CEA, 20/20), thyroglobulin (22/22), TTF1 (15/16) and cytokeratin (17/17). Indeed, these results form the basis for the WHO classification of endocrine tumors for paragangliomas in general: paragangliomas stain positive for antibodies against chromogranin A (the marker for neuroendocrine tumors) in all cases and S-100 (the marker for sustentacular cells of paragangliomas) as well as synaptophysin in nearly all cases, but negative for calcitonin (the marker for medullary thyroid carcinoma), thyroglobulin, and cytokeratin in all cases (DeLellis 2004). Interestingly, two of our paraganglioma patients are males, which is in contrast to the results in the reviewed literature (Basu & Viswanathan 2011, Phitayakorn et al. 2011, Armstrong et al. 2012, Capel 2012, Castelblanco et al. 2012, Costinean et al. 2012,}

Of our five confirmed thyroid paraganglioma patients, four were found very likely to carry germline mutations in SDHx. In contrast, none of the three patients who eventually turned out to have non-paraganglioma thyroid tumors (cases A, B, and C, Table 1, Figs 6, 7, and 8) were found to have germline mutations in the nine known pheochromocytoma-paraganglioma-related genes. Therefore, in addition to IHC, germline genetic analysis could be helpful in differentiating thyroid paraganglioma from other types of thyroid neoplasms that mimic this histology.

Thyroid paraganglioma is a rare entity with approximately 60 cases that are reported, including our series (LaGuette et al. 1997, Yano et al. 2007, Ferri et al. 2009, González Poggioli et al. 2009, Basu & Viswanathan 2011, Phitayakorn et al. 2011, Varsavsky et al. 2011, Armstrong et al. 2012, Capel 2012, Castelblanco et al. 2012, Costinean et al. 2012, Evankovich et al. 2012, Kieu et al. 2012, Mohyuddin et al. 2013, Yu et al. 2013). A major question is whether the rare entity of thyroid paraganglioma is of practical clinical relevance. Our series demonstrates that this diagnosis dictates specific treatment and hence the consequences regarding correct treatment are obvious: one female patient was primarily diagnosed with a papillary thyroid carcinoma. Consequently, the patient was subjected to radioactive iodine radiation. Only the relapse of her tumor 12 years later led to the identification of a paraganglioma, resulting in the retrospective diagnosis of a primary thyroid paraganglioma and not papillary thyroid carcinoma. From an outcome point of view, it is important to know that thyroid paraganglioma may show local invasive growth but confirmed metastases are extremely rare (Massaioli et al. 1979, Mohyuddin et al. 2013).

Finally, it has to be emphasized that we found in most patients with thyroid paraganglioma germline DNA variants, which most probably represent germline mutations. This opens avenues for genetic family screening and preventive medicine. Patients with germline mutations of the SDHB gene, as found in two of our patients, may display adrenal, retroperitoneal, pelvic, or thoracic paragangliomas or metachronous head and neck paragangliomas such as carotid glomus tumors (Haegert et al. 1974, Hughes et al. 1997), which may become malignant and therefore have to undergo lifelong high-risk clinical surveillance (Neumann et al. 2004). Similar risk profiles will be identified once a statistically significant number of patients with mutations of the SDHA gene are available.

In summary, our systematic, population-based series of thyroid paragangliomas indicates that thyroid paraganglioma is an important entity to be differentiated from other thyroid tumors, mainly medullary thyroid carcinoma, despite its prevalence of approximately 0.5% in HNPs, because clinical management is vastly different. The fact that the genetic load of thyroid paraganglioma is seemingly 80% is also significant, with implications for both patient management and family members.

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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