RESEARCH

Head/neck paragangliomas: focus on tumor location, mutational status and plasma methoxytyramine

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Abstract

Head and neck paragangliomas (HNPGLs) are tumors of parasympathetic origin that occur at variable locations and are often secondary to germline mutations in succinate dehydrogenase (SDH) subunit genes. Occasionally, these tumors produce catecholamines. Here, we assessed whether different locations of HNPGLs relate to the presence of SDHx mutations, catecholamine production and other presentations. In this multicenter study, we collected clinical and biochemical data from 244 patients with HNPGLs and 71 patients without HNPGLs. We clarified that jugulotympanic HNPGLs have distinct features. In particular, 88% of jugulotympanic HNPGLs arose in women, among whom only 24% occurred due to SDHx mutations compared to 55% in men. Jugulotympanic HNPGLs were also rarely bilateral, were of a smaller size and were less often metastatic compared to carotid body and vagal HNPGLs. Furthermore, we showed that plasma concentrations of methoxytyramine (MTY) were higher ($P < 0.0001$) in patients with HNPGL than without HNPGL, whereas plasma normetanephrine did not differ. Only 3.7% of patients showed strong increases in plasma normetanephrine. Plasma MTY was positively related to tumor size but did not relate to the presence of SDHx mutations or tumor location. Our findings confirm that increases in plasma MTY represent the main catecholamine-related biochemical feature of patients with HNPGLs. We expect that more sensitive analytical methods will make biochemical testing of HNPGLs more practical in the future and enable

Key Words
- biochemical phenotype
- methoxytyramine
- normetanephrine
- sex-related differences
- succinate dehydrogenase mutations
- tumor size
more than the current 30% of patients to be identified with dopamine-producing HNPGLs. The sex-dependent differences in the development of HNPGLs may have relevance to the diagnosis, management and outcomes of these tumors.

Introduction

Head and neck paragangliomas (HNPGLs) arise from parasympathetic ganglia within the skull base or upper neck. They are highly vascularized but slowly growing tumors that metastatize in 6–13% of cases (Jansen et al. 2000, Lee et al. 2002, Papaspyrou et al. 2012, Mediouni et al. 2014). In contrast to other paragangliomas, symptoms due to HNPGLs are often related to local mass effects rather than catecholamine secretion; some are clinically silent and discovered incidentally during imaging studies (Taieb et al. 2014, Cass et al. 2020). It is well established that germline mutations in succinate dehydrogenase (SDHx) genes predispose to pheochromocytoma and paraganglioma (Amar et al. 2005, Benn et al. 2006, Turkova et al. 2016). Syndromic presentations of HNPGLs are associated with mutations in all SDHx genes and the assembly factor SDHAF2 (Guha et al. 2019). Additionally, epigenetic inactivation of SDHC via promoter methylation has been described in one HNPGL (Bernardo-Castineira et al. 2018). Rare cases of HNPGLs in patients with Von Hippel–Lindau syndrome or multiple endocrine neoplasia type 2 have been reported; however, due to lack of somatic mutation testing, their circumstantial association in patients with these syndromes cannot be excluded (Boedeker et al. 2009).

In 60% of cases, HNPGLs are located at the carotid bifurcation and present as carotid body paragangliomas, often without symptoms (Cass et al. 2020). The most common tumors of the middle ear are tympanic paragangliomas, which may present with symptoms of pulsatile tinnitus, hearing loss or vertigo. In close proximity, HNPGLs from the jugular foramen can arise and cause similar symptoms as tympanic tumors. When lesions extend from the jugular foramen into the tympanic cavity, they are referred to as jugulotympanic paragangliomas. These three groups together account for about 30% of HNPGLs. The rarest group with 5–10% of cases arises along the vagus nerve, commonly at the inferior ganglion (ganglion nodosum). They are mostly asymptomatic, but large tumors can cause dysphagia, hoarseness and vocal cord paralysis, similar to large carotid body HNPGLs.

Biochemical screening to detect HNPGLs is of limited value, since these tumors rarely produce catecholamines (Erickson et al. 2001, van Duinen et al. 2013, Smith et al. 2021). Evidence from these studies suggests that HNPGLs may produce dopamine, which is best assessed from measurements of plasma methoxytyramine (MTY); however, only the study of van Duinen (2013) included measurements of plasma MTY but did not include an appropriate comparison group to assess any significance of observed increases in plasma MTY. So far, no study has examined any relationships of catecholamine biochemical phenotypes of HNPGLs to genotype or specific tumoral locations. Apart from underlying differences in catecholamine biosynthetic machinery, it is unknown why some tumors are biochemically active and others are not.

In the present study, we hypothesized that highly variable biochemical presentations of HNPGLs may depend on both the location and size of the tumors and presence of underlying SDHx mutations. The study also allowed for the evaluation of relationships of different HNPGL locations with other phenotypic features and presentations of patients. Using data from 244 European and USA patients with HNPGLs, we analyzed the presence of SDHx mutations and plasma concentrations of catecholamine O-methylated metabolites MTY, normetanephrine (NMN) and metanephrine (MN) in relation to tumor locations. To establish the clinical relevance of plasma metabolites in patients with HNPGL, we included a cohort of 71 patients in whom HNPGL was excluded and compared increases of urinary and plasma metabolites in a subset of patients with HNPGLs in whom these measurements were available.

Patients and methods

Patients

This observational study included an initial population of 401 patients from centers in Germany (Dresden, Munich and Würzburg), the Netherlands (Nijmegen), Poland (Warsaw), as well as the NIH in Bethesda, USA. Patients were included via two different routes: (1) according to two prospective studies based at Dresden and (2) from a retrospective review of patient records at Nijmegen and the NIH (Fig. 1).
The two studies at Dresden included the multicenter prospective monoamine-producing tumor (PMT) study, which involved the enrolment of patients between September 2010 and October 2019 and a dedicated prospective HNPGL screening study based at the Department of Otorhinolaryngology in Dresden, which involved the enrolment of patients between January 2013 and April 2018. The PMT study included patients with suspicion or risk of pheochromocytoma or paraganglioma based on clinical findings or known germline mutations of tumor susceptibility genes (details at https://pmt-study.pressor.org/). The HNPGL screening study, on the other hand, included patients with imaging findings of a mass in the head and neck region.

Inclusion of patients with HNPGLs from a retrospective review of patient records involved patients with confirmed HNPGLs between January 2000 and June 2020 in Nijmegen and between June 1999 and January 2015 at the NIH. All patients included via studies based on Dresden or the NIH provided signed informed consent under protocols approved by local ethics committees, namely the Ethikkommission an der Technischen Universität Dresden, the Ethikkommission der Ludwig-Maximilians-Universität München, the Ethik-Kommission der Universität Würzburg, the Local Ethics Committee at the National Institute of Cardiology (Warsaw) and the NIH Institutional Review Board. Patients from Nijmegen signed a general consent not specific to this study protocol; for retrospective inclusion of patients, a waiver was obtained from the Medical Ethics Review Committee based on considerations that the study was not subject to the Medical Research Involving Human Subjects Act so that specific patient agreement was not required.

Exclusion criteria were incomplete records, objections against data use and the presence of pheochromocytoma or sympathetic paragangliomas that could contribute to increase in plasma or urinary catecholamine metabolites independently of HNPGLs. Similarly, the presence of metastases simultaneously with HNPGLs and for which the source of metastases could not be determined provided an additional basis for study exclusion. On this basis, 64 of the 253 patients from the retrospective cohort and 22 of the 148 patients from the prospective cohort were excluded from the final study population (Fig. 1).

That final population included 244 patients with HNPGLs and 71 patients in whom HNPGLs and catecholamine-producing tumors had been excluded. The latter patients were included to enable diagnostic comparison of biochemical test results between patients with and without HNPGL. All patients without disease had a clinical need for diagnostic screening for HNPGL, and either had a head and neck tumor for which an HNPGL was excluded based on the pathological review (59 patients from the HNPGL screening study) or had a known hereditary condition predisposing to HNPGLs (12 patients from the PMT study) with HNPGL and catecholamine-producing tumors excluded by patient follow-up as detailed previously (Eisenhofer et al. 2018).

Clinical diagnosis and presentation

Diagnosis of HNPGLs was based on histopathology of resected or biopsied tumors. This followed imaging studies such as sonography, MRI, CT and at some centers functional imaging with radiolabeled somatostatin analogs. Imaging studies were either carried out as part of routine periodic
surveillance due to a genetic predisposition to HNPGLs (e.g. an SDHx mutation) or a clinical presentation that led to suspicion of a head and neck tumor. Such presentations included a noticeable swelling or finding of palpable mass in the skull base, with or without other signs and symptoms of tumors. Such signs and symptoms included difficulties with swallowing, pulsations in the ear and other cranial nerve deficits. In some patients, catecholamine-related signs and symptoms were recorded, such as hypertension, headache, palpitations, excess sweatiness, nausea, dizziness, vomiting and fatigue. For patients who did not undergo surgery or biopsy, a diagnosis of HNPGL was achieved by functional imaging, such as with PET combined with CT and employing (\textsuperscript{68}Ga)Ga-DOTA-TATE as the radiolabeled imaging agent.

HNPGGL locations

Locations of HNPGGLs were based on imaging data. For the purposes of data analyses, specific locations were established according to four groups: (1) carotid body, (2) jugulotympanic, which included tympanic and jugular HNPGGLs, (3) vagal and (4) multifocal, according to the presence of multiple simultaneous HNPGGLs at different locations. Eight patients were presented with solitary HNPGGLs located in parapharyngeal or paratracheal locations, in the tongue, the cervical ganglion, the thyroid or low in the neck. These cases were excluded from the analyses that focused on the four main head and neck locations as outlined above.

Genetic testing

Germline mutations in established susceptibility genes were evaluated by centers of origin or by the Centro Nacional de Investigaciones Oncológicas (CNIO, Madrid, Spain) through a collaborative multicenter study (PMT study) using Sanger sequencing and/or next-generation sequencing, and multiplex ligation-dependent probe amplification or custom array comparative genomic hybridization for deletion detection. Variants detected in SDHx genes met the standards of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (Richards et al. 2015).

Biochemical testing

In all centers except Nijmegen, blood samples were drawn in the morning following an overnight fast and at least 20 min of supine rest. In Nijmegen, supine sampling was performed for 35% of patients. Plasma O-methylated catecholamine metabolites, including MN, NMN and MTY, as well as 24-h urinary-free and total fractionated metanephrines and MTY, were measured by either liquid chromatography with tandem mass spectrometry (LC-MS/MS) or HPLC with electrochemical detection (LC-ECD). For all patients enrolled at Dresden and some patients enrolled at Nijmegen (41%), measurements of plasma and urinary metabolites were performed by LC-MS/MS (Peitzsch et al. 2013a,b, 2015). Comparability of results by LC-ECD and LC-MS/MS assays of plasma metabolites was previously established (Peitzsch et al. 2013b).

Statistics

Statistical analyses were performed with the software package JMP Pro 15. Wilcoxon test and Steel-Dwass all pairs were used for non-parametric comparisons of numeric data in two and multiple groups, respectively. Fisher’s exact test plus analysis of means for proportions was performed for categorical data. Proportions of true- and false-negative results, as well as true- and false-positive results (i.e. sensitivity and specificity), were first established using previously defined cut-offs for metanephrines and MTY optimized for patients with chromaffin cell tumors (Eisenhofer et al. 2019). Thereafter, as described in the ‘Results’ section, cut-offs for plasma MTY were further optimized to establish the best performance for patients with HNPGGLs using receiver operating characteristic (ROC) curve analysis and the derived Youden index. For logistic regression analysis, numeric values were transformed logarithmically before data input.

Results

Study population

As outlined in Fig. 1, after exclusions, the final study population comprised 244 patients with and 71 patients without HNPGGLs. More women than men presented with HNPGGLs, while more male patients were included in the group without HNPGGLs (Table 1). Age was distributed similarly between groups. Germline SDHx mutations were detected in 55% of HNPGGL patients. HNPGGLs associated with SDHA, SDHAF2, SDHB, SDHC and SDHD mutations were present in 9, 12, 17, 9 and 85 patients, respectively. Genetic variants are listed in Table 2.
Phenotypic characteristics of head/neck PGL

Table 1 Characteristics of patients with and without HNPGLs.

<table>
<thead>
<tr>
<th>Gene</th>
<th>HNPGL patients</th>
<th>Patients w/o HNPGL</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDHx</td>
<td>244</td>
<td>71</td>
</tr>
<tr>
<td>SDHAF2</td>
<td>132</td>
<td>10</td>
</tr>
<tr>
<td>VHL</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

*Displayed is median (range); \(^1\)236 of 244 patients were genetically tested; \(^1\)12 patients of 71 were genetically tested as part of the PMT study.

Table 2 SDHx variants in our cohort (n = 132).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant</th>
<th>Cases with HNPGL</th>
<th>Cases with metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDHA</td>
<td>c.91C&gt;T, p.(Arg31*)</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>NM_004168.4</td>
<td>c.553_554insA, p.(Ala186fs)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SDHB</td>
<td>c.778G&gt;A, p.(Gly260Arg)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>SDHAF2</td>
<td>c.1753C&gt;T, p.(Arg58Trp)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>NM_017841.4</td>
<td>c.232G&gt;A, p.(Gly78Arg)</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>SDHD</td>
<td>c.211A&gt;G, p.(Met71Val)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>NM_003000.3</td>
<td>c.268C&gt;T, p.(Arg90*)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>SDHC</td>
<td>c.179G&gt;T, p.(Ser60Ile)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>NM_003001.5</td>
<td>c.214C&gt;T, p.(Arg72Cys)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>SDHD</td>
<td>c.218G&gt;A, p.(Gly73Asp)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>NM_003002.4</td>
<td>c.397C&gt;G, p.(His127Asp)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>SDHC</td>
<td>c.397C&gt;T, p.(Arg133*)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>NM_003001.5</td>
<td>c.410C&gt;A, p.(Glu42fs)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>SDHB</td>
<td>c.428C&gt;T, p.(Glu141Ile)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>SDHC</td>
<td>c.432C&gt;T, p.(Val141Ile)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>SDHD</td>
<td>c.432C&gt;T, p.(Val141Ile)</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Biochemical phenotype of HNPGL patients

Plasma concentrations of free MTY and urinary outputs of total (i.e. deconjugated) NMN were respectively 91% (P < 0.0001) and 50% (P = 0.0325) higher in patients with than without HNPGLs, whereas plasma concentrations of free NMN, as well as urinary outputs of total MTY, free MTY and free NMN, showed no significant differences between patients with and without HNPGL (Fig. 2A, B, C, D, E and F). Examination of plasma concentrations of MTY and NMN according to HNPGL location revealed no significant differences, except for lower plasma concentrations of NMN in carotid body vs multiple and jugulotympanic HNPGLs (Table 3). Similarly, mutations in the SDHx genes were weakly associated with lower plasma NMN (Table 4). Using multivariate linear regression by standard least-squares including age, sex, SDHx mutational status, tumor volume and location, age has been identified as the most significant model parameter (P = 0.0009) and CB location as the second most significant contributor (P = 0.0187). SDHx status, on the other hand, was not significant. In contrast, plasma MTY was only correlated (P = 0.0003) with tumor diameter (Fig. 2G) and not with any other examined parameters. Urine total MTY and MN were somewhat higher in patients with SDHx vs non-SDHx-mutated HNPGLs; however, significance was marginal (Table 4). Similarly, there was a weak difference in urine total MN between patients with jugulotympanic and multiple HNPGLs (Table 3).

Distinct features of jugulotympanic tumors

Initial data analyses were performed with jugular and tympanic HNPGLs as separate groups; however, both entities showed similar features and subsequent statistical analyses combined these groups. Patients with jugulotympanic HNPGLs were diagnosed later in life than patients with carotid body or multiple tumors (Table 3). Jugulotympanic HNPGLs occurred significantly more often in women, while vagal tumors arose at equal proportions in men and women. Multiple HNPGLs were slightly more common in males, and carotid body tumors were somewhat more common in females; however, statistical significance was not reached.
Germline mutations in SDHx genes were found to be in a higher percentage among patients with multiple or carotid body tumors, while less than one-third of jugulotympanic HNPGL patients had SDHx mutations (Table 3). Presentation of multiple HNPGLs was strongly associated with SDHx mutations (Table 3). Metastatic disease occurred less often in patients with jugulotympanic HNPGLs and was not linked to SDHx mutation status in our cohort (Fig. 3B and Tables 3, 4). SDHB mutations were not associated with metastatic disease; among 17 SDHB mutation carriers, only one had metastases. In total, 18 patients suffered from metastatic disease; germline mutations in SDHA, SDHB and SDHC were present in one patient each, while SDHD mutations were found in seven patients (Fig. 3B and Table 2). For eight patients, no germline mutation was identified.

Diagnostic implications of biochemical phenotypes in patients with HNPGL

In our cohort, 14.7 and 11.4% of patients with HNPGL had respective increases in plasma MTY and urine total NMN above previously defined cut-offs for these O-methylated metabolites (Eisenhofer et al. 2019) (Fig. 4A and B). Plasma NMN was not significantly different between patients with and without HNPGLs (Figs 2B and 4C), but 8.8% (19/215) had increases above age-specific upper cut-offs. However, among those 19 patients, eight were sampled in the seated position and had moderate increases of NMN, less than two-fold above age-specific upper cut-offs. Another three patients were sampled supine and had marginal increases in plasma NMN within the range of patients without disease and less than 1.5 times the upper cut-off. Finally, 3.7% (8/215) showed increases of more than 1.5-fold above upper cut-offs with adequate sampling procedures. Similarly, 3.7% (3/79) of patients had strong elevations of urinary total NMN. No patient with HNPGL had increased plasma MN or urine free and total MN above cut-offs (data not shown).

The routinely used upper cut-off for plasma MTY of 107 pmol/L was established from optimized 99.5% percentiles to ensure high diagnostic specificity among
patients with catecholamine-producing chromaffin cell tumors that produced NMN as the main metabolite (Eisenhofer et al. 2019). At this cut-off, 21% (25/119) had positive test results. To establish more appropriate cut-offs for plasma-free MTY in patients with HNPGL, we employed two approaches: (1) use of 97.5 percentiles from our previously published reference population, which provided an upper cut-off of 60 pmol/L and (2) use of the Youden index according to ROC curve analysis (Fig. 5A). For the latter purpose, 51 Nijmegen patients were excluded due to a limit of quantification for MTY of 100 pmol/L for those measurements at that center. Lowering the upper cut-off from 107 to 60 pmol/L yielded a gain in diagnostic sensitivity to 30.3% (30/119) with a drop in specificity to 91.4% (64/70). Use of the Youden index indicated an optimal cut-off of 43 pmol/L with an associated diagnostic sensitivity of 73.9% (88/119) and specificity of 82.9% (58/70).

The area under the ROC curve (AUC) for plasma MTY was 0.775, while that for total urinary NMN was 0.603 (Fig. 5). According to AUCs, other plasma or urinary metabolites had negligible diagnostic utility both alone or in combination with plasma MTY or urinary deconjugated NMN. Combining plasma MTY and NMN resulted in an AUC similar to that of plasma MTY alone (Fig. 5B).

Similarly, combining urine total NMN and MTY resulted in an AUC even lower than that of urinary deconjugated NMN alone (Fig. 5D).

**Discussion**

Although our study did not establish associations of catecholamine-related biochemistry with tumor location or presence of SDHx mutations, we did establish numerous clinical features that differed according to tumor location and SDHx mutation status. Most importantly, we showed for the first time that jugulotympanic HNPGLs without SDHx mutations arise more often in older women and have distinct features compared to other HNPGLs. We also,
Phenotypic characteristics of HNPGLs were assessed, focusing on specific patient and tumor characteristics in respect to SDHx mutational status. Table 4 summarizes these findings:

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MUT</th>
<th>WT</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>132</td>
<td>104</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age at first diagnosis (years)</td>
<td>39 (10–78)</td>
<td>56 (11–89)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age at biochemistry (years)</td>
<td>45 (11–78)</td>
<td>58.5 (16–89)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex (females, %)</td>
<td>57.6 (95%: 49.0–66.7)</td>
<td>85.6 (95%: 77.6–91.1)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Biochemistry

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MUT</th>
<th>WT</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma MTY (pmol/L)</td>
<td>50 (8–14,055)</td>
<td>50 (18–3057)</td>
<td>0.1873</td>
</tr>
<tr>
<td>Plasma NMN (pmol/L)</td>
<td>422 (120–3554)</td>
<td>456 (114–4503)</td>
<td>0.0511</td>
</tr>
<tr>
<td>Plasma MN (pmol/L)</td>
<td>146 (25–378)</td>
<td>151 (39–366)</td>
<td>0.6264</td>
</tr>
<tr>
<td>Urine total MTY (nmol/day)</td>
<td>1601 (184–17,580)</td>
<td>689 (133–25,454)</td>
<td>0.0671</td>
</tr>
<tr>
<td>Urine total NMN (nmol/day)</td>
<td>1210 (242–5536)</td>
<td>1001 (207–8678)</td>
<td>0.5012</td>
</tr>
<tr>
<td>Urine total MN (nmol/day)</td>
<td>532 (87–1380)</td>
<td>351 (120–852)</td>
<td>0.0471</td>
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</table>

Tumor characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MUT</th>
<th>WT</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor volume (cm³)</td>
<td>8.8 (0.03–162.2)</td>
<td>2.8 (0.01–111.9)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Tumor diameter (cm)</td>
<td>2.6 (0.4–6.8)</td>
<td>1.7 (0.3–6.0)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Bilateral (%)</td>
<td>33.3 (95%: 25.9–41.7)</td>
<td>1.9 (95%: 0.5–6.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Metastatic (%)</td>
<td>7.6 (95%: 4.2–13.4)</td>
<td>7.8 (95%: 4.0–14.6)</td>
<td>0.9564</td>
</tr>
</tbody>
</table>

Continuous data are displayed as medians (range), whereas data for proportions are displayed as means (95% CI). Missing data: for age at first diagnosis 1 WT; for age at biochemistry 6 MUT, 8 WT; for plasma MTY 71 MUT, 48 WT; for plasma NMN & MN 13 WT 13 MUT; for urine total MTY 100 MUT, 80 WT; for urine total NMN & MN 82 MUT, 77 WT; for tumor volume/diameter 18 MUT, 25 WT; for metastatic 1 WT.

*Pearson's chi-squared or rank sums test.

MTY, methoxytyramine; MUT, mutant; NMN, normetanephrine.

The findings revealed that the occurrence of HNPGLs is more frequent in women compared to men, with higher tumor diameter and lower metastatic rate in females. Plasma MTY levels were significantly lower in patients with SDHx-mutated tumors, indicating a potential role of SDHx mutations in the pathogenesis of HNPGLs.

For the first time, the nature of catecholamine production in patients with HNPGLs was clarified, with lower plasma MTY in patients with SDHx mutations compared to those without. This finding might be explained by the low percentage of SDHx-related syndromic cases within the group of jugulotympanic HNPGLs, since SDHx-mutated paragangliomas are present in young adults, whereas carotid body tumors are more prevalent in elderly patients. The identification of a distinct mechanism of tumorigenesis for jugulotympanic HNPGLs was observed, which involves a lower growth rate and lower plasma NMN levels.

It is well known that HNPGLs are more common in women than men (Erickson et al. 2001, Smith et al. 2017, Rijken et al. 2019, Singh et al. 2019, Rana et al. 2021) and there was also a reflection in our cohort. We systematically analyzed clinical features of HNPGLs and demonstrated that tumors of jugular and tympanic origin are more common in women (88%) compared to men. On the one hand, this is consistent with carotid body tumors at 62% in women, two-thirds of which were caused by germline SDHx mutations. On the other hand, jugulotympanic tumors in females are rarely associated with germline SDHx mutations, suggesting a distinct mechanism of tumorigenesis for jugulotympanic and tympanic HNPGLs that are not due to SDHx mutations.

It was previously reported that tumors in patients with HNPGLs were assessed differed from those in other locations. Jansen et al. (2019) and also identified a significant relationship between age of presentation and growth rate, with patients below 50 years having higher growth rates. In our cohort, the median age of first presentation for jugulotympanic HNPGLs was 54.5 years, while other HNPGLs presented at younger ages. This finding might be explained by the low percentage of SDHx-related syndromic cases within the group of jugulotympanic HNPGLs, since SDHx-mutated paragangliomas are present at a young age, whereas those in carotid body tumors are more likely to occur in elderly patients. Additionally, the presence of metastatic disease was also reflected in our cohort. We systematically analyzed clinical features of HNPGLs and demonstrated that tumors of jugular and tympanic origin are more common in women (88%) compared to men. On the one hand, this is consistent with carotid body tumors at 62% in women, two-thirds of which were caused by germline SDHx mutations. On the other hand, jugulotympanic tumors in females are rarely associated with germline SDHx mutations, suggesting a distinct mechanism of tumorigenesis for jugulotympanic and tympanic HNPGLs that are not due to SDHx mutations.
In agreement with another study (van Duinen et al. 2013), plasma MTY was the best single parameter for the biochemical evaluation of HNPGLs. Adjusting the upper cut-off from the 99.5 percentile to the 97.5% percentile of a previously published reference population, that is 60 pmol/L (Eisenhofer et al. 2019), increased diagnostic sensitivity with a small loss in specificity. Calculations involving the Youden index suggest even further reduction of the upper cutoff with corresponding increases in sensitivity and decreases in specificity. Combining plasma NMN and MTY has increased sensitivity, which was also observed by van Duinen et al. (2013). However, by including patients without HNPGLs, we showed that the loss in specificity connected to the addition of plasma NMN

Figure 3
Frequency of mutations in SDHx genes in respect to HNPGL location (A) and occurrence of metastatic disease (B). Mosaic plots depict the width of the columns proportional to the number of patients in each group. (A) Mutations in the SDHx genes occur at different frequencies for various locations; Pearson's chi-squared = 0.0001. Especially, SDHD and SDHAF2 mutations are associated with the occurrence of multiple HNPGLs. (B) No statistical difference was found between cases with and without metastatic disease in the number of patients with particular SDHx gene mutations; Pearson's chi-squared = 0.8209. JT, jugulotympanic; CB, carotid body.

Figure 4
Plasma methoxytyramine (MTY, A), urinary total normetanephrine (NMN, B) and plasma NMN (C) relative to previously defined upper cut-offs. Displayed is the respective metabolite concentration in relation to upper cut-offs (UC, dashed line), defined based on age-specific plasma NMN and optimized for pheochromocytomas and non-HNPGLs (Eisenhofer et al. 2019). Significance was calculated by Wilcoxon rank-sum test comparing patients with and without HNPGLs. Sensitivity (Sens) and specificity (Spec) are displayed below the graphs.
abandoned any overall diagnostic benefit. In agreement with current knowledge about HNPGPs, elevations in MN were not detected in any of the patients, while elevations in NMN occurred in only a small number of cases. Hence, we conclude that measurements of plasma MN and NMN have no relevance as diagnostic biomarkers in HNPGPs; only MTY may be useful for diagnosis. Nevertheless, measurements of plasma NMN serve some utility for identifying occasional patients with HNPGPs that produce norepinephrine and in whom preoperative management with alpha-adrenoceptor blockade may be indicated. For patients with HNPGPs that produce only dopamine, management with alpha-adrenoceptor blockade is unlikely to be useful.

Minimal utility of urine measurements of metanephrines for assessing biochemically active HNPGPs is also in agreement with previous studies (Erickson et al. 2001, van Duinen et al. 2010, 2013). However, those previous studies did not include patients without HNPGPs. With the inclusion of such patients in the present study, we established, based on ROC curve analysis, that compared to plasma-free MTY, the diagnostic utility of urine-free or total MTY is negligible. This is also in agreement with emerging concepts about different sources of plasma and urinary MTY, as well as previous findings that measurements of MTY in urine provide an insensitive method for assessing dopamine production by chromaffin cell tumors (Eisenhofer et al. 2018).

Based on measurements of plasma MTY, at least a third of HNPGPs in our cohort were identified as biochemically active, which is similar to previous findings (van Duinen et al. 2013, Smith et al. 2021). However, most current methods of analysis do not reliably measure MTY at the concentration range necessary to establish the production of the metabolite by small tumors typical of HNPGPs (Peitzsch et al. 2021). An exception might be the recently described LC-MS/MS method described by van Faassen et al. by which the use of in situ derivatization enables highly sensitive measurements of plasma MTY and other metabolites in as little as 50 µL of plasma (van Faassen et al. 2020). With such methods it should become possible to measure the production of MTY more precisely in small HNPGPs and thereby establish the true prevalence of biochemically active HNPGPs; as outlined here, this may reach 74%. Such a prevalence is supported by immunohistological examinations of HNPGP tissue, showing that the expression of the dopamine-producing enzyme, L-aromatic amino acid decarboxylase, is present in all samples (Osinga et al. 2015).

A limitation of the present study is that the analysis was carried out with a combination of prospectively and retrospectively included patients, among whom the number of prospective patients amounted to only 55 individuals with HNPGP. Furthermore, measurements of plasma and urine-free and total deconjugated metanephrines were not available for all patients. Another limitation concerns pre-analytical conditions in one of the centers. From 119 patients with measurements of plasma MTY, 48% were not fasted prior to blood withdrawal. Additionally, 34% of patients were not sampled in the supine position. We previously showed that the lack of fasting and supine sampling increases plasma NMN and MTY by up to two-fold in patients without catecholamine-producing tumors (Darr et al. 2014). Nevertheless, the comprehensive collection of other data together with the overall large number of patients with these rare tumors and the inclusion of an appropriate group of patients without disease represent strengths that set our study apart from previous work on catecholamine-related biochemistry of HNPGPs.

In conclusion, we clarify that tumor location and SDHx status do not determine biochemical activity of HNPGPs; rather tumor size appears to be a factor.
About one-third of HNPGLs can be diagnosed biochemically using plasma MTY; however, due to limitations of analytical sensitivity, this proportion is likely underestimated. With the availability of more sensitive analytical methods, biochemical screening for HNPGLs may become more relevant and the true prevalence of dopamine-producing HNPGLs will be established. Additionally, we showed that the female predominance for HNPGLs is largely caused by a higher number of jugulotympanic HNPGLs in women and that these tumors are mostly unrelated to SDHx syndromes, rarely bilateral, typically smaller than other HNPGLs and less often result in metastases.

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 study was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) project number: 314061271 – TRR 205, the AES PI17/01796, co-financed by Fondo Europeo de Desarrollo Regional (FEDER), the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 259735, the Paradifference foundation (FEDER), the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 295735, the Paradifference foundation and the Intramural Research Program of the NIH, NICHD.

References
Oisinga TE, Korpershoek E, Van Den Berg RR, Kersten MN, Dullaart RP, Kema IP, Van Der Laan BE, Van Der Horst-Schippers AN & Links TP 2015 Catecholamine-synthesizing enzymes are expressed in
parasympathetic head and neck paraganglioma tissue.


Smith JD, Ellsperman SE, Basura GJ & Else T 2021 Re-evaluating the prevalence and factors characteristic of catecholamine secreting head and neck paragangliomas. Endocrinology, Diabetes and Metabolism 41 00256. (https://doi.org/10.1002/edm2.256)


van Faassen M, Bischoff R, Rijkenkamp K, De Jong WHA, Van der Ley CP & Kema IP 2020 In matrix derivatization combined with LC-MS/MS results in ultrasensitive quantification of plasma free metanephrines and catecholamines. Analytical Chemistry 92 9072–9078. (https://doi.org/10.1021/acs.analchem.0c01263)

Received in final form 27 January 2022
Accepted 16 February 2022
Accepted Manuscript published online 16 February 2022