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ONCOLOGY: RESEARCH ARTICLE

Pseudohypoxic pheochromocytomas and paragangliomas dominate in children

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Abstract

Objective: Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumors that are associated with cancer predisposition syndromes in up to 80% of affected children. PPGLs can be divided into molecularly defined groups with comparable pathogenesis and biology: (1) pseudohypoxic, (2) kinase signaling, and (3) Wnt-altered.

Methods: We report the data of children and adolescents diagnosed with PPGL who have been registered with the German GPOH-MET registry since 1997.

Results: By December 2019, a total of 88 patients with PPGL were reported. Pheochromocytoma occurred in 56%, paraganglioma in 35%, and synchronous PPGLs in 9.1%. A total of 16% of patients presented with lymph node (5.7%) and distant metastases (10%). Median follow-up was 4.2 years (range 0–17.1). Overall and disease-free survival (DFS) were 98.6% and 54.0%, respectively. Local relapses, metastases, and subsequent PPGLs occurred in 11%, 4.5%, and 15% of patients. Germline mutations were detected in 83% of patients (51% in *VHL*, 21% in *SDHB*, 7.8% in *SDHD*, and one patient each in *RET* and *NF1*). One patient was diagnosed with Pacak–Zhuang syndrome. A total of 96% of patients presented with PPGL of the pseudohypoxic subgroup (34% TCA cycle-related, 66% VHL/EPAS1-related). In multivariate analyses, extent of tumor resection was a significant prognostic factor for DFS.

Conclusions: Most pediatric PPGLs belong to the pseudohypoxia subgroup, which is associated with a high risk of subsequent PPGL events and metastatic disease. Comprehensive molecular profiling of children and adolescents with newly diagnosed PPGLs will open new avenues for personalized diagnosis, treatment, and surveillance.

KEYWORDS
children, germline mutations, paraganglioma, pheochromocytoma, pseudohypoxic

Abbreviations: DFS, disease-free survival; GPOH-MET, German Pediatric Oncology Hematology-Malignant Endocrine Tumor; NF1, neurofibromatosis type 1; OS, overall survival; PCC, pheochromocytoma; PGL, paraganglioma; PPGL, pheochromocytoma and paraganglioma; SDH, succinate dehydrogenase; TCGA, The Cancer Genome Atlas; VHL, Von Hippel Lindau disease.

1 | INTRODUCTION

Pheochromocytomas (PCCs) and paragangliomas (PGLs) (in combination referred to as PPGLs) are rare neuroendocrine tumors that originate from neural crest-derived chromaffin cells of the adrenal medulla and extra-adrenal ganglia. About 10–20% of PPGLs are diagnosed in children.[1–4]

PPGLs have the highest degree of heritability and genetic heterogeneity of any tumor type. Recent studies reported the proportion of children with hereditary disease to be as high as 80%.[1,3,5] Most hereditary PPGLs in children occur as part of the cancer predisposition syndrome Von Hippel Lindau disease (VHL), but also as part of multiple endocrine neoplasia type 2 (MEN2; *RET*), and neurofibromatosis type 1 (NF1).[1,5] Further pathogenic variants include the succinate dehydrogenase (SDHx) genes, SDH assembly factor 2 (*SDHAF2*),[6] *EPAS1*,[7,8] *TMEM127*,[9] and MAX.[10] At least 12 genetic syndromes predisposing to PPGL are currently known. They are associated with various profiles of PPGL localization and paraganglial cell differentiation. Apart from early onset of disease, bilateral, multifocal, and extra-adrenal tumors point toward hereditary disease.[11]

Many insights gleaned over the past few decades into PPGL have enabled improved understanding of the different clinical characteristics of the various PPGL syndromes; this in turn holds considerable promise for the concept of targeted therapy and improved care.

In 2005, Dahia et al. showed a functional link between PPGLs with *VHL* mutations and those with *SDHB* and *SDHD* mutations mediated by the transcription factor hypoxia-inducible factor 1 subunit α (HIF1α).[12] They proposed two major clusters in PCC: cluster 1 (pseudohypoxia) including *VHL*, *SDHD*, and *SDHB* tumors; cluster 2 (kinase signaling) including *RET* and *NF1* tumors. In 2010, Lopez-Jimenez et al. demonstrated within the pseudohypoxia cluster two subclusters related to *SDHx* versus *VHL*.[13] In 2017, The Cancer Genome Atlas (TCGA) project provided an additional Wnt signaling cluster (cluster 3).[14] Subsequently, Crona et al. proposed an enhanced manual for diagnosis and treatment of adult and pediatric patients with PPGL, based on the cluster approach, the TCGA molecular classification and the WHO and AJCC systems (for details see Figure S1).[15]

We herein report the data on children and adolescents with PPGL reported to the German Pediatric Oncology Hematology-Malignant Endocrine Tumor (GPOH-MET) trial center since 1997. We explore the cluster approach and discuss potential avenues of precision medicine decision making in targeted diagnostics, classification, treatment, and surveillance of children with PPGL.

2 | PATIENTS AND METHODS

We included children and adolescents aged 0–18 years with histologically confirmed PPGLs who had been reported to the GPOH-MET study center between 1997 and December 2019. Details on the GPOH-MET 97 study protocol and the GPOH-MET registry and data collection are provided elsewhere.[16] The GPOH-MET 97 protocol and the recommendations of the GPOH-MET registry document were conducted according to the Declaration of Helsinki and approved by the ethical committees of the University of Luebeck (97-125) and Otto von Guericke University Magdeburg (174/12), Germany. Written informed consent was obtained from either the patients themselves, if they were aged 15 years or older, and the child's parents or legal guardians.

Tumors were classified according to tumor number (solitary or multiple in PGLs, unilateral, or bilateral in PCCs) and location (adrenal, extra-adrenal abdominal, thoracic, and head and neck). Synchronous tumors were defined by the simultaneous presence of PCC and PGL. Criteria for malignancy included the presence of PPGLs in nonchromaffin organs including lymph nodes or distant tissues.[15,17] Relapse was classified as local recurrence after resection or metastatic relapse in distant nonchromaffin organs. If chromaffin cell tumors were detected at sites different from the first PPGLs during follow up, they were classified as multifocal PPGLs. Second tumors were classified as tumors other than PPGL.

The GPOH-MET protocol and registry did not provide central biochemistry measurement. Thus, various methods and approaches were used due to the multicenter design of the study and the period of time. It was impossible to make any accurate determination of the catecholamine phenotype. Thus, for the purpose of this analysis, we determined excess production of catecholamines (including metanephrine, normetanephrine, and/or 3-methoxytyramine) or chromogranin A, only if at least one product was at least twice above the upper limit.

The GPOH-MET 97 protocol recommended performing functional imaging with ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphy. Later, ^{68}Ga-DOTATATE and ^{18}F-DOPA PET/CT were occasionally used. For this analysis, we restricted the evaluation of functional imaging to the detection of the primary tumor by at least one functional imaging technique.

Recent comprehensive genetic and molecular analyses allow identification of germline and somatic mutations, but were not available earlier.[14,18] In addition, the study and registry protocol did not define standards for somatic and germline genetic testing. Thus, for this analysis, sporadic cases were defined as patients with negative germline testing for pathogenic variants in *VHL*, *SDHx*, and *RET*, and without suspicious clinical features. Patients without germline testing of *VHL*, *SDHx*, and *RET*, and/or syndromic features were assigned to "unknown" mutational context.

Due to missing data in some patients, the exact number of cases used for the frequency analyses (including only those patients for whom data were available) varied and is given in brackets wherever appropriate.

Overall survival (OS) and disease-free survival (DFS) were determined according to Kaplan–Meier estimates. DFS was defined as the time from the diagnosis until first signs or symptoms of persistent disease, progression/metastatic disease, relapse, and multifocal PPGL, whichever came first. Survivors were censored at the last date of last known follow-up. Groups were compared using the logrank test. The

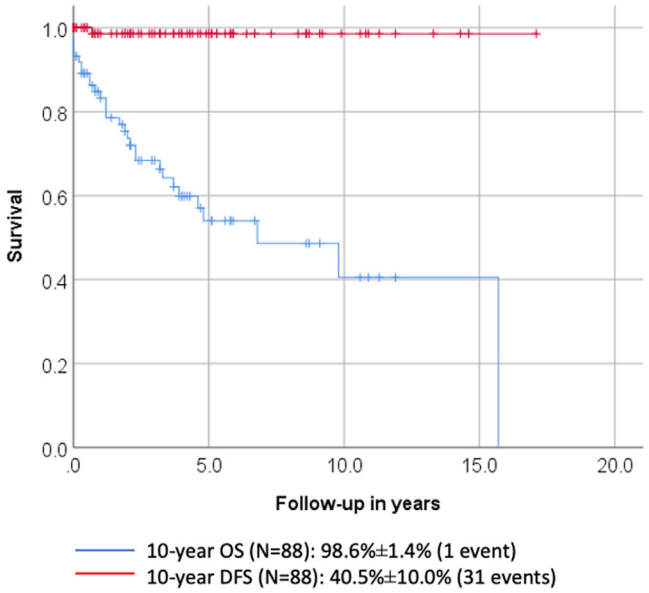

FIGURE 1 Probabilities of overall and disease-free survival calculated using the Kaplan–Meier method. Patients were censored (+) at last follow-up. One patient developed a subsequent pheochromocytomas and paragangliomas (PPGL) 15.7 years after initial diagnosis

Cox proportional hazards model was used to evaluate the impact of prognostic factors on DFS in a univariate and multivariate manner, including the following factors potentially associated with outcome: age (<10 vs. ≥10 years), PGL versus PCC, hereditary versus sporadic, mutations of *SDHB* versus other mutations, metastatic disease (yes/no), metastases at diagnosis (yes/no), and extent of resection (R_0 vs. others). *p*-Values were regarded as significant for ≤.05. Statistical analyses were performed by SPSS version 26.

A PPGL risk score adapted from Crona et al. was calculated considering PCCs with a diameter of >5 cm and 3 cm in *SDHB* carriers, respectively, high risk. Patients were allocated according to their (1) genetic context, and/or (2) disease characteristics. The highest ranking genetic context was counted with 4 points, whereas the lowest ranking was counted with 1 point. The same was applied to disease characteristics. The PPGL risk score was calculated by adding the points for genetic context and disease characteristics summing up to a maximum of 8 points and a minimum of 1 point.

3 | RESULTS

The GPOH-MET database included 88 PPGL patients (59 [67%] boys and 29 [33%] girls). The OS and DFS estimates at 5 years were 98.6% and 54.0%, respectively (Figure 1).

At the time of data analyses, one (1.1%) patient had died due to PPGL (due to malignant ascites and pleural effusion) and eight (9.1%) patients suffered from progression or metastatic disease. The median follow-up was 4.2 years (range 0–17.1). Details on demographic and clinical information are summarized in Table 1.

At initial presentation, 49 patients (56%) presented with PCC and 31 (35%) with PGLs. In eight (9%) patients, synchronous tumors were identified (Figure 2A). In 11 cases (13%), metastases were detected at the time of diagnoses ($n = 3$ lymph node metastases, $n = 6$ distant metastases, $n = 2$ lymph node and distant metastases).

Most (93%; 81/83) patients were symptomatic and presented with hypertension, diaphoresis, or headaches. In six patients, a hypertensive emergency was diagnosed including hypertensive encephalopathy, seizure, and bilateral scotoma. In 39% (23/59) of patients, echocardiography revealed left ventricular load and cardiac hypertrophy. Excess production of catecholamines or chromogranin A was demonstrated in 95% (61/64) of patients. PPGL diagnosis was further clinically supported by functional imaging in 91% (59/65) of patients.

Tumor resection was performed in all patients. Eighty-two percent (68/83) of patients received pharmaceutical blockade preoperatively; this included alpha-adrenoceptor blockade as a monotherapy in 29% (20/68) of patients or combined with beta-adrenoceptor blockade in 50% (34/68) of patients. Intraoperative blood pressure fluctuation was reported in 40% (30/75), of those 24 patients had received preoperative blockade. No intraoperative death was reported.

Total 5.7% (5/88) of patients underwent radiotherapy and 10.2% (9/79) received adjuvant chemotherapy (due to various reasons including incomplete resection, inoperability, initial misdiagnosis of neuroblastoma, metastases) (Table 2).

Two patients suffered from distant relapse 0.3 and 4.8 years after achieving complete remission. Subsequent multifocal PPGLs were reported in 13 patients after a mean interval of 3.9 years (range 0.2–15.7). Extra-paraganglial tumors were reported in four patients. Of those, two patients (*NF1* $n = 1$, *SDHB* $n = 1$) developed acute myeloid leukemia (AML) as second malignancy after 1.2 and 1.6 years, respectively; one of them died subsequently. Both patients received chemotherapy for PPGL, one additionally underwent MiBG and radiotherapy. Two children were diagnosed with simultaneous papillary (*SDHB* related)[19] and medullary thyroid carcinoma (*RET* codon 634), respectively.

3.1 | Genetic testing

Pathogenic variation screening was sufficient for further analyses in 72% (63/88) of patients. Of these, 51% ($n = 32$) had germline mutations in *VHL*, 21% ($n = 13$) in *SDHB*, 7.9% ($n = 5$) in *SDHD*, and one patient each in *RET* and *NF1*. In addition, syndromic features including polycythemia and somatostatinoma were present in one patient with a somatic *EPAS1* mutation, highly suggestive of Pacak–Zhuang syndrome due to mosaicism. In 61% (31/51) of patients with germline mutations, there was an established family history. Genetic testing for mutations in *VHL*, *SDHx*, and *RET* was negative in 17% (11/88) of patients (without syndromic features and a negative family history). In 27% (24/88) of patients, genetic testing did not comprise necessary susceptibility genes or was not performed at all (subsequently termed "unknown").

TABLE 1 Demographic and clinical characteristics of 88 patients with pheochromocytomas and paragangliomas (PPGL) at initial presentation

Syndrome	Total	Unknown	Sporadic	Hereditary Von Hippel Lindau	Pacak–Zhuang	PGL1	PGL4	MEN2	NF1
Gene				***VHL***	***EPAS1***	***SDHD***	***SDHB***	***RET***	***NF1***
Number of patients	*n* = 88	*n* = 24	*n* = 11 17%	*n* = 32 50%	*n* = 1 1.6%	*n* = 5 7.8%	*n* = 13 20%	*n* = 1 1.6%	*n* = 1 1.6%
Gender, *n* (%) Male Female	59 (67) 29 (33)	14 (58) 10 (42)	9 (82) 2 (18)	21 (66) 11 (34)	1 (100) 0	2 (40.0) 3 (60.0)	12 (92) 1 (78)	0 1 (100)	0 1 (100)
Age at diagnosis, median years (range)	12.6 (4.0–18.7)	12.5(4.0–16.6)	13.3(7.8–17.6)	11.6(4.3–18.7)	12.6	14.2 (11.9–16.6)	13.4(8.6–17.6)	14.5	17.2
Family history positive, *n* (%)	32 (39)	1 (5.0)	0	21 (66)	0	4 (80.0)	4 (36.4)	1 (100)	1 (100)
Pheochromocytoma, *n* (%) Unilateral, *n* (%) Bilateral	49 (56.1) 47 8 (9.1)	16 (67.5) 63 1 (4.2)	5 (45.5) 5 (45.5) 0	23 (72.6) 50 7 (20.0)	0	0	3 (20.0) 3 (20.0) 0	1 (100) 1 (100) 0	1 (100) 1 (100) 0
Paraganglioma, *n* (%) Solitary Head/neck (HN) Thoracic Extra-adr. abdominal Multiple HN and abdominal Abdominal	31 (35.6) 30 4 (4.5) 2 (2.3) 20 (23) 5 (5.7) 2 (2.3) 3 (3.4)	8 (33.3) 8 (33.3) (4.2) 0 7 (29.2) 0 0 0	6 (54.5) (45.5) (9.1) 0 4 (36.4) 1 (9.1) 0 1 (9.1)	3 (9.4) 2 (6.3) 1 (3.1) 0 1 (3.1) 1 (3.1) 0 1 (3.1)	1 (100) 0 0 0 0 1 (100) 0 1 (100)	4 (80.0) 2 (40.0) 0 1 (20.0) 1 (20.0) 2 (40.0) 2 (40.0) 0	9 (69.2) 9 (69.2) (7.7) 1 (7.7) 7 (53.8) 0 0 0	0	0
PCC + PGL, *n* (%) Adrenal and abdominal Adrenal and thoracic	8 (9.1) 5 (5.7) 3 (3.4)	0	0	6 (18.8) 3 (9.4) 3 (9.4)	0	1 (20.0) 1 (20.0) 0	1 (7.7) 1 (7.7) 0	0	0
Primary tumor size, cm, Median (range)	4.4 (1.3–10.5)	4.4 (2.0–9.0)	4.7 (3.2–6.0)	4.0 (1.3–6.6)	1.8	4.4 (2.5–7.0)	5.0 (2.5–7.3)	2.0	7.0
Capsule invasion	18 (29)	4 (19.0)	1 (12.5)	6 (30.0)	0	1 (25.0)	5 (62.5)	0	1 (100)
Blood vessel invasion	15 (30)	3 (23.1)	0	5 (29.4)	0	0	6 (75.0)	0	1 (100)
Lymph vessel invasion	5 (5.7)	1 (7.7)	0	2 (11.8)	0	0	1 (12.5)	0	1 (100)
Staging pN1 pM1	5 (5.7) 9 (10.2)	0 5 (20.8)	0 0	2 (6.3) 0	0 0	0 0	3 (23.1) 3 (23.1)	0 0	0 1 (100)
Metastatic disease	15% (13/88)	20.8% (5/24)	0% (0/11)	6.3% (2/32)	0% (0/1)	0% (0/5)	38.5% (5/13)	0% (0/1)	100% (1/1)

Abbreviations: MEN2, multiple endocrine neoplasia type 2; PPGL, pheochromocytomas and paragangliomas.

TABLE 2 Treatment and clinical outcome of 88 patients with PPGL

				Hereditary					
Syndrome	**Total**	**Unknown**	**Sporadic**	**Von Hippel Lindau**	**Pacak–Zhuang**	**PGL1**	**PGL4**	**MEN2**	**NF1**
Treatment, *n* (%)	88 (100)	24 (100)	11 (100)	32 (100)	1 (100)	5 (100)	13 (100)	1 (100)	1 (100)
Surgery	72 (82)	22 (92)	8 (72.7)	29 (91)	1 (100)	4 (80.0)	7 (53.8)	1 (100)	0
R0	8 (9.1)	1 (4.2)	1 (9.1)	3 (9.4)	0	1 (20.0)	3 (23.1)	0	0
R1	6 (6.8)	0	2 (18.2)	0	0	0	2 (15.4)	0	1 (100)
R2	2 (2.3)	1 (4.2)	0	0	0	1 (20.0)	1 (7.7)	0	0
Rx	5 (5.7)	1 (4.2)	0	1 (3.1)	0	0	1 (7.7)	0	1 (100)
Radiotherapy	9 (10.2)	4 (16.7)	0	2 (6.3)	0	0	3 (23.1)	0	1 (100)
Chemotherapy	5 (5.7)	1 (4.2)	0	0	0			0	1 (100)
MIBG therapy									
Mean follow-up, years	4.2 (4.0)	2.1 (2.5)	4.8 (3.7)	5.5 (4.5)	2.1	4.4 (2.3)	2.8 (2.1)	17.1	9.2
Mean (SD)	3.2 (0–17.1)	1.2 (0–8.6)	4.7 (0.3–10.6)	4.0 (0–14.6)	2.1	5.3 (0.5–6.4)	2.2 (0.4–6.7)	17.1	9.2
Median (range)									
Events, *n* (%)	57 (65)	5 (20.8)	2 (18.2)	12 (38)	0	3 (60.0)	7 (53.8)	1 (100)	1 (100)
Non-CR	10 (11)	2 (8.4)	0	3 (9.4)	0	1 (20.0)	0	0	0
Relapse	4 (4.5)	2 (8.4)	2 (18.2)	0	0	2 (40.0)	2 (15.4)	0	0
Metastases	13 (15)	1 (4.2)	0	10 (31)	0	0	0	0	1 (100)
Multifocal PPGL	2 (2.3)	0	0	0	0	1 (20.0)	1 (7.7)	1 (100)	
Second malignancy					0	0		0	
Outcome, *n* (%)	68 (77)	20 (83)	10 (91)	27 (84)	1 (100)	3 (60.0)	6 (46.2)	1 (100)	0
CR	1 (1.1)	0	1 (9.1)	0	0	2 (40.0)	0	0	0
PR	10 (11)	1 (4.2)	0	3 (9.4)	0	0	4 (30.8)	0	1 (100)
SD	8 (9.1)	2 (8.3)	0	0	0	0	3 (23.1)	0	
PD	1 (1.1)	1 (4.2)	0				0		
DoD									

Abbreviations: CR, complete remission; DoD, death of disease; MEN2, multiple endocrine neoplasia type 2; PD, progressive disease; PPGL, pheochromocytomas and paragangliomas; PR, partial remission; SD, stable disease.

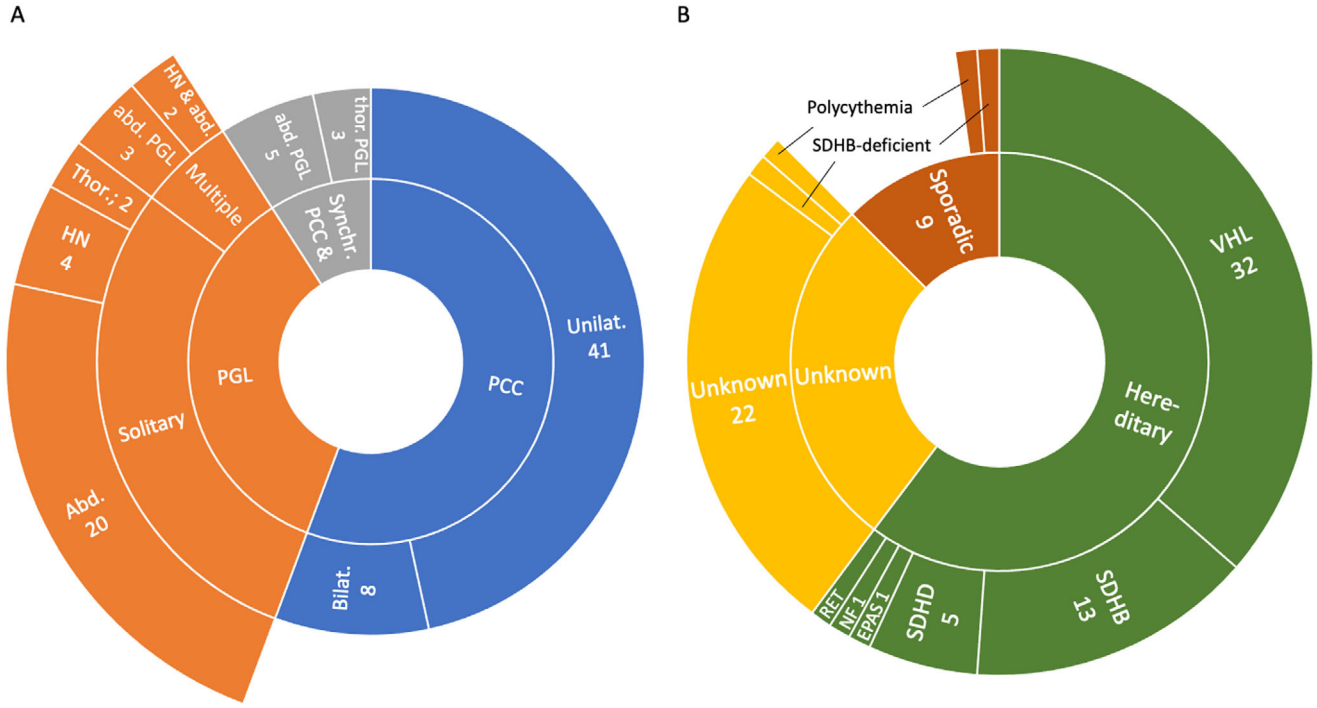

FIGURE 2 (A) Distribution and anatomic location of pheochromocytoma (PCC) and paraganglioma (PGL) at initial presentation among 88 patients registered in the GPOH-MET registry. The numbers listed indicate the number of patients. Legend: abd, abdominal; bilat, bilateral; HN, head and neck; thor, thoracic; unilat, unilateral. (B) Results of molecular genetic analysis in 88 children and adolescents with pheochromocytomas and paragangliomas (PPGL). Of those 53 patients with proven hereditary disease, the distribution of germline mutations is indicated. Two patients each in the "sporadic" and "unknown" group presented with SDHB-deficient tumors and polycythemia. Numbers of patients are indicated in subgroups with more than one patient

SDHB-deficient tumors (i.e., from immunohistochemistry data) were identified in two patients presenting with PGLs (one each in the "sporadic" and "unknown" groups). Two children were identified as additionally having polycythemia and, thus, were suspicious of Pacak–Zhuang syndrome. Genetic testing in these two children, however, did not include analysis of *EPAS1* (one each in the "sporadic" and "unknown" groups, too). Of note, all other patients without appropriate molecular genetic characterization did not show any syndromic component or positive family history (Figure 2B).

3.2 | Prognostic factors for outcome

Differences in DFS were observed among diagnosis of PPGL (PCC vs. PGL), extent of tumor resection (R_0 vs. incomplete resection), metastatic status at diagnosis (Figure S2A–C). A statistically nonsignificant trend toward inferior DFS was observed in *SDHB* mutation carriers (Figure S2D). Lymph node status and age (<10 vs. ≥10 years, $p = .817$) had no impact on DFS.

In multivariate analysis of DFS (including age [<10 vs. ≥10 years], PGL vs. PCC, hereditary vs. sporadic, SDHB vs. others, metastases at diagnosis [yes/no], and extent of resection [R_0 vs. incomplete]), incomplete tumor resection was identified as significant adverse prognostic factor (hazard ratio [HR] 3.610, 95% confidence interval [CI] 1.042–12.500, $p = .043$). Hereditary disease (HR 3.279, CI 0.659–16.393, $p = .147$) and in particular *SDHB* mutation carrier status (HR 1.269, CI 0.381–4.219, $p = .698$) had no significant influence on outcome.

3.3 | Exploring the cluster approach to stratify PPGLs in children and adolescents

We next explored the new classification and pathways proposed by Crona et al., which are based on the previously recognized cluster groups. To this end, we first applied the cluster molecular taxonomy of PPGL, and assigned patients with hereditary PPGLs to one cluster: (1) pseudohypoxic ($n = 51$), (1a) TCA cycle-related (*SDHx*, *FH*) ($n = 18$), (1b) VHL/EPAS1-related (*VHL*, *EPAS1*) ($n = 33$); (2) Wnt signaling (*CSDE1*, *MAML3*) ($n = 0$); and (3) kinase signaling (*NF1*, *HRAS*, *RET*, *TMEM127*, *MAX*) ($n = 2$). In addition, we allocated the two patients with polycythemia to cluster (1b).

We next explored anatomic location in hereditary diseases (Figure 3). The frequency of PCC was highest in *VHL*, *RET*, and *NF1* mutation carriers, whereas in the context of *EPAS1* only abdominal PGLs and PCC occurred. The frequency of metastatic disease in *SDHB* mutation carriers approached nearly 40%.

To elucidate the algorithm for personalized follow-up of PPGLs proposed by Crona et al. in children and adolescents, we calculated a PPGL risk score (Figure 4). We reassessed the calculated PPGL risk score

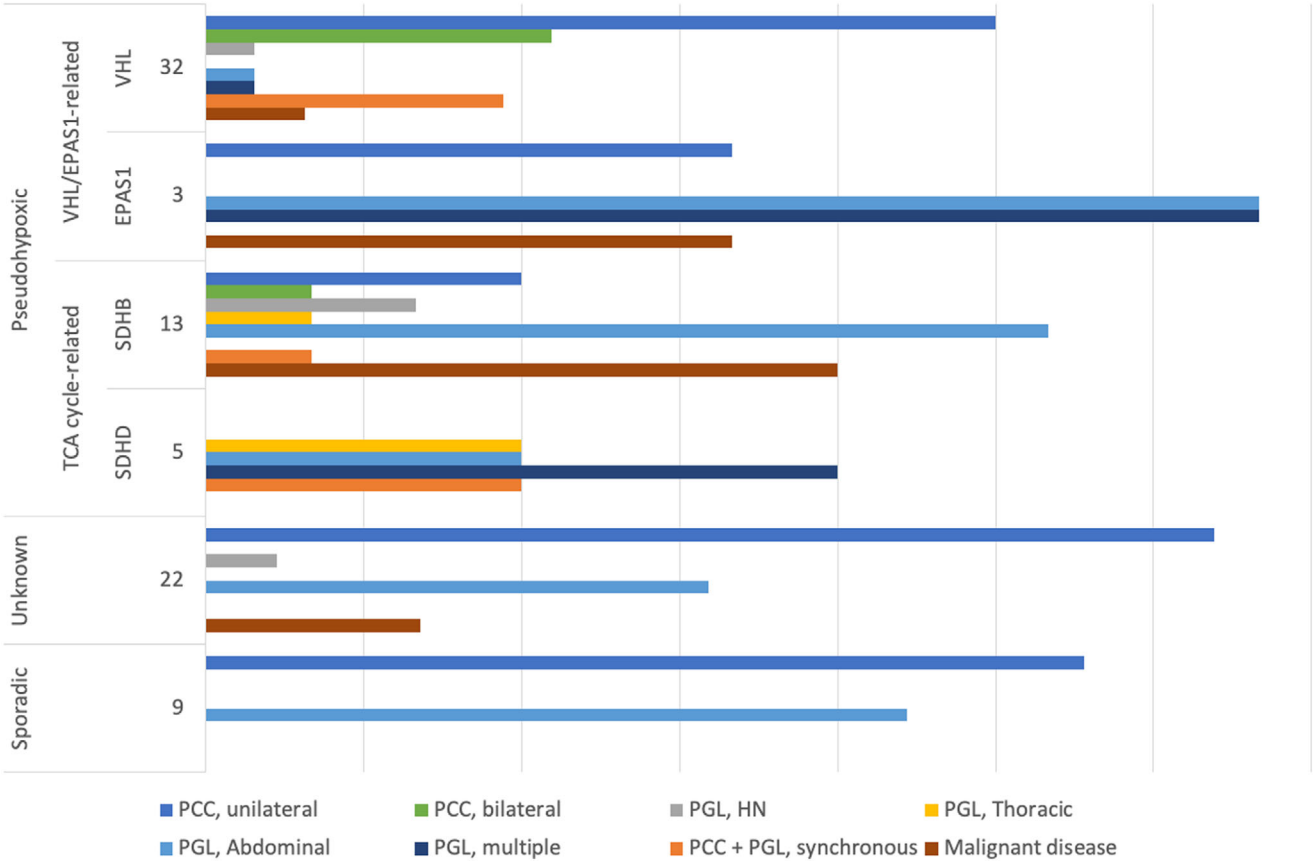

FIGURE 3 Bar diagram indicates frequency of pheochromocytomas and paragangliomas (PPGLs) at different anatomic locations as well as metastatic disease in different molecular contexts, sporadic disease and in patients with uncertain mutational context. The two patients with polycythemia are assigned to the VHL/EPAS1-related subgroup. Frequencies are given related to the absolute number of patients in the subgroup indicated on the *y*-axis. Each scale mark on the *x*-axis indicates 10%. The *RET* and *NF1* cases are excluded from this diagram as frequencies for single cases cannot be validly considered

in relation to the number of new PPGL events and the frequency of metastatic disease in each risk group. Expectedly, the highest number of new PPGL and metastatic disease events occurred in the high-risk group.

4 | DISCUSSION

Our data on children with PCC and PGL confirm a high percentage of children (83%) affected by germline mutations in one of the various susceptibility genes.[1,3,11,20] The prevalence of *SDHB* mutations, however, was lower than previously reported (20% vs. 39.1%[3]). This may account for the lower number of patients with extra-adrenal (35%), multifocal (15%), and recurrent (11%) PPGLs.[3,21] In line with a recent report on 64 children and adolescents with *SDHB*-related PPGLs,[4] 92% of patients initially presented with a solitary tumor. On the other hand, only 13% of patients developed metastases compared to 70% in the study on *SDHB*-related PPGLs. This study, however, demonstrated a second peak of the development of metastases after 12–18 years after initial diagnosis. King et al. previously reported *SDHB* mutations in 71.9% of patients with metastatic PPGLs related to primary tumor development in childhood and adolescence.[21] Thus, with a median follow-up of 4.2 years, we potentially lost a number of metastatic events occurring at a later time. This may explain the nonsignificant trend toward inferior DFS in *SDHB* mutation carriers in our study.

The French Pediatric Rare Tumors Database on children with PPGLs demonstrated incomplete tumor resection as significant adverse prognostic factor for events.[22] This is confirmed by our data. In the Tumori Rari in Etá Pediatrica (TREP) study, however, completeness of surgery showed no statistically significant differences in EFS.[23]

When applying the cluster approach[13–15,24] to our patients, most patients (96%) with known mutational context presented with PPGLs of the pseudohypoxia group (either VHL/EPAS1- or TCA cycle-related), whereas 50–60% of PPGLs in adults belong to the kinase signaling cluster.[15] A high prevalence of cluster 1 mutations in children was previously reported by Pamporaki et al.[3] This has important implications for diagnosis, treatment, and surveillance in children as pseudohypoxic PPGLs are associated with a higher risk of subsequent PPGL events and metastatic disease. We acknowledge that our data were mostly restricted to germline mutations in *VHL*, *SDHx*, and *RET* and did not systematically capture somatic mutations including *CSDE*, *MAML3*, and *HRAS* in sporadic cases and may thereby have missed some cases of

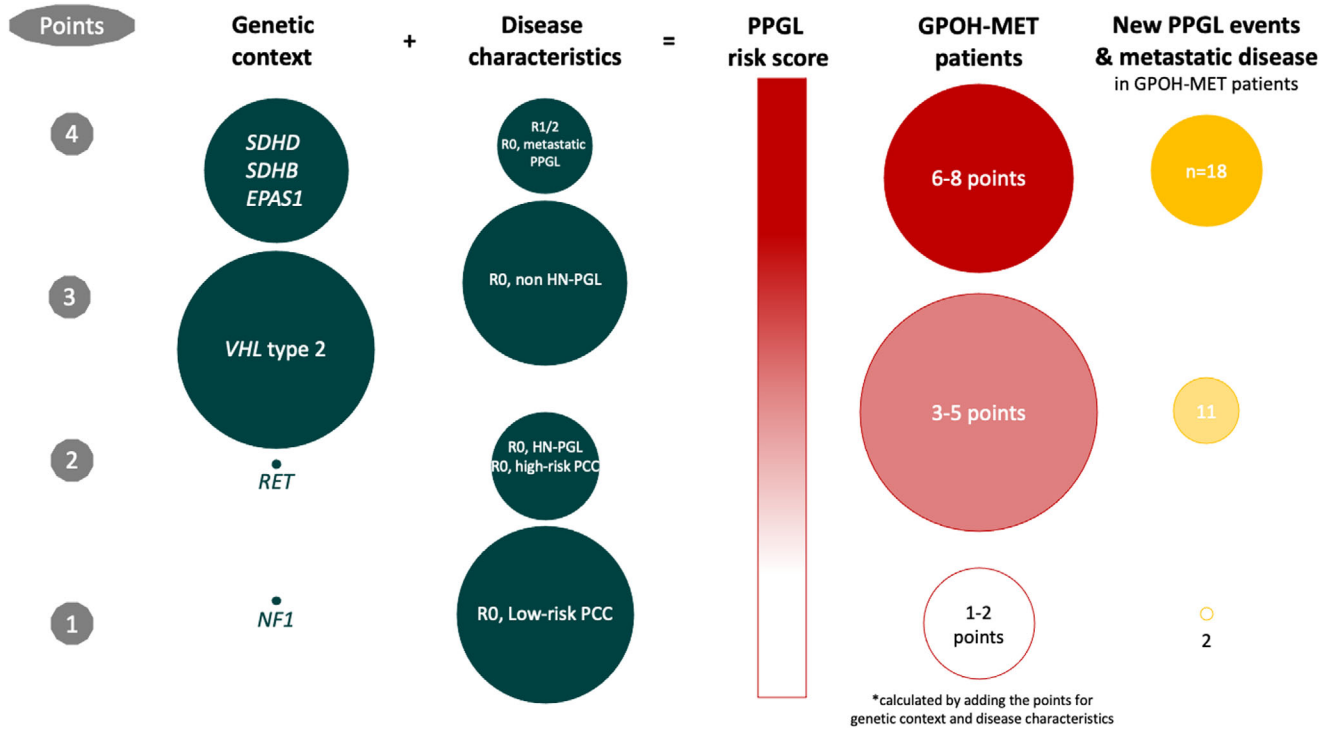

FIGURE 4 Pheochromocytoma and paraganglioma (PPGL) surveillance algorithm adapted from Crona et al.[15] The size of each circle indicates the number of children assigned to each group. High-risk PCC was defined as PCC with a diameter of >5 and >3 cm in SDHB carriers. A PPGL risk score was calculated by adding the risk from genetic subtype (from 4 points [*SDHD*, *SDHB*, *EPAS1*] to 1 point [*NF1*]), and disease characteristics (from 4 points [R1/2 resection and/or R0 resection but metastatic PPGL] to 1 point (R0 resection in low-risk PCC]). The red circles give the number of patients with high risk of new PPGL events and/or metastatic disease (6–8 points), middle risk (3–5 points), and low risk (1–2 points). The yellow circles indicate the frequency of new PPGL events and metastatic disease in the high-, middle- and low-risk groups

Wnt and kinase signaling.[14,15] We aim at subsequent comprehensive genetic testing in these patients.

Pseudohypoxic TCA cycle-related PPGLs often show a decrease or absence of key enzymes in catecholamine metabolism.[25] Adults with TCA cycle-related PPGLs are more frequently diagnosed with larger tumors due to lacking signs and symptoms of catecholamine excess as well as metastatic disease.[26,27] And indeed, children and adolescents with TCA cycle-related PPGLs had larger tumor sizes and presented with distant metastases more frequently compared to other subtypes ($p = .035$ and .042, respectively).

The paramount implication of genetic testing and cluster analyses is the great hope for increasing life expectancy and improving quality of life by tailored therapies and gene-specific surveillance. Gene-specific differences include recurrence risk, potential transformation to malignant behavior, and frequency of subsequent PPGLs. Life expectancy in children is mainly influenced by malignant behavior of the disease including metastases.[1] Thus, particularly but not only *SDHB* carriers need to be followed carefully.[1,21] When employing the PPGL risk score proposed by Crona et al., the majority of children and adolescents were assigned to high and middle risk of new PPGL events and metastatic disease. And indeed, 94% of events occurred in these patients. Thus, comprehensive surveillance including metanephrine analysis every 6–12 months and MRI every 12–24 months is recommended.[28,29] Of note, recently, successful screening identifying tumors before becoming secretory/symptomatic in a pediatric cohort was reported.[30]

Future diagnostic workups of children with PPGL need to include biochemical workup, functional imaging as appropriate, and molecular profiling including germline, somatic, and methylation analysis.[31] It may not only aid in diagnosis and staging but also provide important information for surveillance and in case of progressive metastatic or unresectable disease.[32–35]

The unique distribution of PPGL subgroups in children and adolescents, namely the predominance of pseudohypoxic PPGLs, will open new therapeutic avenues for children and adolescents with PPGL in the future. For example, targeting hypermethylation and glutamine synthesis in TCA cycle-related PPGLs and targeting the hypoxiome in pseudohypoxic PPGLs by new drugs such as the HIF2α inhibitor PT2385 may constitute attractive therapeutic options, which need to be evaluated in pediatric trials.

Our study has several limitations, which deserve to be mentioned. Incomplete genetic testing and short follow-up may result in lower proportions of hereditary, metastatic, and recurrent disease. The substances (plasma vs. urine), the metabolites (e.g., 3-methoxythyramine), and the methods used for the assessment of the secretory profiles as well as functional imaging techniques varied substantially between laboratories and over the time, particularly in patients diagnosed before

and after 2010. In some patients, biochemical and functional imaging data were not available at all.

5 | CONCLUSION

Most pediatric PPGLs belong to the pseudohypoxia subgroup including *VHL*/*EPAS1*-related and TCA cycle-related (*SDHx*) PPGLs. These are associated with high risk of new PPGL events and metastatic disease, and necessitate thorough follow-up including clinical examination, biochemical workup, and imaging. Of note, incomplete tumor resection is an adverse prognostic factor for subsequent events.

Comprehensive molecular profiling of children and adolescents with newly diagnosed PPGLs will add in our understanding of sporadic cases and open new avenues for personalized diagnosis, treatment, and surveillance.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the finding of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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