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Genotype–phenotype correlations in pheochromocytoma and paraganglioma: a systematic review and individual patient meta-analysis

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Abstract

Pheochromocytoma and paraganglioma (PPGL) can be divided into at least four molecular subgroups. Whether such categorizations are independent factors for prognosis or metastatic disease is unknown. We performed a systematic review and individual patient meta-analysis aiming to estimate if driver mutation status can predict metastatic disease and survival. Driver mutations were used to categorize patients according to three different molecular systems: two subgroups (*SDHB* mutated or wild type), three subgroups (pseudohypoxia, kinase signaling or Wnt/unknown) and four subgroups (tricarboxylic acid cycle, *VHL/EPAS1*, kinase signaling or Wnt/unknown). Twenty-one studies and 703 patients were analyzed. Multivariate models for association with metastasis showed correlation with *SDHB* mutation (OR 5.68 (95% CI 1.79–18.06)) as well as norepinephrine (OR 3.01 (95% CI 1.02–8.79)) and dopamine (OR 6.39 (95% CI 1.62–25.24)) but not to PPGL location. Other molecular systems were not associated with metastasis. In multivariate models for association with survival, age (HR 1.04 (95% CI 1.02–1.06)) and metastases (HR 6.13 (95% CI 2.86–13.13)) but neither paraganglioma nor *SDHB* mutation remained significant. Other molecular subgroups did not correlate with survival. We conclude that molecular categorization accordingly to *SDHB* provided independent information on the risk of metastasis. Driver mutations status did not correlate independently with survival. These data may ultimately be used to guide current and future risk stratification of PPGL.

Key Words

- ▶ pheochromocytoma
- ▶ paraganglioma
- ▶ molecular genetics
- ▶ driver mutations
- ▶ meta-analysis

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Introduction

The Cancer Genome Atlas (TCGA) proposed that neuroendocrine tumors of adrenal paraganglia, pheochromocytomas (PCCs) and extra-adrenal paraganglia paragangliomas (PGLs, together denoted PPGL) can be

divided into three main molecular subgroups that have been linked to distinct driver genes (Fishbein *et al.* 2017): pseudohypoxia (*SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *FH*, *VHL*, *EPAS1* and *EGLN1*), Wnt-altered (*CSDE1* or *MAML3*)

and kinase signaling (*RET*, *NF1*, *TMEM127*, *MAX*, *HRAS*, *FGFR1* and *MET*) (Letouze *et al.* 2013, Castro-Vega *et al.* 2015, Toledo *et al.* 2016, Bausch *et al.* 2017, Fishbein *et al.* 2017, Welander *et al.* 2018). Previous data also support that the pseudohypoxic group can be further divided into two subclusters: tricarboxylic acid (TCA) cycle-related (*SDHA-SDHD*, *SDHAF2* or *FH*) and those with *VHL/EPAS1*-related (*VHL/EPAS1/EGLN1*) PPGLs (Burnichon *et al.* 2011, Letouze *et al.* 2013, Flidner *et al.* 2016). Each subgroup is named after their molecular hallmarks and are thought to be associated with distinct biochemical and clinical phenotypes (reviewed in Crona *et al.* 2017, Neumann *et al.* 2018). All pseudohypoxic PPGLs secrete norepinephrine and those related to the TCA cycle are more predominantly PGLs with relatively high proportion having dopamine secretion. The TCA cycle subgroup and particularly *SDHB* carriers are associated with the highest proportion of metastatic disease (Eisenhofer *et al.* 2011a,b). On the other end of the spectrum is the kinase signaling subgroup that has a more well-differentiated phenotype with epinephrine secretion, predominantly adrenal location and rarely develop metastatic disease. PPGLs related to the Wnt-altered subgroup are thought to display intermediate characteristics in terms of catecholamine secretion (mixed noradrenergic and adrenergic) and frequency of metastatic or recurrent disease (Fishbein *et al.* 2017). It has also been proposed that PPGL with somatic aberrations in genes related to telomere maintenance (inactivation of *ATRX* or transcriptional activation of *TERT*) as well as chromatin maintenance (*SETD2*) could have more aggressive features and may thus be disease modifiers (Fishbein *et al.* 2015, 2017, Job *et al.* 2018).

The predominant cause of death in patients with PPGL is metastasis that occurs in about 10–20% of cases (Timmers *et al.* 2008, Hamidi *et al.* 2017a). Tumor location (PGL versus PCC), germline *SDHB* mutations (*SDHB* mutated versus *SDHB* wild type), *ATRX* mutation, *TERT* overexpression, catecholamine secretion (noradrenergic or dopaminergic versus adrenergic) and large size of the primary tumor have all been independently associated with metastasis (Ayala-Ramirez *et al.* 2011, Welander *et al.* 2011, Eisenhofer *et al.* 2012, Turkova *et al.* 2015, Assadipour *et al.* 2017, Cho *et al.* 2018, Job *et al.* 2018). The disease course of those with metastatic disease is heterogeneous in terms of tumor aggressiveness and overall survival (Hamidi *et al.* 2017b). Size of the primary tumor, gender, *SDHB* mutation, catecholamine profile, *ATRX* mutation and *TERT* overexpression are suggested to be prognostic factors for survival (Amar *et al.* 2007, Ayala-Ramirez *et al.* 2011, Zelinka *et al.* 2011, Hamidi *et al.* 2017b,

Job *et al.* 2018). Recent data published after submission of this article interestingly showed that among patients with metastatic disease, *SDHB* was not a negative prognostic factor for survival (Hescot *et al.* 2019).

Although at least 16 common driver genes have been identified in PPGL, the only disease driver that showed a robust correlation to metastatic disease and outcome has been *SDHB*. We and others have proposed that the improved characterization of PPGL driver mutations provide additional information beyond the dichotomous categorization based on *SDHB*. However, due to disease rarity and extensive genetic heterogeneity, interpretation of findings are currently restricted due to low statistical power. We hypothesized that a systematic review and individual patient meta-analysis could overcome these challenges and provide information on correlation between driver mutation status and clinical parameters. We particularly focused on predictive factors for metastatic disease and prognostic factors for survival.

Methods

This study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) workflow (Liberati *et al.* 2009). The study reviewed and analyzed published data, these activities fall under an approval by the Regional Ethics Committee in Uppsala, Sweden (Dnr 2015/544).

Search strategy

One investigator (J C) performed a systematic search of PubMed to identify relevant reports published between 2007-01-01 and 2017-12-01. The following search terms were used: ‘pheochromocytoma’ and ‘paraganglioma’. Reports were initially screened by title for relevance and potentially relevant reports had its abstract reviewed. Case reports, review articles and editorials as well as those publications in other languages than English were not considered. Potentially relevant studies were assessed for eligibility through review of the full-text article.

Study selection and data extraction

Studies fulfilling the following eligibility criteria were included. Criteria (1), genetic sequencing and reporting of PPGL disease drivers: germline mutations, *SDHA*, *SDHB*, *SDHD*, *TMEM127*; germline and somatic mutations,

VHL, *RET*, *NF1*, *MAX*; and somatic mutations, *HRAS*. Criteria (2), shared data on genetic mutations and clinical characteristics on the individual patient level for both mutation-positive and mutation-negative cases. Criteria (3), patient identification numbers for cross-validation between different studies from the same study site. Publications were grouped into cohorts based on the study site to allow for reconstruction of each study cohort. Two investigators (J C and S G) reviewed the papers independently and transferred the data into one study database. Values that did not overlap between the two investigators were re-assessed to reach a common conclusion.

Data collection and cleaning

Patients without available PPGL tissue for analysis were excluded. For patients with multiple primary tumors, the one that occurred at the earliest age was selected or if the same time point, the row that occurred first in the original data was chosen. In patients with a conflict of data between multiple publications, the most recent value was used. Collected data points and definitions are provided in the Supplementary Methods and Results (see section on [supplementary data](#) given at the end of this article).

Definition of PPGL driver gene subgroups

With the TCGA publication as a starting point and taking into account the available literature, we selected three different systems for driver gene categorization. Firstly, a two-molecular subgroup system accordingly to *SDHB* mutation status: *SDHB* mutated or *SDHB* wild type. Secondly, a system with three-molecular subgroups categorized according to the presence of germline/somatic driver mutations/gene fusions: pseudohypoxia (*SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *FH*, *VHL*, *EPAS1* or *EGLN1*), kinase signaling (*NF1*, *RET*, *TMEM127*, *MAX*, *HRAS*, *MET* or *FGFR1*) and Wnt/unknown (*CSDE1* or *MAML3*). PPGL with driver mutations associated with different molecular subgroups as well as those without a driver mutation was classified as Wnt/unknown. The cortical admixture subgroup, originally reported by the TCGA project is thought to be defined by non-tumoral cells and was not included (Crona *et al.* 2017, Fishbein *et al.* 2017). Thirdly, a system with four-molecular subgroups was used to take into account the distinct features of TCA-cycle-related (*SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2* and *FH*) and *VHL/EPAS1*-related (*VHL*, *EPAS1* and *EGLN1*) PPGLs (Burnichon *et al.* 2011, Flynn *et al.* 2015, Fliedner *et al.* 2016).

Risk of bias assessment

Risk of bias was determined by two investigators (J C, S G), and in cases of discrepant assessment, the original papers were re-evaluated to reach a common conclusion. Bias assessment was designed based on a modified Newcastle–Ottawa tool for bias assessment adopted by Hamidi *et al.* (2017a) that was further modified to this study. Criteria for bias assessment is available in the Supplementary Methods and Results.

Statistical analyses

Nominal data are presented as number of patients and percentages and were analyzed with chi-square test. Scaled data were presented as median and range or 95% confidence interval (CI) and were analyzed with Mann–Whitney *U* or Kruskal–Wallis tests. Logistic regression (univariate/multivariable) was used as appropriate. Survival analysis was performed using log-rank, Kaplan–Meier and Cox regression analyses. *P* values <0.05 were defined as statistically significant. Variables identified as significant in univariate analysis were included in multivariable analysis (applicable for logistic and for Cox regression). Statistical analyses were performed using SPSS version 22 (IBM) and Stata version 12. Figures were drawn with Prism 6.0h (GraphPad Software Inc) and Stata version 12.

Results

A PubMed search generated 7689 results, and 118 manuscripts were selected for review of eligibility (Supplementary Fig. 1). A total of 97 publications did not meet the criteria on method for genetic sequencing (*n*=82) or individual patient data availability (*n*=13). Two studies were excluded as individual patients could not be matched to previous studies. Twenty-one publications matched study criteria and allowed reconstruction of seven cohorts (Table 1). These seven cohorts represented 948 individual patients, 32 had data on multiple tumor lesions. Two hundred forty-five patients were excluded as there was no tumor tissue available. Seven hundred three patients remained, 274 were analyzed with exome sequencing and 429 with a targeted re-sequencing approach.

Risk of bias assessment

Risk of bias assessments was performed for each study cohort, and it is presented in Supplementary Fig. 2. All studies performed retrospective characterization of case series.

Table 1 Study cohorts.

References	Patients (n)	Study site	WES (n)	Targeted re-sequencing (n)
Welander <i>et al.</i> (2012, 2018), Welander <i>et al.</i> (2014a,b), Juhlin <i>et al.</i> (2015), Stenman <i>et al.</i> (2016a,b)	137	Karolinska University Hospital, Stockholm, Sweden; Linköping University Hospital, Linköping, Sweden; Haukeland University Hospital, Bergen, Norway	19	118
Wilzen <i>et al.</i> (2016)	9	Sahlgrenska University Hospital, Gothenburg, Sweden	9	0
Flynn <i>et al.</i> (2015, 2016, 2017), Dwight <i>et al.</i> (2018)	48	The Peter MacCallum Cancer Centre and University of Melbourne, Australia; Kolling Institute and University of Sydney, Australia; Royal Brisbane Hospital, Brisbane, Australia; Royal North Shore Hospital, Sydney, Australia; The Children's Hospital at Westmead, Sydney, Australia	44	4
Burnichon <i>et al.</i> (2011, 2012), Favier <i>et al.</i> (2012), Letouze <i>et al.</i> (2013), Castro-Vega <i>et al.</i> (2014, 2015)	190	INSERM, Hôpital Europe' en Georges Pompidou and Université Paris Descartes, Sorbonne Paris Cité French, Paris, France. Cortico et Médullosurrénale: les Tumeurs Endocrines (COMETE) Network, France	29	161
Fishbein <i>et al.</i> (2017)	173	National Institutes of Health, United states and multiple collaborating institutions of the Cancer Genome Atlas, Pheochromocytoma and Paraganglioma project	173	0
Curras-Freixes <i>et al.</i> (2015)	118	Centro Nacional de Investigaciones Oncológicas, Madrid, Spain and multiple collaborating institutions throughout Spain	0	118
Toledo <i>et al.</i> (2016)	28	University of Texas Health Science Center at San Antonio, San Antonio, United States	28	0

WES, whole-exome sequencing.

Assessment of genetic results (7/7 studies low risk) and method coverage (5/5 studies low risk) showed relatively low risk of bias. Clinical data and particularly hormone assessment (6/7 studies high or unclear risk) and follow-up time (7/7 studies high or unclear risk) had a high risk of bias.

Baseline characteristics

Clinical characteristics of the reviewed patients are presented in Table 2. PPGL-related driver mutations were detected in 437 patients (62.6%, 95% CI 58.5–65.7, Supplementary Fig. 3 and Supplementary Table 1) that were confirmed as germline in 178 (25.3%, 95% CI 22.3–28.7) and somatic in 237 (33.7%, 95% CI 30.3–37.3). The frequency of mutations in the different driver genes are shown in the Supplementary Methods and Results, and Supplementary Table 1.

Patients were categorized into three different molecular systems. Two-molecular subgroups: *SDHB* mutated 58 patients (8.3%, 95% CI 6.4–10.5, Table 2) and *SDHB* wild type 645 patients (91.8%, 95% CI 89.5–93.6). Three molecular subgroups: pseudohypoxia, 177 patients (24.9%, 95% CI 21.8–28.2); kinase signaling, 245 patients (34.9%, 95% CI 31.4–38.5); and Wnt/unknown, 281 patients (39.9%, 95% CI 36.4–43.6). In the four-molecular

subgroup system, the pseudohypoxia subgroup was further divided into TCA cycle, 79 patients (11.2%, 95% CI 9.1–13.8) and *VHL/EPAS1* related, 98 patients (13.9%, 95% CI 11.6–16.7).

Clinical correlations to molecular subgroups

An overview of clinical correlations to the different molecular systems are presented in Fig. 1, Supplementary Fig. 3 and Supplementary Table 2. Gender, catecholamine profile, WHO classification (PCC versus PGL), metastatic stage, age at diagnosis as well as tumor size were all differently distributed (*P* values <0.05) among subgroups of all three molecular systems. Detailed descriptive data are available in the Supplementary Methods and Results.

Predictive factors of metastatic disease

Frequency of metastatic disease in the cohort was 12.1% (85/703 patients). Categorization accordingly to the two-, three- and four-molecular subgroup systems as well as catecholamine profile, WHO classification and *ATRX* mutation status correlated with metastatic disease in univariate Cox regression analyses (Fig. 1 and Table 3). Those with *SDHB*-mutated PPGLs had metastatic disease in 46.6% (27/58 patients, OR 8.81 (95% CI 4.92–15.78);

Table 2 Clinical characteristics of the reviewed patients.

Patients (n = 703)		Frequency	%
Gender	Male	307	43.7
	Female	392	55.7
	Data not available	4	0.6
Age at diagnosis	Median (range)	46 (7–84)	
Tumor size (mm)	Median (range)	45 (10–160)	
Stage	Non-metastatic	618	87.9
	Metastatic	85	12.1
Catecholamine profile	Epinephrine	161	22.9
	Norepinephrine	139	19.8
	Dopamine	24	3.4
	Data not available	324	53.9
WHO 2004	Pheochromocytoma	572	81.4
	Paraganglioma	127	18.1
	Data not available	4	0.5
WHO 2017	PCC	572	81.4
	Sympathetic PGL	96	13.7
	Head and neck PGL	27	3.8
	Data not available	8	1.1
Time on follow-up (months)	Median (range)	33 (0–316)	
Status at the end of follow-up	Alive	494	70.3
	Dead	40	5.7
	Data not available	169	24.0
ATRX mutation status	ATRX mutated	450	63.9
	ATRX wild type	17	2.4
	Data not available	237	33.7
2-molecular subgroups	SDHB wild type	645	91.7
	SDHB mutated	58	8.3
3-molecular subgroups	Pseudohypoxia	177	25.2
	Kinase signaling	245	34.9
4-molecular subgroups	Wnt/unknown	281	39.9
	Pseudohypoxia	79	11.3
	TCA-cycle		
	Pseudohypoxia	98	13.9
	VHL/EPAS1		
	Kinase signaling	245	34.9
	Wnt/unknown	281	39.9

Data on age was missing in 3 patients, on tumor size on 291 patients and on follow-up length in 167 patients.

DA, dopamine; E, epinephrine; F, female; HNPGL, head and neck PGL; M, male; NA, not available; NE, norepinephrine; PCC, pheochromocytoma; PGL, paraganglioma; sPGLs, sympathetic PGL; TCA, tricarboxylic acid.

$P < 0.001$) that was higher compared to *SDHB* wild type 8.9% (58/645 patients) PPGLs. In the three-molecular subgroup system, metastasis was more common in pseudohypoxia 24.3% (43/177 patients, OR 2.49 (95% CI 1.51–4.13) $P < 0.001$) and less frequent in kinase signaling 4.1% (10/245 patients, OR 0.33 (95% CI 0.16–0.69)

$P = 0.003$) compared to Wnt/unknown 11.4% (32/281 patients). In the four-molecular subgroups classification, metastatic PPGLs occurred more often in TCA cycle 40.5% (32/79 patients, OR 5.29 (95% CI 2.96–9.47)) but was not different in *VHL/EPAS1*-related PPGLs 11.2% (11/98 patients, OR 0.98 (95% CI 0.78–2.04)) compared to the Wnt/unknown group.

The three different molecular systems were analyzed separately for association with metastatic disease in multivariate models. Each model included other significant variables in univariate analyses: catecholamine profile (norepinephrine or dopamine compared to epinephrine) and WHO classification (PGL compared to PCC). While *ATRX*-mutated PPGL showed a positive correlation with metastases in the univariate analysis, information on *ATRX* mutation status was only available in a subset of patients (467/703) that also lacked complete clinical annotations. As such, *ATRX* mutation status was not included in the multivariate models. In model 1 (exploring the role of two-molecular subtype; Table 3, Column B), *SDHB* mutation (OR 5.68 (95% CI 1.79–18.06); $P = 0.003$) as well as norepinephrine (OR 3.01 (95% CI 1.02–8.79); $P = 0.045$) and dopamine (OR 6.39 (95% CI 1.62–25.24); $P = 0.008$) secretion but not WHO classification were associated with metastatic disease. In model 2 (exploring the role of three-molecular subtype; Table 3, Column C), dopamine secretion (OR 7.86 (95% CI 2.03–30.4), $P = 0.003$), PGL (OR 3.09 (95% CI 1.20–7.97); $P = 0.019$) but not the three-molecular subgroup system were associated with metastatic disease. In model 3 (exploring the role of four-molecular subtype; Table 3, Column D), norepinephrine (OR 3.12 (95% CI 1.02–9.56) $P = 0.046$), dopamine (OR 6.32 (95% CI 1.58–25.3) $P = 0.009$) but not the four-molecular classification system nor WHO classification showed association with metastasis. Thus, in the context of clinical characteristics, the only relevant molecular biomarker for predicting metastasis was categorization accordingly to *SDHB* mutation status.

Prognostic information

Median survival time for the entire cohort was 240 months (95% CI 202–not reached). Age at diagnosis (hazard ratio (HR) 1.04 (95% CI 1.01–1.05); $P = 0.019$), metastatic stage (HR 6.63 (95% CI 3.46–12.7); $P < 0.001$), PGL (HR 2.6 (95% CI 1.32–5.15); $P = 0.006$), *SDHB* mutation (HR (95% CI 1.32–5.94); $P = 0.007$), pseudohypoxia TCA cycle (HR 2.28 (95% CI 1.03–5.08); $P = 0.043$) and *ATRX* mutation (HR 9.44 (95% CI 3.29–27.15); $P < 0.001$) correlated with worse survival in univariate Cox regression analyses

Figure 1

Clinical correlations to modified molecular subgroups from the Cancer Genome Atlas. Epi, epinephrine; kinase, kinase signaling subgroup; Norepi, norepinephrine; PCC, pheochromocytoma; PGL, paraganglioma; TCA, tricarboxylic acid; UK, unknown; *VHL/EPAS1*, pseudohypoxia *VHL/EPAS1* related. ***Chi-square test including all four molecular subgroups had a significance level <0.001.

(Table 4 and Supplementary Fig. 4). In multivariate model 1 (exploring the role of two-molecular subtype; Table 4, Column B), age (HR 1.04 (95% CI 1.02–1.06); $P=0.001$) and metastases (HR 6.13 (95% CI 2.86–13.13); $P<0.001$) but not PGL nor *SDHB* mutation remained significant for survival. In multivariate model 2 (exploring the role of four-molecular subtype; Table 4, Column C), age (HR 1.04 (95% CI 1.02–1.06); $P<0.001$) and metastases (HR 5.85 (95% CI 2.69–12.71); $P<0.001$) but not PGL or categorization according to the four-molecular subgroup system remained significant for survival.

A subgroup analysis of patients with metastatic disease ($n=57$) did not show any clinical or molecular factors associated with survival in univariate Cox regression analysis (Supplementary Table 3). Even though there was a trend toward worse overall survival on patients with PPGLs classified as pseudohypoxia TCA cycle related and Wnt/unknown identified in Kaplan–Meier curves (Supplementary Fig. 5), such differences did not reach statistical significance due to limited power and number of events (log-rank test, $P=0.1620$).

Discussion

We performed a meta-analysis on data from a systematic review of 703 PPGL patients published by 21 genome sequencing studies, and this is to our knowledge, the largest review in the literature. We focused on identifying predictive factors of metastatic disease, the major determinant of outcome from PPGL disease. While tumor location, biochemical phenotype and the driver gene classifications all showed different frequencies of metastatic disease in the univariate analyses, the only categorization accordingly to a driver gene that remained significant in the multivariate models was *SDHB* mutation status. In the univariate analyses, age, tumor location, metastatic disease, *SDHB* and TCA cycle-related PPGL showed difference in survival. But, no molecular information remained significant for survival in the multivariate model.

The aggregated frequency of driver mutations presented in our review was 62.2% – 24.6% in germline and 32.9% on the somatic level. This number is slightly

Table 3 Factors related with increased risk of metastatic disease.

		Logistic regression (univariate analysis)		Logistic regression (multivariable analysis)	Logistic regression (multivariable analysis)	Logistic regression (multivariable analysis)
		metastatic disease patients (%)	OR (95% CI); <i>P</i> value	Model 1	Model 2	Model 3
Gender	Female	11.2%	1 (Ref)	–	–	–
	Male	13.4%	1.22 (0.77–1.92); 0.393	–	–	–
Age at diagnosis	Continuous variable	NA	0.99 (0.98–1.01); 0.307	–	–	–
Tumor size (mm)	≤50	10.9%	1 (Ref)	–	–	–
	>50	15.7%	1.52 (0.76–3.05); 0.236	–	–	–
Hormone	Epinephrine	3.1%	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
	Norepinephrine	12.9%	4.64 (1.68–12.86); 0.003	3.01 (1.02–8.79); 0.045	2.88 (0.94–8.82); 0.065	3.12 (1.02–9.56); 0.046
	Dopamine	29.2%	12.85 (3.67–44.93); <0.001	6.39 (1.62–25.24); 0.008	7.86 (2.03–30.4); 0.003	6.32 (1.58–25.30); 0.009
WHO 2004	PCC	7.9%	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
	PGL	29.1%	4.81 (2.95–7.85); <0.001	1.52 (0.52–4.43); 0.436	3.09 (1.20–7.97); 0.019	1.76 (0.57–5.42); 0.324
WHO 2017	PCC	7.9%	1 (Ref)	*	*	*
	Sympathetic PGL	29.2%	4.82 (2.82–8.23); <0.001	*	*	*
	Head and Neck PGL	25.9%	4.09 (1.65–10.21); 0.002	*	*	*
ATRX mutation status	ATRX mutated	58.5%	13.18 (4.78–36.36); <0.001	*	*	*
2-Molecular subgroups	ATRX wild type	9.8%	1 (Ref)	*	*	*
	SDHB wild type	8.9%	1 (Ref)	1 (Ref)	£	&
	SDHB mutated	46.6%	8.81 (4.92–15.78); <0.001	5.68 (1.79–18.06); 0.003	£	&
3-Molecular subgroups	Wnt/unknown	11.4%	1 (Ref)	\$	1 (Ref)	&
	Pseudohypoxia	24.3%	2.49 (1.51–4.13); <0.001	\$	0.92 (0.35–2.43); 0.861	&
	Kinase signaling	4.1%	0.33 (0.16–0.69); 0.003	\$	0.49 (0.13–1.91); 0.305	&
4-Molecular subgroups	Wnt/unknown	11.4%	1 (Ref)	\$	£	1 (Ref)
	Pseudohypoxia	40.5%	5.29 (2.96–9.47); <0.001	\$	£	2.65 (0.83–8.48); 0.101
	TCA-cycle	11.2%	0.98 (0.78–2.04); 0.965	\$	£	!
	Pseudohypoxia VHL/EPAS1-Kinase signaling	4.1%	0.33 (0.19–0.69); 0.003	\$	£	0.45 (0.12–1.74); 0.246

ATRX mutation status correlated with increased frequency of metastasis in univariate analysis. But, it was not included in the multivariate model due to a lack of clinical annotations in cases that had analysis of ATRX mutation status. Identification of threshold for tumor size is described in the Supplementary Materials and Results

*Not included, WHO 2004 classification included in the multivariable analysis; *three- and four-molecular subgroup systems not included in model 1 of the multivariable analysis (two-molecular subgroups included instead); ‡two- and four-molecular subgroups not included in model 2 of the multivariable analysis (three-molecular subgroups classification included instead); §two- and three-molecular subgroups not included in model 3 of the multivariable analysis (four-molecular subgroups classification included instead); †could not be calculated due to lack of observations.

95% CI, 95% confidence interval; DA, dopamine; E, epinephrine; HNPGL, head and neck PGL; NA, not available; NE, norepinephrine; OR, odds ratio; PCC, pheochromocytoma; PGL, paraganglioma; Ref, reference; sPGLs, sympathetic PGL; TCA, tricarboxylic acid.

Table 4 Survival analysis.

		Median survival (months)	Cox regression (univariate analysis)	Cox regression (multivariable analysis)	Cox regression (multivariable analysis)
		(95% CI)	HR (95% CI); <i>P</i> value	HR (95% CI); <i>P</i> value Model 1	HR (95% CI); <i>P</i> value Model 2
Gender	Female	240 (202–nr)	1 (Ref)	–	–
	Male	Nr (!)	1.14 (0.59–2.19); 0.686	–	–
Age at diagnosis	Continuous variable	NA	1.02 (1.01–1.05); 0.019	1.04 (1.02–1.06); 0.001	1.04 (1.02–1.06); <0.001
Tumor size (mm)	≤50	240 (nr–nr)	1 (Ref)	–	–
	>50	Nr (!)	0.82 (0.34–1.99); 0.654	–	–
Stage	Non-metastatic	240 (202–nr)	1 (Ref)	1 (Ref)	1 (Ref)
	Metastatic	156 (84–nr)	6.63 (3.46–12.70); <0.001	6.13 (2.86–13.13); <0.001	5.85 (2.69–12.71); <0.001
Catecholamine	Epinephrine	240 (nr–nr)	1 (Ref)	–	–
	Norepinephrine	Nr (192–nr)	1.15 (0.39–3.35); 0.800	–	–
	Dopamine	168 (156–nr)	3.06 (0.85–11.04); 0.088	–	–
WHO 2004	PCC	240 (202–nr)	1 (Ref)	1 (Ref)	1 (Ref)
	PGL	192 (156–nr)	2.60 (1.32–5.15); 0.006	1.56 (0.64–3.81); 0.332	1.47 (0.56–3.87); 0.440
WHO 2017	PCC	240 (202–nr)	1 (Ref)	*	*
	Sympathetic PGL	192 (117–nr)	2.76 (1.37–5.58); 0.005	*	*
	Head and neck PGL	Nr (!)	1.54 (0.21–11.53); 0.672	*	*
ATRX mutation status	ATRX mutated	100 (3–nr)	9.44 (3.29–27.15); <0.001	*	*
	ATRX wild type	Nr (!)	1 (ref)	*	*
2-Molecular subgroups	SDHB wild type	240 (202–nr)	1 (Ref)	1 (Ref)	£
	SDHB mutated	168 (117–nr)	2.80 (1.32–5.94); 0.007	1.45 (0.47–4.44); 0.514	£
3-Molecular subgroups	Wnt/unknown	Nr (!)	1 (Ref)	–	–
	Pseudohypoxia	202 (156–nr)	1.66 (0.79–3.48); 0.180	–	–
4-Molecular subgroups	Kinase signaling	240 (nr–nr)	0.66 (0.28–1.55); 0.335	–	–
	Wnt/unknown	Nr (!)	1 (Ref)	\$	1 (Ref)
	Pseudohypoxia TCA cycle	168 (117–nr)	2.28 (1.03–5.08); 0.043	\$	1.43 (0.45–4.55); 0.543
	Pseudohypoxia VHL/EPAS1	202 (192–nr)	0.86 (0.25–2.98); 0.814	\$	0.88 (0.24–3.24); 0.851
	Kinase signaling	240 (nr–nr)	0.66 (0.28–1.56); 0.341	\$	0.79 (0.33–1.95); 0.616

ATRX mutation status correlated to survival in univariate analysis. But, it was not included in the multivariate model due to a lack of clinical annotations in cases that had analysis of ATRX mutation status.

!Could not be calculated due to lack of observations; *not included, WHO 2004 classification included in the multivariable analysis; \$four-molecular subgroups system not included in model 1 of the multivariable analysis (two-molecular subgroups included instead); £two-molecular subgroups system not included in model 2 of the multivariable analysis (four-molecular subgroups classification included instead).

95% CI, 95% confidence interval; DA, dopamine; E, epinephrine; HNPGL, head and neck PGL; HR, hazard ratio; NA, not available; NE, norepinephrine; Nr, not reached; PCC, pheochromocytoma; PGL, paraganglioma; Ref, reference; sPGLs, sympathetic PGL; TCA, tricarboxylic acid.

lower than the frequencies observed in the included TCGA study (27% germline and 39% somatic driver mutations) that used the most comprehensive genetic analysis of all included studies (Fishbein *et al.* 2017). Major driver genes

in the reviewed studies were *NF1*, *VHL*, *RET*, *SDHB* and *HRAS* that were mutated in 45.2% in of PPGL. A second group of driver genes, *EPAS1*, *SDHD*, *SDHA*, *MAML3*, *MAX* and *TMEM127* occurred less frequently and had a

cumulative frequency of 8.8%. A third group of genes were only found to be mutated in a minority of patients, cumulative frequency 2.8%: *CSDE1*, *FGFR1*, *MET*, *SDHC*, *SDHAF2*, *FH* and *EGLN1*. It should be noted that *MAML3*, *CSDE1*, *FGFR1* and *MET* were recently discovered in this disease and were therefore only partially included in the sequencing analyses of the reviewed studies.

In order to correlate these findings to patient phenotype, we categorized PPGLs into subgroups accordingly to the biological hallmarks of the tumor as per driver mutation status. A novel category, Wnt/unknown, was created to allow for groups with adequate patient numbers for the statistical analyses. We recognize that Wnt/unknown represents a diverse group of PPGLs that is likely to be dissected as investigators employ more comprehensive methods for genome sequencing in a near future. Such improved categorization could include additional data on newly discovered PPGL driver genes, such as *EGLN2* (Yang *et al.* 2015), *SLC25A11* (Buffet *et al.* 2018), *MDH2* (Cascon *et al.* 2015), *DNMT3A* (Remacha *et al.* 2018), *H3F3A* (Toledo *et al.* 2016) as well as information on disease-modifying genes related to telomere maintenance as well as chromatin modification (Fishbein *et al.* 2015, 2017, Job *et al.* 2018).

Tumor location, biochemical phenotype and molecular subgroup are three interconnected factors that are all known to be associated with PPGL metastasis (reviewed in Crona *et al.* 2017). Welander *et al.* reviewed the frequency of metastatic disease in patients with hereditary PPGL: *RET*, 2.9%; *VHL*, 3.4%; *SDHD*, 3.5%; and *SDHB*, 30.7% (Welander *et al.* 2011). A systematic review later showed that metastasis occurred in 17% of *SDHB* and 8% of *SDHD* carriers (van Hulsteijn *et al.* 2012). The findings in our review and meta-analysis corroborate these studies that define *SDHB* (46.6%), pseudohypoxia (24.3%) and pseudohypoxia TCA-cycle-related (40.5%) PPGL as having a relatively high risk of metastatic disease. Different from previous studies, PPGLs related to either *VHL/EPAS1* (11.2%) or Wnt/unknown (11.4%) subgroups had an intermediate frequency of metastasis, whereas the kinase signaling subgroup was validated as having a relatively low frequency of metastatic disease (4.1%). However, only molecular categorization according to *SDHB* mutation status, but not other molecular systems or mutations, was associated with metastasis in the multivariate models.

SDHB has been proposed as a negative prognostic factor for survival in metastatic PPGL (Amar *et al.* 2007, Turkova *et al.* 2015, Assadipour *et al.* 2017).

However, the recent study showed that *SDHB* mutation was not an independent prognostic factor for survival in a large cohort of metastatic PPGL (Hescot *et al.* 2019). Although our survival analysis did not show significant results for the molecular subgroups, Kaplan–Meier curves clearly indicate trend toward worse outcome on both TCA cycle and Wnt/unknown-related PPGL. Remarkably, no deaths occurred in patients with pseudohypoxia *VHL/EPAS1* as well as kinase signaling PPGLs. This information must be considered with caution since the number of events was very low but indicate that patient stratification beyond *SDHB* mutation status should be investigated in future studies.

Our review and analysis has a number of limitations: clinical annotations in general, and hormone evaluations in particular, showed a high risk of bias. Lack of data on *ATRX* inactivation or *TERT* expression is also a relevant limitation as it has been associated with higher frequency of metastasis as well as poor survival (Fishbein *et al.* 2015, 2017, Job *et al.* 2018). Selection bias is also likely as a majority of reviewed manuscripts comes from well-recognized groups at tertiary centers. Another bias may have been incorporated from our exclusion of patients without available tumor tissue, which could include a selection bias that excludes a relevant subgroup of patients (Roman-Gonzalez *et al.* 2018). The analysis of survival in the whole study cohort is likely skewed by the higher age in patients with sporadic PPGLs, which are less likely to have metastasis, compared to the pseudohypoxia group that develop disease earlier mainly due to genetic predisposition. Disease-related survival would be a preferred measurement, even though it could not be explored due to lack of data. Finally, there was a significant loss of patients for the multivariate analysis due to incomplete clinical annotations, which the subsequent limited statistical power that this implies.

Our findings demonstrated *SDHB* as independently associated with PPGL metastasis and do not favor the use of information on other driver genes as it was not independently correlated to metastatic disease. Due to relatively low patient number and various risks of bias, we predict that the observed trends for both metastasis and survival still indicate that there is a potential of molecular information to yield relevant information on PPGL outcome in future. To test this hypothesis large, preferably prospective, series with very complete clinical and genetic annotation will be required (Kimura *et al.* 2014, Turkova *et al.* 2015, Koh *et al.* 2017).

Conclusion

Our review and individual patient meta-analysis validated previous phenotype correlations including different frequencies of metastasis in-between PPGL driver genes. However, only *SDHB* mutation status remained significant in the multivariate model. Instead, the biochemical profile including dopamine secretion emerged as a more useful predictor of metastatic disease. Categorization accordingly to a driver gene mutation was not an independent factor associated with survival in this study.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/ERC-19-0024>.

Declaration of interest

J C received lecture honoraria from Novartis. The other authors have nothing to disclose.

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