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Lack of Utility of SDHB Mutation Testing in Adrenergic Metastatic Phaeochromocytoma

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Abstract

Objective: Testing for succinate dehydrogenase subunit B (*SDHB*) mutations is recommended in all patients with metastatic phaeochromocytomas and paragangliomas (PPGLs), but may not be required when metastatic disease is accompanied by adrenaline production. This retrospective cohort study aimed to establish the prevalence of *SDHB* mutations among patients with metastatic PPGLs characterised by production of adrenaline compared to those without production of adrenaline, and to establish genotype-phenotype features of metastatic PPGLs according to underlying gene mutations.

Design & Methods: Presence of *SDHB* mutations or deletions was tested in 205 patients (114 males) aged 42±16 yrs (range 9 to 86 yrs) at diagnosis of metastatic PPGLs with and without adrenaline production.

Results: Twenty-three of the 205 patients (11%) with metastatic PPGLs had disease characterized by production of adrenaline, as defined by increased plasma concentrations of metanephrine larger than 5% of the combined increase of both normetanephrine and metanephrine. None of these 23 patients had *SDHB* mutations. Of the other 182 patients with no tumoural adrenaline production, 51% had *SDHB* mutations. Metastases in bone were 36% to 41% more prevalent among patients with *SDHB* mutations or extra-adrenal primary tumours than those without mutations or with adrenal primary tumours. Liver metastases were 81% more prevalent among patients with adrenal than extra-adrenal primary tumours.

Conclusion: *SDHB* mutation testing has no utility among patients with adrenaline-producing metastatic PPGLs, but is indicated in other patients with metastatic disease. Our study also reveals novel associations of metastatic spread with primary tumour location and presence of *SDHB* mutations.

Introduction

Phaeochromocytomas and paragangliomas (PPGLs) are catecholamine-producing tumours that respectively arise from adrenal medullary or paraganglial chromaffin cells (1, 2). Over 30% of PPGLs have a hereditary basis due to mutations of more than 11 tumour-susceptibility genes identified to date (3, 4). In a significant proportion of cases mutations are found without any clear family history or syndromic features suggesting a hereditary basis (5-10). Identification of mutations in such patients is important since this impacts subsequent patient management. Mutation testing can also lead to identification of other family members with the same mutation, who are at risk for PPGLs and other tumours and who can benefit from routine screening to identify disease at an early stage.

Due to the above considerations, it has been suggested that all patients with PPGLs should undergo testing for germline mutations of tumour susceptibility genes regardless of family history or syndromic features (5). Although costs of mutation testing may be significantly reduced with next generation sequencing, currently the testing of all genes in every patient with PPGLs is costly and not recommended (11, 12). Rather the decision to test and selection of genes to be tested should be based on genotype-phenotype considerations (9, 11, 12). High risk of metastatic disease in patients with mutations of the succinate dehydrogenase subunit B (*SDHB*) gene (7), leading to high prevalence of *SDHB* mutations in patients with metastatic PPGLs (13), has in particular led to agreement that all patients with metastatic PPGLs should be considered for testing of that gene (6, 7, 9, 11). This recommendation is now supported by Endocrine Society Guidelines on PPGLs (12).

PPGLs in patients with *SDHB* mutations are characterised by production of noradrenaline and/or dopamine, without significant production of adrenaline (14). This suggests that testing for *SDHB* mutations may not be required if metastatic disease is associated with significant adrenaline production. There are, however, limited data concerning biochemical phenotypic features in metastatic PPGLs according to *SDHB* mutation status. Consequently, as indicated by the recently published guidelines (12), recommended testing of *SDHB* mutations in patients with malignant PPGLs remains independent of any consideration concerning biochemical phenotypes.

The primary objective of this study was therefore to establish the prevalence of *SDHB* mutations among patients with metastatic PPGLs with and without adrenaline production. Secondary objectives included characterisation of other phenotypic features in patients with metastatic PPGLs according to *SDHB* mutation status and primary tumour location.

Patients and Methods

Patients

The study involved retrospective analysis of data from 205 patients with metastatic PPGLs diagnosed on imaging evidence of metastatic disease combined with either or both a past history of pathologically proven PPGLs or biochemical evidence of excess catecholamine production. Imaging evidence of metastatic lesions at sites where chromaffin cells are normally absent involved a combination of computed tomography or magnetic resonance imaging with one or more of several functional imaging modalities: ^{123}I-metaiodobenzylguanidine scintigraphy, ^{68}Ga-DOTATATE positron emission tomography (PET), ^{18}F-fluoro-2-deoxy-D-glucose PET, ^{18}F-3,4-dihydroxyphenylalanine PET, and ^{18}F-fluorodopamine PET. Patients were investigated at the National Institutes of Health or at seven European centres under the multicentre prospective monoamine-producing tumour protocol (https://pmt-study.pressor.org/jsp/home.jsp). Written informed consent was obtained from all patients.

Inclusion criteria and data collection

Apart from presence of metastases at locations where chromaffin cells are normally absent (e.g., bones, lungs, liver, lymph nodes), consecutive inclusion of patients into the analysis required two key criteria: 1. chromatography-based measurements of plasma concentrations of normetanephrine and metanephrine performed on blood samples taken when metastatic disease was present; and 2. assessment for presence of *SDHB* mutations determined by Sanger sequencing and multiplex ligation-

dependent probe amplification (MLPA). The latter was commonly performed using the p226 SDH kit from MRC-Holland (Amsterdam, The Netherlands).

Other required data were restricted to gender, date of birth, date of first diagnosis of primary tumours and metastatic disease and locations of primary tumours and metastases. Dimensions of primary tumours were available from 176 of the 205 patients. In line with current recommendations, testing of tumour susceptibility genes other than *SDHB* was not routinely performed unless indicated by disease presentation or other considerations.

Definition of adrenaline-producing PPGLs

As described previously (15), adrenaline-producing tumours were defined by both increased plasma concentrations of metanephrine above the upper cut-offs (88 pg/mL, 0.45 nmol/L) and increases of metanephrine larger than 5% of the combined increases of both plasma normetanephrine and metanephrine. The latter was defined by the equation $\%MN_t = (MN_t/NMN_t+MN_t).100$ where MN_t and NMN_t are tumour-derived plasma concentrations of metanephrine and normetanephrine. As described previously (15), tumour-derived concentrations were determined by subtracting mean concentrations in a reference population of patients without PPGLs from measured concentrations in patients with PPGLs.

Statistics

Statistical analysis was performed using JMP Pro10 (10.0.1.1), with significance established by Chi-squared, Wilcoxon, Kruskal-Wallis, Steel-Dwass nonparametric multiple comparison nominal logistic multivariate tests as appropriate. Principal components analysis was carried out for 3 principal components defining tumoural adrenaline production that clustered data in 3 dimensional space separately.

Results

The 205 patients with metastatic PPGLs (114 males) were aged 36±16 yrs (range 6-83 yrs) at initial diagnosis of PPGLs and 42±16 yrs at diagnosis of metastatic disease (range 9-86 yrs). Sixty-three patients (31%) had primary tumours at adrenal locations, 132 (64%) at extra-adrenal locations and 10 (5%) at both locations with multifocal tumours. Metastases in bones, liver, lungs and lymph nodes were respectively identified in 75%, 40%, 36% and 56% of patients, mostly showing multiple locations. Ninety-three of the 205 patients with metastatic PPGLs (45%) harboured *SDHB* mutations (Supplemental Table). Mutation testing, when clinically indicated among the other 112 patients, revealed mutations of subunit D of succinate dehydrogenase (*SDHD)* in 13 patients, subunit A of succinate dehydrogenase (*SDHA*) in 1 patient, the von Hippel-Lindau gene in 5 patients and the *RET* (rearranged during transfection) gene in 5 patients.

Among all 205 patients, 30 patients presented with elevations of metanephrine (Fig 1). Among these 30 patients, 23 had increases of tumour-derived metanephrine higher than 5% of the combined increases of both normetanephrine and metanephrine, defining these patients as adrenaline-producing metastatic PPGLs. The other 7 patients with elevations of metanephrine had much larger elevations in normetanephrine, so that this did not reach the 5% criterion for defining significant adrenaline production. The twenty-three patients (11%) with adrenaline-producing metastatic PPGLs, none of who had *SDHB* gene mutations, were clearly distinguished from all other patients. Thus, of the other 182 patients with no tumoural adrenaline production, a higher proportion of 93 patients had *SDHB* mutations compared to those exhibiting an adrenaline-producing phenotype (51 % vs. 0% $P<0.0001$) (Table 1). Twenty-two of the 23 patients with an adrenergic biochemical phenotype had primary tumours localised to the adrenals, a higher proportion ($P<0.0001$) than for patients with metastatic PPGLs without adrenaline production (96% vs. 28%). Adrenergic metastatic disease was characterised by 27-fold higher ($P<0.0001$) plasma concentrations of metanephrine compared to disease without adrenaline production. No differences in locations of metastases were apparent according to differences in catecholamine biochemical phenotypes.

Patients with *SDHB* gene mutations were on average 9 and 11 years younger at respective diagnosis of primary tumours and metastatic disease compared to those without *SDHB* mutations (Table 2). Twenty-three of 34 paediatric patients diagnosed with primary tumours before reaching 18 years (range 6-18 yrs) had *SDHB* mutations, a higher proportion than the 70 of 171 adult cases with *SDHB* gene mutations (68% vs. 41%, P= 0.0043). Male gender was more prevalent among patients with *SDHB* gene mutations compared to those without *SDHB* gene mutations (65% vs. 48%, P=0.0193). This difference reflected 17 of 20 paediatric male patients who had *SDHB* gene mutations compared to 43 of 94 adult male patients with *SDHB* gene mutations (85% vs. 46%, P=0.0016).

Metastatic disease secondary to adrenal phaeochromocytomas was associated with a higher (P<0.0001) proportion of adrenergic phenotypic features and a lower proportion (P<0.0001) of *SDHB* mutations than disease secondary to extra-adrenal paragangliomas (Table 3). Age at first diagnosis of primary tumours and metastatic disease was higher (P<0.001) for patients with adrenal than extra-adrenal primary tumours. Thirty-four paediatric patients were characterised by a higher (P=0.0087) prevalence of tumours at extra-adrenal and multifocal adrenal and extra-adrenal locations (88%) compared to adults (65%).

Patients with *SDHB* mutations showed a 36% higher (P=0.0002) prevalence of metastases in bone compared to those without *SDHB* mutations (Table 2). There was also a 41% higher (P=0.0006) prevalence of bone metastases among patients with extra-adrenal than adrenal primary tumours, whereas liver metastases were 81% more prevalent (P<0.001) among patients with adrenal than extra-adrenal primary tumours (Table 3). Multivariate analysis (with *SDHB* mutation status, tumour location and catecholamine biochemical phenotype as independent variables) indicated that for bone metastases *SDHB* gene mutation status (P=0.0145), and location of primary tumours (P=0.0370), but not catecholamine phenotype (P=0.7902) remained the critical determinants. Similarly, location of the primary tumour remained the only significant (P=0.0041) determinant for liver metastases when both *SDHB* mutation status (P=0.9655) and catecholamine biochemical phenotype (P=0.6093) were considered as additional variables by multivariate analysis.

Discussion

This study, involving one of the largest cohorts of patients with metastatic PPGLs yet described, establishes adrenergic phenotype-related differences in disease-causing *SDHB* mutations, not only relevant to testing of the gene, but also to interpretation and follow-up of test results. The study also provides confirmatory evidence for relationships between presence of *SDHB* mutations, tumour location and differences in ages and gender of disease presentation, but importantly presents novel findings relating the former two variables to occurrence of metastasis at different locations.

The testing of every tumour-susceptibility gene in every PPGL patient is not supported by any published evidence to date relating cost-effectiveness to clinical outcome (11, 12). Nevertheless, the high prevalence of *SDHB* mutations among patients with metastatic PPGLs leaves little doubt that targeted *SDHB* testing is warranted for this presentation (6, 7, 9, 11-13). Findings of an *SDHB* mutation in such patients may be particularly important for identification of other family members with mutations who may benefit from routine screening and therapeutic interventions at an early stage before metastasis occurs.

As we now show here, complete lack of *SDHB* mutations among 23 patients with metastatic PPGLs characterised by adrenergic biochemical features indicates that testing of the *SDHB* gene is of no benefit when disease is associated with significant production of adrenaline, as indicated by increases in plasma metanephrine. Guidelines for *SDHB* testing, such as those recently published by the Endocrine Society (12), should thus be modified to exclude testing when disease is accompanied by increases in metanephrine. For those patients presenting with adrenaline-producing metastatic PPGLs, testing for *RET*, *MAX*, *TMEM127* or *NF1* gene mutations could be considered. However, we would not recommend such testing unless patients present with a family history consistent with hereditary PPGLs, evidence of syndromic features, young age or bilateral adrenal tumours.

The above considerations and recommendations are consistent with current guidelines that genetic testing should be prioritised according to clinical features, with specific genes targeted according to those features (12). As also outlined in those guidelines such selective approaches to genetic testing

may be become obsolete with introduction of next-generation sequencing (NGS) methods that allow rapid and low-cost analysis of all PPGLs susceptibility genes. Nevertheless, whether NGS should be applied indiscriminately to all patients with PPGLs, including those with adrenaline-producing metastatic tumours, requires evidence from carefully designed prospective outcome studies clearly establishing that benefits to patients and their families outweigh costs and potential harms. Such harms include wrongful designation of non-functional polymorphisms as pathogenic mutations. As outlined elsewhere (16) this problem is likely to become highly relevant with NGS, for which interpretation of pathogenicity among detected variants of unknown significance can be a major challenge. Such problems have already surfaced in several case reports of test results in patients with PPGLs initially interpreted to indicate a mutation, but subsequently determined to reflect a non-pathogenic variant (17, 18). In such cases biochemical findings that do not fit the genotype can provide useful information to review relative to potential pathogenicity before any subsequent actions may adversely affect patients or their family.

The 23 patients in the present study with adrenaline-producing malignant PPGLs included one case with a retroperitoneal paravertebral extra-adrenal tumour. Extra-adrenal paragangliomas very rarely produce significant amounts of adrenaline. This patient therefore represents an exception to the rule and it is important to note that all 22 of the other cases of adrenaline-producing metastatic PPGLs included patients with adrenal primary tumours. From this it might be surmised that patients with metastatic PPGLs due to adrenal primary tumours might also not benefit from testing for *SDHB* mutations. Indeed, while the prevalence of *SDHB* mutations among patients with primary tumours localized to the adrenals is much lower than that for patients with extra-adrenal tumours (13% vs. 62%), it is nevertheless the presence or absence of adrenaline production that better defines likelihood of an underlying *SDHB* mutation.

Our findings of associations of *SDHB* mutations with extra-adrenal locations of primary tumours and young age at diagnosis are in agreement with several previous studies (5-7, 9-11, 13, 19). Bausch *et al.* showed *SDHB* gene mutations were highly prevalent among paediatric patients with hereditary and malignant disease (20). Higher prevalence of *SDHB* mutations in children than in adults with

metastatic disease is also consistent with other findings in a paediatric series establishing a high proportion of cases with *SDHB* mutations and metastatic disease, (21); it was concluded that testing for *SDHB* mutations and follow-up to check for metastasis is particularly important in paediatric cases of PPGLs. We extend these findings by now showing that male gender is more prevalent among paediatric than adult patients with *SDHB* mutations who develop metastatic PPGLs. Male gender has been previously described by Neumann *et al.* (22) as an independent risk factor for *SDHx* germline mutations among patients with head and neck paragangliomas, but the basis of this observation and how it relates to the present findings are not established.

The higher prevalence of bone metastasis in patients with *SDHB* mutations compared to those without *SDHB* mutations represents novel unexpected findings. The higher prevalence of liver metastasis associated with adrenal than extra-adrenal tumours and the reverse higher prevalence of bone metastasis associated with extra-adrenal compared to adrenal tumours represent other novel findings. By multivariate analysis we show that the catecholamine biochemical phenotype appears irrelevant to these differences in metastatic spread. It remains unclear why the presence of an *SDHB* mutation or extra-adrenal tumour predisposes to bone metastasis whereas presence of an adrenal primary tumour predisposes to liver metastasis.

In summary, lack of *SDHB* mutations among patients with adrenaline-producing metastatic PPGLs indicates that it is unnecessary to test for *SDHB* mutations among such patients. High prevalence of *SDHB* mutations among other patients with metastatic PPGLs supports recommendations that these patients should all be considered for *SDHB* mutation testing. Our data also indicate associations of metastatic spread with primary tumour location and presence of *SDHB* mutations.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

All authors contributed equally to this publication.

282 **Figure Legend**

283 **Figure 1.**

284 Principal components analysis 3 dimensional scatter plot illustrating clustering of patients with
285 metastatic PPGLs according to presence or absence of tumoural adrenaline production and *SDHB*
286 gene mutations. Values shown on the axis are in log scale, whereas values of metanephrine and
287 normetanephrine are in units of nanomoles per litre. %MNt is defined as the increase of tumour-
288 derived plasma metanephrine as a per cent of both normetanephrine and metanephrine as described in
289 methods. Patients with tumours producing adrenaline are indicated by triangles, whereas patients with
290 tumours without adrenaline production are indicated by dots or circles, with patients with *SDHB*
291 mutations are shown by circles, and those without *SDHB* mutations are shown by dots or triangles.

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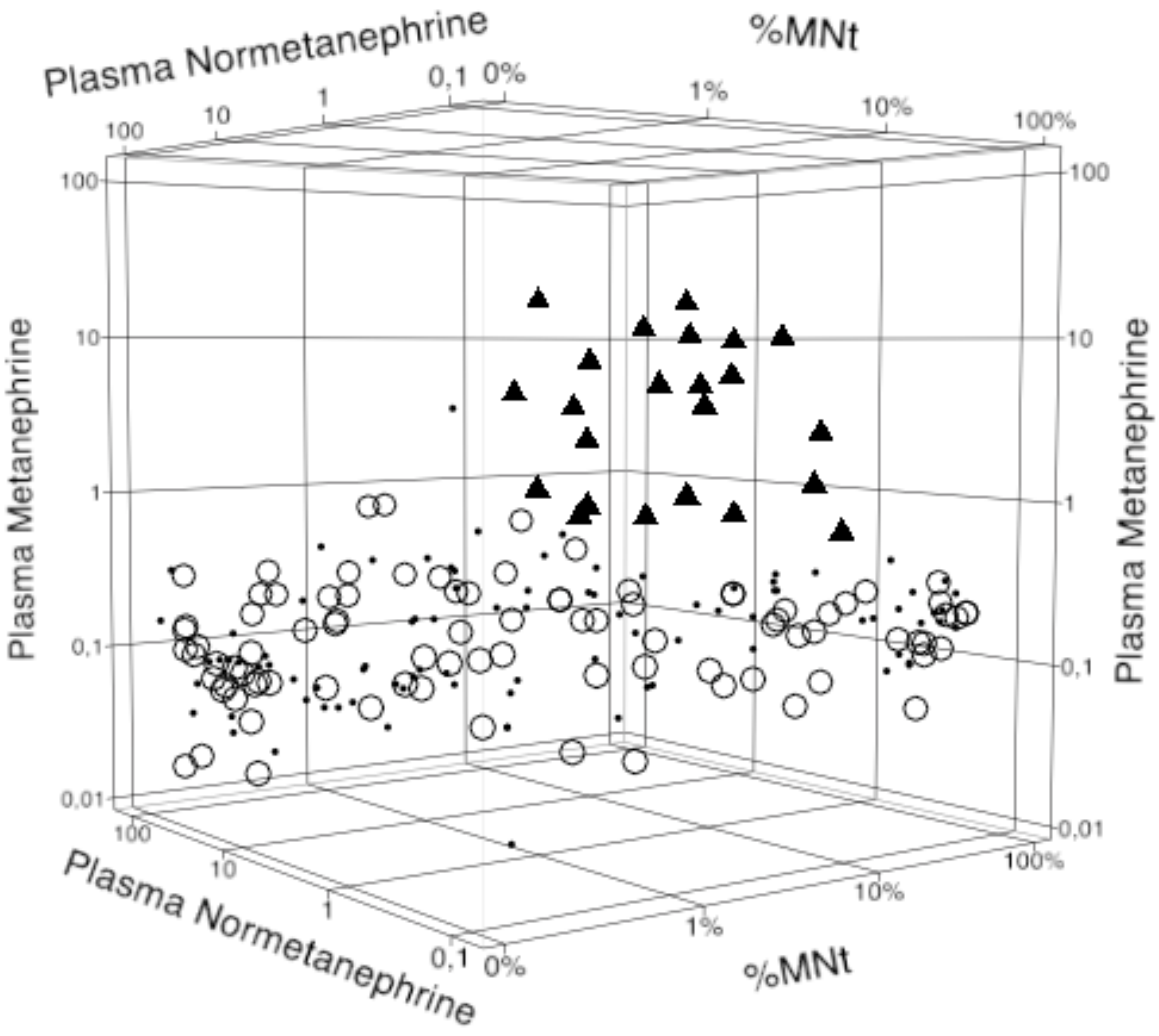
Figure 1.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10
1
0,1
0%
1%
10%
100%
Plasma Metanephrine
0,01
Plasma Normetanephrine
%MNt

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TABLE 1. Disease-associated characteristics of metastatic PPGLs according to production or lack of production of adrenaline.

	Adrenaline production	No Adrenaline production	P value
N	23	182	
Male gender	8 (35%)	106 (58%)	0.0329
SDHB mutation	0 (0%)	93 (51%)	<0.0001
Adrenal primary tumour*	22 (96%)	51 (28%)	<0.0001
Primary tumour volume (mL)†	178±56	132±20	0.1812
Age at first diagnosis (yrs)§	39±12	35±16	0.2316
Age at metastasis (yrs)§	48±13	41±16	0.0664
Plasma MN (nmol/L)†	5.3±1.1	0.2±0.1	<0.0001
Plasma NMN (nmol/L)†	14.0±3.7	11.3±1.6	0.0079
Location of metastases			
Bone	14 (61%)	139 (77%)	0.1074
Liver	12 (52%)	70 (38%)	0.2059
Lungs	6 (26%)	67 (37%)	0.3114
Lymph nodes	12 (52%)	102 (56%)	0.7249

Abbreviations: mL, milliliter; nmol/L, nanomoles per litre; MN, metanephrine; NMN, normetanephrine. *Primary tumors designated with an adrenal location include 10 with multifocal adrenal and extra-adrenal locations; † means±SE; § means±SD.

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TABLE 2. Disease-associated characteristics of metastatic PPGLs according to presence or absence of SDHB mutations.

	SDHB **mutation**	**No *SDHB*** **mutation**	**P value**
N	93	112	
Male gender	60 (65%)	54 (48%)	0.0193
Adrenergic disease	0 (0%)	23 (21%)	<0.0001
Adrenal primary tumour*	11 (12%)	62 (55%)	<0.0001
Primary tumour volume (mL)†	131±20	142±29	0.1548
Age at first diagnosis (yrs)§	31±15	40±16	<0.0001
Age at metastasis (yrs)§	36±14	47±15	<0.0001
Plasma MN (nmol/L)†	0.2±0.1	1.3±0.3	0.0008
Plasma NMN (nmol/L)†	14.0±2.6	9.6±1.7	0.7140
Location of metastases			
Bone	81 (87%)	72 (64%)	0.0002
Liver	32 (34%)	50 (45%)	0.1365
Lungs	30 (32%)	43 (39%)	0.3611
Lymph nodes	52 (56%)	62 (55%)	0.9363

Abbreviations: mL, milliliter; nmol/L, nanomoles per litre; MN, metanephrine; NMN, normetanephrine. *Primary tumors designated with an adrenal location include 10 with multifocal adrenal and extra-adrenal locations; † means±SE; § means±SD.

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TABLE 3. Disease-associated characteristics of metastatic PPGLs according to adrenal and extra-adrenal locations of primary tumors.

	Adrenal	Extra-Adrenal	Adrenal & extra-adrenal	P value*
N	63	132	10	
Male gender	28 (44%)	80 (61%)	6 (60%)	0.1005
Adrenergic phenotype	21 (33%)***	1 (1%)	1 (10%)	<0.0001
SDHB mutation	8 (13%)***	82 (62%)	3 (30%)	<0.0001
Primary tumour volume (mL)†	195±46	105±15	193±405	0.3374
Age at first diagnosis (yrs)§	43±16***	33±15	31±21	0.0003
Age at metastasis (yrs)§	50±13***	38±15	37±20	<0.0001
Plasma MN (nmol/L)†	2.0±0.5	0.2±0.1	0.4±0.3	0.0676
Plasma NMN (nmol/L)†	14.3±3.2**	10.7±1.8	5.8±2.0	0.0052
Location of metastases				
Bone	37 (59%)***	110 (83%)	6 (60%)	0.0006
Liver	35 (56%)***	41 (31%)	6 (60%)	0.0020
Lungs	24 (38%)	43 (33%)	6 (60%)	0.1926
Lymph nodes	36 (57%)	72 (55%)	6 (60%)	0.9055

Abbreviations: mL, milliliter; nmol/L, nanomoles per litre; MN, metanephrine; NMN, normetanephrine. *P value indicates overall significance by Kruskal-Wallis multiple comparison tests. ** and *** designates significant difference (P<0.01) and (P<0.001) from extra-adrenal group by post hoc testing; † means±SE; § means±SD.

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Supplemental Table 1. Germline mutations of SDHB identified among malignant PPGLs patients.

Exon / intron	Mutation cDNA nucleotide change	Mutation protein change	Coding effect	Number of cases
1 to 8	c.1-c.843del	Exon 1-8 Deletion	Whole Gene Deletion	3
1	c.1A>T	p.Met1?	Start Loss	1
1	c.1_72del	Exon 1 Deletion	Deletion	6
1	c.26T>A	p.Leu9*	Nonsense mutation	1
IVS1	c.72+1G>T	Splice site mutation	Splice site mutation	8
IVS1	c.73-9A>G (IVS1-9A>G)	Splice site mutation	Splice site mutation	1
2	c.136C>T	p.Arg46*	Nonsense mutation	8
2	c.137G>A	p.Arg46Gln	Missense mutation	3
3	c.268C>T	p.Arg90*	Nonsense mutation	4
3	c.271A>T	p.Arg91*	Nonsense mutation	1
3	c.274T>C	p.Ser92Pro	Missense mutation	1
3	c.275C>A	p.Ser92*	Nonsense mutation	1
3	c.277T>C	p.Cys93Arg	Missense mutation	1
IVS3	c.286+1G>A	Splice site mutation	Splice site mutation	2
IVS3	c.286+2T>A	Splice site mutation	Splice site mutation	3
IVS3	c.287-1G>C	Splice site mutation	Splice site mutation	1
4	c.287G>A	p.Gly96Asp	Missense mutation	1
4	c.330delTC	p.Leu111Serfs*7	Frameshift mutation	1
4	c.343C>T	p.Arg115*	Nonsense mutation	1
4	c.369_370insA	p.Val124Serfs*39	Frameshift mutation	1
4	c.380T>G	p.Ile127Ser	Missense mutation	6
4	c.392delC	p.Pro131Hisfs*5	Frameshift mutation	1
4	c.395A>C	p.His132Pro	missense mutation	1
4	c.418G>T	p.Val140Phe	Missense mutation	7
IVS4	c.423+1G>A	Splice site mutation	Splice site mutation	2
5	c.445-447delCAinsGGTATCT	p.Gln149Glyfs*11	Frameshift mutation	1
5	c.445C>T	p.Gln149*	Nonsense mutation	1
IVS5	c.541-2A>G(IVS5-2A>G)	Splice site mutation	Splice site mutation	2
6	c.553G>T	p.Glu185*	Nonsense mutation	1
6	c.574T>C	p.Cys192Arg	Missense mutation	2
6	c.587G>A	p.Cys196Tyr	Missense mutation	3
6	c.590C>G	p.Pro197Arg	Missense mutation	1
6	c.600G>T	p.Trp200Cys	Missense mutation	1
6	c.626C>T	p.Pro209Leu	Missense mutation	1
6	c.642G>C	p.Gln214His	Missense mutation	1
IVS6	c.642+1G>A	Splice site mutation	Splice site mutation	3
7	c.683_684delAG	p.Glu228Glyfs*27	Frameshift mutation	1
7	c.688C>T	p.Arg230Cys	Missense mutation	2
7	c.689G>A	p.Arg230His	Missense mutation	2
7	c.689G>T	p.Arg230Leu	Missense mutation	2
7	c.725G>A	p.Arg242His	Missense mutation	1
7	c.727T>A	p.Cys243Ser	Missense mutation	1
7	c.761dup	p.Lys255*	Frameshift mutation	1

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