

Lack of utility of SDHB mutation testing in adrenergic metastatic pheochromocytoma

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1 **Lack of Utility of SDHB Mutation Testing in Adrenergic Metastatic Pheochromocytoma**

2

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40

41 **Abstract**

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

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

51 Results: Twenty-three of the 205 patients (11%) with metastatic PPGLs had disease characterized by
52 production of adrenaline, as defined by increased plasma concentrations of metanephrine larger than
53 5% of the combined increase of both normetanephrine and metanephrine. None of these 23 patients
54 had *SDHB* mutations. Of the other 182 patients with no tumoural adrenaline production, 51% had
55 *SDHB* mutations. Metastases in bone were 36% to 41% more prevalent among patients with *SDHB*
56 mutations or extra-adrenal primary tumours than those without mutations or with adrenal primary
57 tumours. Liver metastases were 81% more prevalent among patients with adrenal than extra-adrenal
58 primary tumours.

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

63

64 **Introduction**

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

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

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

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

94

95 **Patients and Methods**

96 **Patients**

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

108

109 **Inclusion criteria and data collection**

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

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

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

122

123 **Definition of adrenaline-producing PPGLs**

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

132

133 **Statistics**

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

139

140

141

142 Results

143 The 205 patients with metastatic PPGLs (114 males) were aged 36 ± 16 yrs (range 6-83 yrs) at initial
144 diagnosis of PPGLs and 42 ± 16 yrs at diagnosis of metastatic disease (range 9-86 yrs). Sixty-three
145 patients (31%) had primary tumours at adrenal locations, 132 (64%) at extra-adrenal locations and 10
146 (5%) at both locations with multifocal tumours. Metastases in bones, liver, lungs and lymph nodes
147 were respectively identified in 75%, 40%, 36% and 56% of patients, mostly showing multiple
148 locations. Ninety-three of the 205 patients with metastatic PPGLs (45%) harboured *SDHB* mutations
149 (Supplemental Table). Mutation testing, when clinically indicated among the other 112 patients,
150 revealed mutations of subunit D of succinate dehydrogenase (*SDHD*) in 13 patients, subunit A of
151 succinate dehydrogenase (*SDHA*) in 1 patient, the von Hippel-Lindau gene in 5 patients and the *RET*
152 (rearranged during transfection) gene in 5 patients.

153 Among all 205 patients, 30 patients presented with elevations of metanephrine (Fig 1). Among these
154 30 patients, 23 had increases of tumour-derived metanephrine higher than 5% of the combined
155 increases of both normetanephrine and metanephrine, defining these patients as adrenaline-producing
156 metastatic PPGLs. The other 7 patients with elevations of metanephrine had much larger elevations in
157 normetanephrine, so that this did not reach the 5% criterion for defining significant adrenaline
158 production. The twenty-three patients (11%) with adrenaline-producing metastatic PPGLs, none of
159 who had *SDHB* gene mutations, were clearly distinguished from all other patients. Thus, of the other
160 182 patients with no tumoural adrenaline production, a higher proportion of 93 patients had *SDHB*
161 mutations compared to those exhibiting an adrenaline-producing phenotype (51 % vs. 0% $P<0.0001$)
162 (Table 1). Twenty-two of the 23 patients with an adrenergic biochemical phenotype had primary
163 tumours localised to the adrenals, a higher proportion ($P<0.0001$) than for patients with metastatic
164 PPGLs without adrenaline production (96% vs. 28%). Adrenergic metastatic disease was
165 characterised by 27-fold higher ($P<0.0001$) plasma concentrations of metanephrine compared to
166 disease without adrenaline production. No differences in locations of metastases were apparent
167 according to differences in catecholamine biochemical phenotypes.

168 Patients with *SDHB* gene mutations were on average 9 and 11 years younger at respective diagnosis
169 of primary tumours and metastatic disease compared to those without *SDHB* mutations (Table 2).
170 Twenty-three of 34 paediatric patients diagnosed with primary tumours before reaching 18 years
171 (range 6-18 yrs) had *SDHB* mutations, a higher proportion than the 70 of 171 adult cases with *SDHB*
172 gene mutations (68% vs. 41%, $P=0.0043$). Male gender was more prevalent among patients with
173 *SDHB* gene mutations compared to those without *SDHB* gene mutations (65% vs. 48%, $P=0.0193$).
174 This difference reflected 17 of 20 paediatric male patients who had *SDHB* gene mutations compared
175 to 43 of 94 adult male patients with *SDHB* gene mutations (85% vs. 46%, $P=0.0016$).
176 Metastatic disease secondary to adrenal pheochromocytomas was associated with a higher
177 ($P<0.0001$) proportion of adrenergic phenotypic features and a lower proportion ($P<0.0001$) of *SDHB*
178 mutations than disease secondary to extra-adrenal paragangliomas (Table 3). Age at first diagnosis of
179 primary tumours and metastatic disease was higher ($P<0.001$) for patients with adrenal than extra-
180 adrenal primary tumours. Thirty-four paediatric patients were characterised by a higher ($P=0.0087$)
181 prevalence of tumours at extra-adrenal and multifocal adrenal and extra-adrenal locations (88%)
182 compared to adults (65%).

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

194

195 **Discussion**

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

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

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

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

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

234 The 23 patients in the present study with adrenaline-producing malignant PPGLs included one case
235 with a retroperitoneal paravertebral extra-adrenal tumour. Extra-adrenal paragangliomas very rarely
236 produce significant amounts of adrenaline. This patient therefore represents an exception to the rule
237 and it is important to note that all 22 of the other cases of adrenaline-producing metastatic PPGLs
238 included patients with adrenal primary tumours. From this it might be surmised that patients with
239 metastatic PPGLs due to adrenal primary tumours might also not benefit from testing for *SDHB*
240 mutations. Indeed, while the prevalence of *SDHB* mutations among patients with primary tumours
241 localized to the adrenals is much lower than that for patients with extra-adrenal tumours (13% vs.
242 62%), it is nevertheless the presence or absence of adrenaline production that better defines likelihood
243 of an underlying *SDHB* mutation.

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

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

256 The higher prevalence of bone metastasis in patients with *SDHB* mutations compared to those without
257 *SDHB* mutations represents novel unexpected findings. The higher prevalence of liver metastasis
258 associated with adrenal than extra-adrenal tumours and the reverse higher prevalence of bone
259 metastasis associated with extra-adrenal compared to adrenal tumours represent other novel findings.

260 By multivariate analysis we show that the catecholamine biochemical phenotype appears irrelevant to
261 these differences in metastatic spread. It remains unclear why the presence of an *SDHB* mutation or
262 extra-adrenal tumour predisposes to bone metastasis whereas presence of an adrenal primary tumour
263 predisposes to liver metastasis.

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

269

270 **Declaration of interest**

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

273

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278

279 **Author contribution statement**

280 All authors contributed equally to this publication.

281

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.

292

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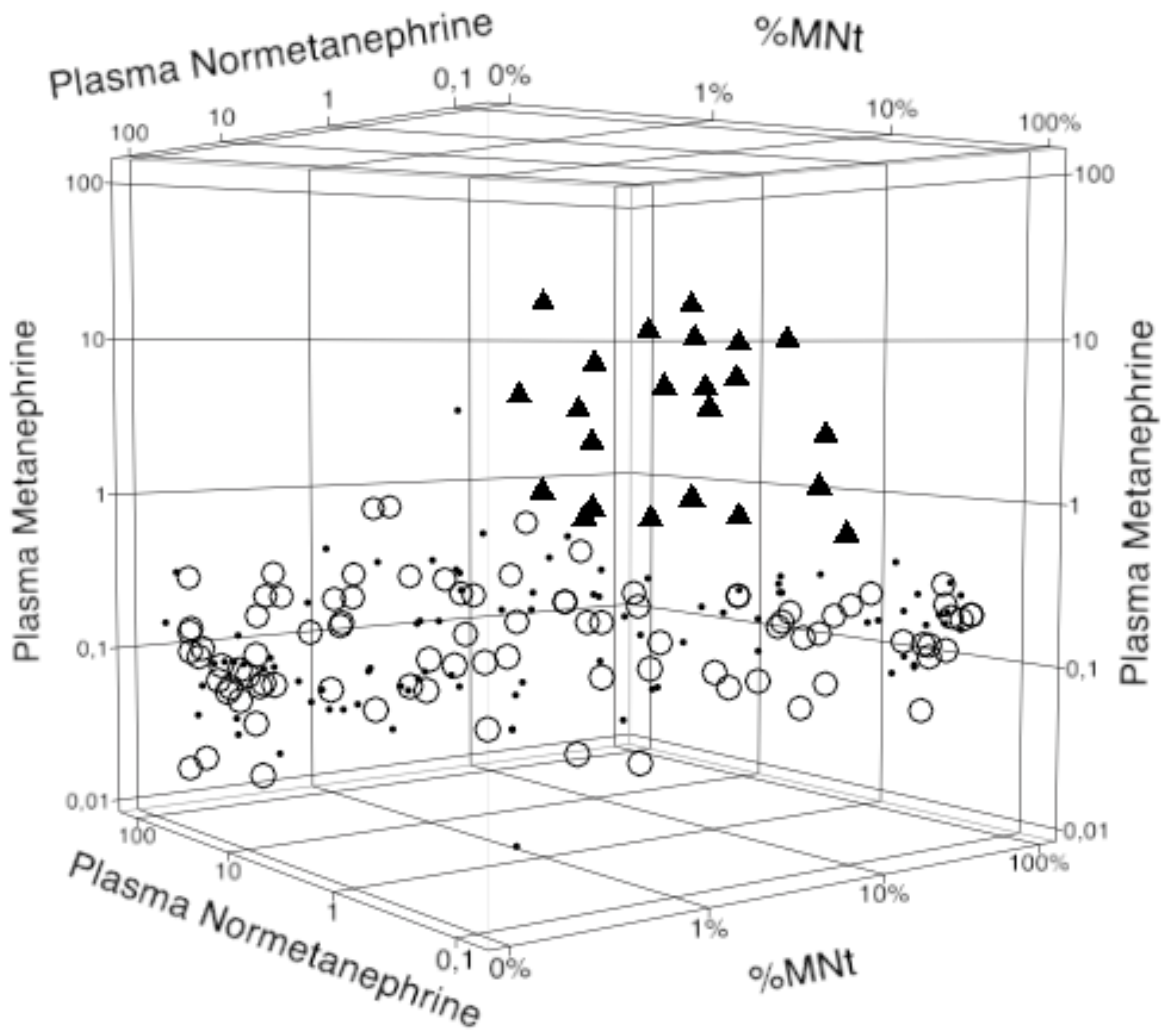
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Figure 1.



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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
<i>SDHB</i> 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
<i>SDHB</i> 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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