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 with those without production of adrenaline, and to establish genotype–phenotype features of metastatic PPGLs according to underlying gene mutations.

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

Results: Twenty-three of the 205 patients (11%) with metastatic PPGLs had disease characterised by production of adrenaline, as defined by increased plasma concentrations of metanephrine larger than 5% of the combined increase in 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 36–41% 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, 6, 7, 8, 9, 10). Identification of mutations in such patients is important because 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 (NGS), 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 in 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: 123I-metaiodobenzylguanidine scintigraphy, 68Ga-DOTATATE positron emission tomography (PET), 18F-fluoro-2-deoxy-D-glucose PET, 18F-3,4-dihydroxyphenylalanine PET, and 18F-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 the 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: i) chromatography-based measurements of plasma concentrations of normetanephrine and metanephrine in blood samples taken when metastatic disease was present and ii) assessment for the 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 in metanephrine larger than 5% of the combined increases in both plasma normetanephrine and metanephrine. The latter was defined by the equation $\%MN_t = \left(\frac{MN_t}{NMN_t + MN_t}\right) \times 100$ 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.

**Statistical analyses**

Statistical analysis was performed using JMP Pro10 (10.0.1.1), with significance established by $\chi^2$, Wilcoxon, Kruskal–Wallis, Steel–Dwass nonparametric multiple comparison nominal logistic multivariate tests as appropriate. Principal components analysis was carried out for three principal components defining tumoural adrenaline production that clustered data in three-dimensional space separately.

**Results**

The 205 patients with metastatic PPGLs (114 males) were aged 36 ± 16 years (range 6–83 years) at initial diagnosis of PPGLs and 42 ± 16 years at diagnosis of metastatic disease (range 9–86 years). 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 (Supplementary Table 1, see section on supplementary data given at the end of this article). 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 one patient, the von Hippel–Lindau gene in five patients and the rearranged during transfection (RET) gene in five patients.

Among all 205 patients, 30 patients presented with elevations of metanephrine (Fig. 1). Among these 30 patients, 23 had increases in tumour-derived metanephrine higher than 5% of the combined increases in both normetanephrine and metanephrine, defining these patients as adrenaline-producing metastatic PPGLs. The other seven patients with elevations of metanephrine had much larger elevations of normetanephrine, so that this did not reach the 5% criterion for defining significant adrenaline production. The 23 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 with those exhibiting an adrenaline-producing phenotype.

**Figure 1**

Principal components analysis three-dimensional scatter plot illustrating clustering of patients with metastatic PPGLs according to presence or absence of tumoural adrenaline production and SDHB gene mutations. Values shown on the axes are in log scale. Values of metanephrine and normetanephrine are in units of nanomoles per litre. $\%MN_t$ is defined as the increase in tumour-derived plasma metanephrine as a per cent of both normetanephrine and metanephrine as described in methods. Patients with tumours-producing adrenaline are indicated by triangles, whereas patients with tumours without adrenaline production are indicated by dots or circles, with patients with SDHB mutations shown by circles, and those without SDHB mutations are by dots or triangles.
Metastatic disease secondary to adrenal pheochromocytomas 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 with adults (65%).

Patients with SDHB mutations showed a 36% higher \((P=0.0002)\) prevalence of metastases in bone compared with 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

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

<table>
<thead>
<tr>
<th></th>
<th>Adrenaline production</th>
<th>No adrenaline production</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>23</td>
<td>182</td>
<td>0.0329</td>
</tr>
<tr>
<td>Male gender</td>
<td>8 (35%)</td>
<td>106 (58%)</td>
<td>0.0032</td>
</tr>
<tr>
<td>SDHB mutation</td>
<td>22 (96%)</td>
<td>178 ± 56</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Primary tumour volume</td>
<td>39 ± 12</td>
<td>35 ± 16</td>
<td>0.2316</td>
</tr>
<tr>
<td>Age at first diagnosis</td>
<td>48 ± 13</td>
<td>41 ± 16</td>
<td>0.0664</td>
</tr>
<tr>
<td>Location of metastases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>14 (61%)</td>
<td>139 (77%)</td>
<td>0.1074</td>
</tr>
<tr>
<td>Liver</td>
<td>12 (52%)</td>
<td>70 (38%)</td>
<td>0.2059</td>
</tr>
<tr>
<td>Lungs</td>
<td>6 (26%)</td>
<td>67 (37%)</td>
<td>0.3114</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>12 (52%)</td>
<td>102 (56%)</td>
<td>0.7249</td>
</tr>
</tbody>
</table>

\(a^{Primary tumors designated with an adrenal location include ten with multifocal adrenal and extra-adrenal locations.}

\(b^{Means \pm S.E.M.}

\(c^{Means \pm t.d.}

\(\text{MN, metanephrine; NMN, normetanephrine.}

(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 that 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 with disease without adrenaline production. No differences in the 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 with those without SDHB mutations (Table 2). Twenty-three of 34 paediatric patients diagnosed with primary tumours before reaching 18 years (range 6–18 years) 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 with 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 with 43 of 94 adult male patients with SDHB gene mutations (85% vs 46%, \(P=0.0016\)).

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

<table>
<thead>
<tr>
<th></th>
<th>SDHB mutation</th>
<th>No SDHB mutation</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>93</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td>60 (65%)</td>
<td>54 (48%)</td>
<td>0.0193</td>
</tr>
<tr>
<td>Adrenergic disease</td>
<td>0 (0%)</td>
<td>23 (21%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adrenal primary tumour(a)</td>
<td>11 (12%)</td>
<td>62 (55%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Primary tumour volume(b)</td>
<td>131 ± 20</td>
<td>142 ± 29</td>
<td>0.1548</td>
</tr>
<tr>
<td>Age at first diagnosis(c)</td>
<td>31 ± 15</td>
<td>40 ± 16</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age at metastasis(c)</td>
<td>36 ± 14</td>
<td>47 ± 15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plasma MN (nmol/l)(b)</td>
<td>0.2 ± 0.1</td>
<td>1.3 ± 0.3</td>
<td>0.0008</td>
</tr>
<tr>
<td>Plasma NMN (nmol/l)(b)</td>
<td>14 ± 2.6</td>
<td>9.6 ± 1.7</td>
<td>0.7140</td>
</tr>
</tbody>
</table>

\(a^{Primary tumors designated with an adrenal location include ten with multifocal adrenal and extra-adrenal locations.}

\(b^{Means \pm S.E.M.}

\(c^{Means \pm t.d.}

\(\text{MN, metanephrine; NMN, normetanephrine.}

\(GZ\text{S.D.}

\(0.1548\) for patients with adrenal 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
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 the introduction of 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

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Adrenal</th>
<th>Extra-adrenal</th>
<th>Adrenal and extra-adrenal</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>63</td>
<td>132</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td>28 (44%)</td>
<td>80 (61%)</td>
<td>6 (60%)</td>
<td>0.1005</td>
</tr>
<tr>
<td>Adrenergic phenotype</td>
<td>21 (33%)†</td>
<td>1 (1%)</td>
<td>1 (10%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SDHB mutation</td>
<td>8 (13%)†</td>
<td>82 (62%)</td>
<td>3 (30%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Primary tumour volume (ml)b</td>
<td>195±46</td>
<td>105±15</td>
<td>193±405</td>
<td>0.3374</td>
</tr>
<tr>
<td>Age at first diagnosis (years)c</td>
<td>43±16c</td>
<td>33±15</td>
<td>31±21</td>
<td>0.0003</td>
</tr>
<tr>
<td>Age at metastasis (years)c</td>
<td>50±13c</td>
<td>38±15</td>
<td>37±20</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plasma MN (nmol/l)b</td>
<td>2.0±0.5</td>
<td>0.2±0.1</td>
<td>0.4±0.3</td>
<td>0.0676</td>
</tr>
<tr>
<td>Plasma NMN (nmol/l)b</td>
<td>14.3±3.2c</td>
<td>10.7±1.8</td>
<td>5.8±2.0</td>
<td>0.0052</td>
</tr>
</tbody>
</table>

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

MN, metanephrine; NMN, normetanephrine. * and †Significant difference (P<0.01) and (P<0.001) from extra-adrenal group by post hoc testing.

*P value indicates overall significance by Kruskal–Wallis multiple comparison tests.

*Means ± s.e.m.

*Means ± s.d.
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 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 localised 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 the 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, 6, 7, 9, 10, 11, 13, 19). Bausch et al. (20) showed SDHB gene mutations were highly prevalent among paediatric patients with hereditary and malignant disease. 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 among 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 with those without SDHB mutations represents novel unexpected findings. The higher prevalence of liver metastasis associated with adrenal than that with extra-adrenal tumours and the reverse higher prevalence of bone metastasis associated with extra-adrenal compared with 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.

**Supplementary data**
This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-14-0756.

**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.

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