The prevalence of *SDHB*, *SDHC*, and *SDHD* mutations in patients with head and neck paraganglioma and association of mutations with clinical features

R F Badenhop, J C Jansen, P A Fagan, R S A Lord, Z G Wang, W J Foster, P R Schofield


Paraganglioma (PGL) is a rare disorder (MIM 168000) characterised by tumours of the paraganglia, a collection of neuroendocrine tissues and small organs which are distributed throughout the body. The normal paraganglia play an important role in homeostasis either by acting directly as chemical sensors or by secreting catecholamines in response to stress. PGL is broadly categorised into two groups, those occurring in the head and neck region and those occurring elsewhere, with the adrenal medulla being the major site. Tumours in the head and neck have been detected in nearly 20 distinct locations including the jugular, vagal, tympanic, and aortic paraganglia, however the carotid body is the major site. The paraganglia of the head and neck region have sensory innervation and function as chemoreceptors. They are associated with the parasympathetic nervous system and are located in the vicinity of major arteries and nerves. The tumours usually present as an asymptomatic slow growing mass, lacking endocrine activity. The tumours are mostly benign but local expansion can cause cranial nerve deficit, invasion of the skull base, and eventually compression of the brain stem.

The incidence of head and neck PGL is difficult to determine, however estimates range from 1 in 30 000 to 1 in 100 000 in the general population. To date, four genetic loci have been implicated in the pathogenesis of head and neck PGL. *PGL1* was mapped to the long arm of chromosome 11 at 11q23 in several Dutch and North American families. Candidate gene analysis in the region revealed germline mutations in the succinate dehydrogenase subunit D (SDHD) gene in families carrying the *PGL1* locus. Linkage analysis of another unrelated Dutch pedigree revealed the presence of a second more proximal locus (*PGL2*) on 11q13. This locus remains unconfirmed. *PGL3* was independently mapped to 1q21 and a mutation in the succinate dehydrogenase subunit C (*SDHC*) gene was identified in a large German pedigree. Mutations in the succinate dehydrogenase subunit B (*SDHB*) gene (*PGL4*), mapped to 1p36, were identified by candidate gene analysis in both pedigrees with familial paraganglioma and non-syndromic familial phaeochromocytomas. In familial PGL, the disease is inherited in an autosomal dominant fashion and subject to not only age-dependent penetrance but also complete maternal imprinting in most pedigrees.

The *SDHD*, *SDHB*, and *SDHC* genes encode subunits of mitochondrial complex II (succinate dehydrogenase). Mitochondrial complex II is a heterotetrameric complex involved in the aerobic electron transport chain and catalyses the oxidation of succinate to fumarate (Krebs cycle) with transport of electrons to the ubiquinone pool. The complex is encoded by nuclear genes and consists of four subunits. These include a 70 kDa flavoprotein (*SDHA*), a 30 kDa iron-sulfur protein (*SDHB*), the 15 kDa large subunit of cytochrome *b* (*SDHC*), and the 12 kDa small subunit of cytochrome *b* (*SDHD*). *SDHA* and *SDHB* are the hydrophilic catalytic part of the complex and are highly conserved. *SHDC* and *SDHD* are hydrophobic integral membrane proteins which form cytochrome *b* and link the catalytic subunits to the matrix side of the mitochondrial inner membrane.

It has been estimated from recorded family history studies that the proportion of patients with glomus tumours attributed to genetic causes varies from 7–10% up to 50%. The aim of the current study is to determine the prevalence of genetic mutations in the *SDHD*, *SDHC*, and *SDHD* genes in an unbiased cohort of probands who have undergone examination or surgery for paragangliomas (rare tumours arising in the paraganglia) and to compare the clinical features in patients with familial paraganglioma with those in patients with sporadic tumours. We screened a total of 34 patients and identified mutations in 14 patients. The majority (79%) of these patients had mutations in *SDHD*, while the remaining 21% had mutations in *SDHB*. No mutations were detected in *SHDC*. Mutations in the *SDHD* and *SDHB* genes accounted for paraganglioma in 41% of this cohort, 91% of the familial cases, and 17% of the sporadic cases.

There were several differences in the clinical features between those patients with familial paraganglioma compared to those with sporadic tumours. Patients with familial paraganglioma had a significantly lower age of onset and were more likely to have carotid body or vagal tumours. Females were more likely than males to develop sporadic tumours.

A positive family history, bilateral tumours, early age of onset, and tumour type can help identify genetically determined cases of paraganglioma, thus facilitating targeted screening of affected patients and their relatives.

**Key points**

- This study aimed to determine the prevalence of *SDHB*, *SDHC*, and *SDHD* mutations in a population cohort of patients who had undergone examination or surgery for paragangliomas (rare tumours arising in the paraganglia) and to compare the clinical features in patients with familial paraganglioma with those in patients with sporadic tumours.
- We screened a total of 34 patients and identified mutations in 14 patients. The majority (79%) of these patients had mutations in *SDHD*, while the remaining 21% had mutations in *SDHB*. No mutations were detected in *SHDC*. Mutations in the *SDHD* and *SDHB* genes accounted for paraganglioma in 41% of this cohort, 91% of the familial cases, and 17% of the sporadic cases.
- There were several differences in the clinical features between those patients with familial paraganglioma compared to those with sporadic tumours. Patients with familial paraganglioma had a significantly lower age of onset and were more likely to have carotid body or vagal tumours. Females were more likely than males to develop sporadic tumours.
- A positive family history, bilateral tumours, early age of onset, and tumour type can help identify genetically determined cases of paraganglioma, thus facilitating targeted screening of affected patients and their relatives.

**Abbreviations:** PGL, paraganglioma
We identified all PGL patients who were treated at St Vincent’s public or private hospitals, Sydney between 1991 and 2001 and contacted all patients from New South Wales and the Australian Capital Territory. Where multiple cases from the same pedigree were identified, only the index case was included in this study. All patients recruited into the study were asked to provide details of their symptoms, family history of glomus tumours, and a blood sample. All patients who participated in the study provided appropriate informed written consent. The study was approved by the Human Research Ethics Committee of St Vincent’s Hospital.

**Mutation analysis**

DNA was extracted from whole blood using a standard salting-out method. Primers used for amplification of the eight exons of the SDHB gene and the six exons of the SDHC gene, were as described in Astuti et al. Intronic primers were designed from the genomic sequence of the SDHD gene region to amplify the four exons of the SDHD gene as previously described previously in Badenhop et al. PCR of all exons was carried out in 15 μl containing 60 ng DNA, 250 μM dNTPs, 0.33 μM of each primer (forward primer labelled with 6-FAM fluorescent dye), 2.5 mM MgCl₂, 1 × PCR buffer (Applied Biosystems, Foster City, CA, USA) and 0.6 U AmpliTaq Gold polymerase (Applied Biosystems). PCR reactions were carried out on a Hybaid OmniGene thermal cycler (Hybaid, Middlesex, UK) using the following protocol: 95°C for 12 min, 30 cycles of 95°C for 30 s, either 55°C, 58°C, or 60°C for 30 s, 72°C for 30 s, and 72°C for 5 min. Products were purified using Millipore Multiscreen-PCR membrane plates (Millipore, Sydney, Australia). DNA cycle sequencing was performed on a Hybaid OmniGene thermal cycler (Hybaid, Middlesex, UK) using the ABI PRISM BigDye Terminator Ready Reaction Kit (Applied Biosystems), 50 ng PCR product and the following protocol: 25 cycles of 95°C for 30 s, 50°C for 15 s, and 60°C for 4 min. Sequence products were purified using Millipore Multiscreen-HV membrane plates (Millipore, Sydney, Australia), loaded onto 4% polyacrylamide gels and electrophoresis was carried out on a ABI PRISM 377 DNA Sequencer (Applied Biosystems). Products were detected using the Sequencing Analysis program, version 3.3 (Applied Biosystems). Point mutations were detected using SeqMan, version 5.0 (DNASTAR, Madison, WI, USA).

**RESULTS**

A search by the medical records department of both St Vincent’s private and public hospitals identified 63 patients who were treated or followed up for PGL between 1991 and 2001 and lived in NSW or the ACT. From the 63 patients eligible for the study, 44 were successfully contacted, of whom two declined to be involved in the study. Twelve patients were members of four pedigrees reported in our previous study and only the proband from each pedigree was included in the current cohort. Blood samples were obtained from the remaining 30 patients, bringing the total number of patients in the study to 44.

Of the 34 patients recruited into the study, 17 were diagnosed with single glomus jugulare tumours, four with single glomus vagale tumours, and nine with single carotid body tumours. Four patients had multiple tumours (table 1). Patient PG075 had bilateral carotid body tumours, PG086 had bilateral carotid body tumours and a glomus vagale tumour, PG079 had a right-side jugulare and a left-side vagale tumour, and PG085 had bilateral carotid body tumours, a glomus jugulare tumour, and a phaeochromocytoma. The average (SD) age of onset of the entire cohort was 45.2 (16.4) years and ranged from 14 to 74 years. A total of 27 patients had undergone surgery and three had received radiotherapy, while the remaining four had received no treatment. Tumours were histologically confirmed in 29 patients. Of the patients 23 were female and 11 male (table 1).

Eleven of the 34 patients were considered to have familial PGL as they had at least one first degree family member diagnosed with PGL or had a diagnosis of bilateral or multifocal tumours. The remaining 23 patients had no family history of PGL and a diagnosis of a single tumour (table 1).

Mutations were detected in 10 of the 11 familial cases (91%). Nine patients had mutations in SDHD (table 1). One of these mutations had not previously been reported, a T deletion at +296 from the translation start site, resulting in a frameshift mutation (1996). One patient had a novel mutation in SDHB, a C deletion at +79 from the translation start site, resulting in a frameshift (S26fs).

Mutations were also detected in four of the 23 patients (17%) with no family history and single tumours. The P81L mutation in SDHD was identified in two of these patients. The remaining two patients had novel SDHB mutations, the S26fs mutation and a C to T substitution at +724 resulting in the substitution of Cys-242 for Arg-242 (R242C).

The majority of mutations detected in this study were in SDHD, with mutations in this gene accounting for 79% of all mutations and mutations in SDHB accounting for the remaining 21%. No mutations were detected in SDHC. As expected, mutations were more prevalent in those patients with a positive family history or bilateral tumours (91%) compared to those patients with apparent sporadic tumours (17%). There were 20 patients (59%) for whom no mutations were detected in the SDHB, SDHC, or SDHD genes.

There were several differences in the clinical features between those patients with familial PGL (including bilateral or multifocal tumours) compared to those with sporadic tumours. The average (SD) age of onset in patients with familial PGL was significantly younger than those with sporadic tumours, being 38.3 (14.3) years in familial cases compared to 47.7 (16.7) years in sporadic cases (t test, p = 0.04; non-parametric permutation test, p = 0.04). All individuals with multiple tumours had complex II mutations. In those individuals with single tumours, complex II mutations were more prevalent in those patients with either carotid body (67%, six out of nine) or vagale (50%, two out of four) tumours compared to those patients with jugulare tumours (12%, two out of 17), although these numbers are too small to provide a meaningful statistical analysis. The female to male ratio also differed in those patients with familial PGL compared to those with sporadic disease. The female to male ratio in familial cases was approximately 1:2, whereas in cases with sporadic tumours the female to male ratio was 4:1. When the data were reanalysed by the mutation status of the individual, the difference in age of onset became more pronounced. Mutation carriers had a significantly younger average (SD) age of onset of 34.2 (13.2) years compared to 52.3 (14.4) years in non-mutation carriers (t test, p = 0.008; non-parametric permutation test, p = 0.009) (table 2).

**DISCUSSION**

We screened a cohort of 34 patients with head and neck PGL and identified 10 mutations (three of which are novel) in 14 patients. Of those patients with mutations, 10 had a positive family history of glomus tumours and/or bilateral tumours and four were apparently sporadic cases. Mutations in SDHD accounted for the majority of mutations (79%), while mutations in SDHB accounted for the remaining 21%. No mutations were detected in SDHC. Mutations in the SDHD
and SDHB genes account for PGL in 41% of this cohort, 91% of the familial cases, and 17% of the sporadic cases.

The proportion of PGL cases due to genetic causes in our study (41%) is consistent with the heritability estimates from Dutch and North American populations. Based on recorded family history, the proportion of hereditary cases of PGL in the Netherlands was estimated to be approximately 50%. Subsequent studies in the Dutch population identified SDHD mutations in 34–40% of isolated or sporadic cases. We observed one SDHD D92Y mutation in a patient of Dutch ancestry. A study of 55 patients from two otolaryngology clinics in the US found 50% of familial head and neck PGL cases and 3% of sporadic cases had SDHD mutations with the sporadic cases all carrying the P81L mutation. The P81L mutation has been suggested to result from a founder effect among US patients, although this effect could also arise as a result of recurrent mutation. Of the four apparently sporadic cases with mutations in the present study, two carried the SDHD P81L mutation. This suggests that a more likely explanation may be frequent C>T transitions that occur at methylated CpG nucleotides. We have previously shown that the P81L CpG site is methylated.

The remaining 21% of the mutations identified in the present study were in the SDHB gene. SDHB mutations were present in 10% of the familial cases and 9% of the sporadic cases with SDHB mutations also had multiple tumours. There were four patients in our study with multiple tumours, one of whom had no family history. All these patients had SDHD mutations. Taken together, these studies indicate that multiple tumours in the same patient with or without a positive family history are indicative of mitochondrial complex II gene mutations.

In all studies of complex II gene mutations in head and neck PGL, SDHD is reported as the most commonly mutated gene. To date over 26 distinct mutations have been described. In the Netherlands, two founder SDHD mutations, D92Y and L139P, explain 94–96% of familial paraganglioma cases and 34–36% of non-familial cases. We observed one SDHD D92Y mutation in a patient of Dutch ancestry. A study of 55 patients from two otolaryngology clinics in the US found 50% of familial head and neck PGL cases and 3% of sporadic cases had SDHD mutations with the sporadic cases all carrying the P81L mutation. The P81L mutation has been suggested to result from a founder effect among US patients, although this effect could also arise as a result of recurrent mutation. Of the four apparently sporadic cases with mutations in the present study, two carried the P81L mutation. This suggests that a more likely explanation may be frequent C>T transitions that occur at methylated CpG nucleotides. We have previously shown that the P81L CpG site is methylated.

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Table 2  Genetic findings and correlations to patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SDHD mutation (n = 11)</th>
<th>SDHB mutation (n = 3)</th>
<th>No mutation (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) age of onset</td>
<td>36.4 (14.3)</td>
<td>27 (5)</td>
<td>52.3 (14.4)</td>
</tr>
<tr>
<td>Carotid body tumours</td>
<td>82%</td>
<td>0%</td>
<td>15%</td>
</tr>
<tr>
<td>Jugular tumours</td>
<td>18%</td>
<td>67%</td>
<td>75%</td>
</tr>
<tr>
<td>Vagal tumours</td>
<td>27%</td>
<td>33%</td>
<td>10%</td>
</tr>
<tr>
<td>Pheochromocytoma</td>
<td>9%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Multiple or bilateral tumours</td>
<td>36%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Family history</td>
<td>73%</td>
<td>33%</td>
<td>5%</td>
</tr>
<tr>
<td>Sporadic</td>
<td>27%</td>
<td>67%</td>
<td>95%</td>
</tr>
</tbody>
</table>

cases. These results are comparable to those of Baysal et al., who found that SDHB mutations accounted for 20% of familial PGL cases and 3% of sporadic cases. However the number of patients in this study and our study are small and the results need to be confirmed in a larger cohort. We did not find any SDHC mutations in our cohort, a finding which is consistent with the US study. To date mutations of the SDHC gene are restricted to the report of a single pedigree, and the contribution of the SDHC gene to the aetiology of PGL remains unclear.

While a positive family history and presence of multiple tumours are the primary indicators of complex II gene mutations, factors such as age of onset and tumour type may also be important. In the present study the age of onset was significantly lower in those cases with familial PGL compared to those with sporadic tumours. This finding is consistent with trends reported in other studies and is in accord with earlier onset being a characteristic feature of inherited versus sporadic disease. In our study, mutations were more prevalent in those patients with either carotid body (six out of nine) or vagal (two out of four) tumours compared to those patients with jugular tumours (two out of 17). Other studies have reported that carotid body tumours are more common in familial cases. Another interesting feature observed in our study and others is the difference in female to male ratio between heritable and sporadic cases. While there are more males than females with inherited PGL, females in the general population are more likely than males to develop sporadic PGL. The reason for the difference in female to male ratio remains unclear.

This study has important implications for the clinical management of head and neck PGL. The identification of potential heritable cases of PGL based on the presence of a positive family history and/or bilateral tumours, early age of onset and perhaps also tumour type will allow targeted screening of affected patients and their relatives.

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