Prevalence of SDHB, SDHC, and SDHD germline mutations in clinic patients with head and neck paragangliomas

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Background: Paragangliomas are rare and highly heritable tumours of neuroectodermal origin that often develop in the head and neck region. Germline mutations in the mitochondrial complex II genes, SDHB, SDHC, and SDHD, cause hereditary paraganglioma (PGL).

Methods: We assessed the frequency of SDHB, SDHC, and SDHD gene mutations by PCR amplification and sequencing in a set of head and neck paraganglioma patients who were previously managed in two otolaryngology clinics in the USA.

Results: Fifty-five subjects were grouped into 10 families and 37 non-familial cases. Five of the non-familial cases had multiple tumours. Germline SDHD mutations were identified in five of 10 (50%) familial and two of 37 (∼5%) non-familial cases. R38X, P81L, H102L, Q109X, and L128fsX134 mutations were identified in the familial cases and P81L was identified in the non-familial cases. Both non-familial cases had multiple tumours. P81L and R38X mutations have previously been reported in other PGL families and P81L was suggested as a founder mutation. Allelic analyses of different chromosomes carrying these mutations did not show common disease haplotypes, strongly suggesting that R38X and P81L are potentially recurrent mutations. Germline SDHB mutations were identified in two of 10 (20%) familial and one of 33 (∼3%) non-familial cases. P131R and M71fsX80 were identified in the familial cases and Q59X was identified in the one non-familial case. The non-familial case had a solitary tumour. No mutations could be identified in the SDHC gene in the remaining four families and 20 sporadic cases.

Conclusions: Mutations in SDHD are the leading cause of head and neck paragangliomas in this clinic patient series. SDHD and SDHB mutations account for 70% of familial cases and ∼8% of non-familial cases. These results also suggest that the commonness of the SDHD P81L mutation in North America is the result of both a founder effect and recurrent mutations.

Head and neck, extra-adrenal and adrenal paraganglia comprise a diffuse neuroendocrine system with similar embryogenesis and histology and are dispersed from the middle ear and the skull base to the pelvic floor. Whereas the paraganglia in the head and neck region are located in close association with the parasympathetic nervous system along the cranial nerves and the arterial vasculature, the adrenal medulla and other extra-adrenal paraganglia are associated with the sympathetic nervous system (that is, the sympathetic adrenal system). Paraganglioma refers to rare and mostly benign tumours that arise from any component of this neuroendocrine system. In the head and neck region, the carotid body (CB) is the largest of all paraganglia and is also the most common site of the tumours. Other tumour locations in the head and neck include jugulotympanic, vagal, laryngeal, aorticopulmonary, and minor locations such as the orbit and the thyroid.

The aetiology of head and neck paragangliomas has been linked to the chemoreceptor function of the normal paraganglia. There is an increased incidence of the tumours in people living at high altitude, presumably caused by the reduced atmospheric oxygen levels. Certain medical conditions with chronic arterial hypoxaemia are also thought to be associated with hyperplastic growth of paraganglia. In the absence of chronic hypoxic stimuli, paragangliomas often develop as a result of a genetic predisposition. At least four genetic loci have been implicated in the pathogenesis of hereditary paraganglioma (PGL). Three of the loci, PGL1 on chromosome 11q23, PGL3 on chromosome 1q21, and PGL4 on chromosome 1p36, encode the mitochondrial complex II (succinate dehydrogenase, succinate:ubiquione oxidoreductase) subunits SDHD, SDHC, and SDHB, respectively. The PGL2 gene at 11q13 has yet to be confirmed and identified. PGL1 is transmitted as an autosomal dominant trait with age dependent penetrance liabilities when transmitted through fathers, whereas maternal transmission does not cause tumour development. This parent of origin effect, which is most consistent with genomic imprinting, suggests that expression of SDHD is altered through sex specific epigenetic modifications during gametogenesis, although the exact molecular mechanisms remain unknown. Interestingly, so far only maternal transmissions have been observed in the limited number of PGL families with SDHC and SDHB mutations.

Identifying whether paraganglioma is heritable in a patient is of utmost significance for clinical management. The proportion of heritable cases has been variably estimated from 10% to as high as 50%. A recent analysis of an unbiased sample of clinic patients with head and neck paragangliomas uncovered the presence of a positive family history in ∼25% of patients. When heritability was defined as the presence of a positive family history or tumour multifocality, this ratio increased to ∼35% in the same sample. The identification of the underlying genes enables us to explore the molecular basis of this heritability, as the relative role of each of the
mitochondrial complex II subunits is unknown. In a recent study, SDHD mutations were identified in 30 of 32 (∼94%) PGL families and in 20 of 55 (∼36%) isolated cases in The Netherlands. Twenty-four of the 32 (75%) Dutch PGL families were the result of a single founder mutation. However, given the genetic (locus) and non-genetic heterogeneity in the aetiology of paragangliomas, it is unknown whether SDHD plays such a prominent role in other populations. It is conceivable that a fraction of paraganglioma patients without a family history could be carriers of germline SDHD mutations, because of imprinting and age dependent penetrance that obscure the familial nature of the PGL1 tumours. Accordingly, previous analyses of patients without a family history of head and neck paragangliomas and those with phaeochromocytomas (that is, adrenal paragangliomas) showed germline mutations in the SDHD gene. To determine the relative frequencies of SDHB, SDHC, and SDHD mutations in the aetiology of head and neck paragangliomas, we performed mutation analysis in a group of patients ascertained from two clinic patient populations in the United States.

MATERIALS AND METHODS

Head and neck paraganglioma patients

The subjects were patients diagnosed with head and neck paragangliomas, originating in the carotid body, jugular foramen, vagal nerve, temporal bone, or middle ear, at the Department of Otolaryngology at the University of Pittsburgh School of Medicine, Pittsburgh, PA and at the House Ear Institute, Los Angeles, CA. The recruitment of the subjects was performed either for the ongoing PGL linkage studies or through a more recent study aimed at establishing the proportion of heritable cases of paragangliomas by clinical criteria. The latter study followed a procedure that involved mail questionnaires and structured telephone interviews and has been described in detail elsewhere. Family 33 has been independently ascertained from Canada and only one family member was available for the research study. Both studies were approved by the Institutional Review Board of the University of Pittsburgh.

Classification of subjects on the basis of family history

The subjects were classified as familial if two or more subjects of an extended pedigree were diagnosed with paragangliomas as documented either by hospital records or by the family history reported in the questionnaires. On the basis of positive family history, 18 subjects were classified into 10 families. A total of 37 subjects who were recruited to the study had no family history. Five of the 37 non-familial cases had multiple tumours. Pedigrees for families 1, 3, 4, 9, and 13, which had multiple affected subjects, have been described previously. Maternal transmission was observed in family 9. None of the five new families (fig 1) showed maternal disease transmission.

Genotyping and haplotype analysis

DNA was isolated either from peripheral blood by standard phenol-chloroform extraction (PGL linkage study) or from cheek swabs using a commercial kit (Puregene D-500A) following the manufacturer’s protocol (clinical study). The genotyping was performed only in the DNA obtained from peripheral blood. The simple tandem repeat polymorphisms (STRPs) used in genotyping and the haplotype analysis have been described elsewhere, except for the new polymorphic tetranucleotide repeat marker, D11S5030, which was located approximately 40 kb telomeric to the 3’-SDHD. This STRP had five alleles in 20 unrelated chromosomes with a calculated

Figure 1 The new PGL families constructed from the subjects with head and neck paragangliomas. Affected subjects available for the study are shown with an asterisk. Maternal transmission is not observed in the pedigrees. The unaffected subjects’ symbols in each pedigree have been collapsed to preserve anonymity. The subject depicted with a question mark in family 27 was reported to have a small solitary mass in the skull base by radiological analysis. However, this subject does not carry the SDHD mutation (table 1) detected in her affected relatives and is under further clinical investigation.
An amplified with the originally described primers. Preamplified samples were diluted 20-fold and subsequently first round amplifications for each exon were as follows: exon 1, F: 5′-TTCGCTGCTGTTGGTGGGAA-3′ and R: 5′-CTGGTGGAGGCTACGCTA-3′; exon 2, F: 5′-CACTCTGGTTAAGAGGAGGCT-3′ and R: 5′-CCCTACAGGTTAGAAGTCC-3′; exon 3, F: 5′-GAGTGGTGGTCATCAGT-3′ and R: 5′-GATCCAAACCTTCTTCACTCCCA-3′; exon 4: F: 5′-GGAGAGCGACCTCGGGGTT-3′, R: 5′-GGAGGCTACGCTA-3′, and 5′-GAGGCTACGCTA-3′; exon 5, F: 5′-GAGTGGTGGTCATCAGT-3′ and R: 5′-GATCCAAACCTTCTTCACTCCCA-3′; exon 6, F: 5′-GGAGAGCGACCTCGGGGTT-3′ and R: 5′-CAGCCAAGTTATC-3′. The primer sequences for PCR product was subsequently used to amplify the gene sequence bridges the AP002007 and AP001781 sequences as obtained by the analyses of fully sequenced regional genomic clones in Genbank. D11S5016, D11S5025, D11S5027, and D11S1347. Mutation analysis was performed by SSCP analyses and mutation analyses of the paraganglioma patients. Mutations in SDHD were detected in five of the 10 (50%) PGL families and two of the 37 (∼5%) subjects without any family history (table 1). The identified mutations were predicted to truncate or dramatically alter the conformation of the SDHD protein product, cybS. Family 3 and the disease causing mutation H102L have been reported previously. The mutations in family SP64 and family 27 introduce premature stop codons that remove most of exon 4 from the mature cybS. R38X and P81L were identified in family 1 and family SP52, respectively. Subjects SP59 and SP77 who had no family history also carried the P81L mutation. Both subjects had multiple tumours in the head and neck region. The mutations R38X and P81L were previously reported in other families linked to the 11q23 locus and their potential impact on cybS has been discussed.

Mutations in SDHD were identified in two of the 10 (20%) PGL families and in one of the 33 (∼3%) subjects (including the two cases with SDHD P81L mutations) without any family history. P131R and M71fsX80 mutations were identified in families 9 and 13, respectively. Both mutations segregated with the disease phenotype in three affected subjects in family 9 and in two affected subjects in family 13. The P131R mutation alters an amino acid residue conserved among five eukaryotic species and was not detected in 200 normal chromosomes by PCR amplification of exon 4 and 5′I restriction enzyme digestion, which recognizes the mutant allele. The M71fsX80 mutation of family 13 is predicted to cause a very early truncation of the SDHD protein product. A premature stop codon mutation, Q59X, was identified in one subject without a positive family history. This subject did not have multiple paraganglioma tumours.

No mutations could be identified in the SDHC gene in the remaining PGL families 4, SP48, SP56, and in 20 subjects (including the three cases with multiple tumours) who had no family history and no mutations in the SDHD and SDHB genes. Availability of multiple affected subjects in family 4 enabled us to show that the markers at chromosome 11q23 and chromosome 1p36 did not segregate in the affected subjects, thus confirming the exclusion of the SDHD and SDHB genes by linkage. However, several markers at the SDHC containing region on chromosome 1q21 showed cosegregation with the disease phenotype, thus precluding the exclusion of SDHC by linkage (data not shown). Additional members of families SP48 and SP56 were not available to assess the segregation of the three PGL loci further.

**Table 1 Mutations identified in SDHD and SDHB**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Family/subject</th>
<th>Mutation</th>
<th>cDNA</th>
<th>Protein</th>
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</thead>
<tbody>
<tr>
<td>SDHD</td>
<td>Family 1</td>
<td>c.112C&gt;T</td>
<td>R38X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Family 27</td>
<td>c.381-383delG</td>
<td>L128fsX134</td>
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<td></td>
<td>Family SP52</td>
<td>c.242C&gt;T</td>
<td>P81L</td>
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<tr>
<td></td>
<td>Family SP64</td>
<td>c.325C&gt;T</td>
<td>Q109X</td>
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<tr>
<td>Subjects SP59 and SP77</td>
<td>c.242C&gt;T</td>
<td>P81L</td>
<td></td>
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<tr>
<td>SDHB</td>
<td>Family 9</td>
<td>c.392C&gt;G</td>
<td>P131R</td>
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<td></td>
<td>Family 13</td>
<td>c.207-210insC</td>
<td>M71fsX80</td>
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<tr>
<td></td>
<td>Subject SP36</td>
<td>c.174-175G&gt;C&gt;T</td>
<td>Q59X</td>
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</tr>
</tbody>
</table>

**RESULTS**

**Mutation analyses of the paraganglioma patients**

Mutations in SDHD were detected in five of the 10 (50%) PGL families and two of the 37 (∼5%) subjects without any family history (table 1). The identified mutations were predicted to truncate or dramatically alter the conformation of the SDHD protein product, cybS. Family 3 and the disease causing mutation H102L have been reported previously. The mutations in family SP64 and family 27 introduce premature stop codons that remove most of exon 4 from the mature cybS. R38X and P81L were identified in family 1 and family SP52, respectively. Subjects SP59 and SP77 who had no family history also carried the P81L mutation. Both subjects had multiple tumours in the head and neck region. The mutations R38X and P81L were previously reported in other families linked to the 11q23 locus and their potential impact on cybS has been discussed.

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**Haplobery analyses of the disease chromosomes carrying the P81L and R38X mutations**

Previously, haplobery analyses had shown extensive haplotype sharing in four families with P81L mutations, suggesting the presence of a common ancestral mutation in these US.
families. The same mutation was subsequently identified in three other families from the USA, one family from the UK, and one family from Australia. Similarly, the R38X germline mutation was independently reported in two families from the USA and in one subject with extra-adrenal paraganglioma. To test whether P81L and R38X cases were the result of founder mutations, we performed haplotype analyses using polymorphic markers located very close to the SDHD gene.

A visual inspection of the haplotypes comprising nine STRPs distributed within an approximate 450 kb region around the SDHD gene did not show a single haplotype for each mutation (fig 2). The identical alleles observed for the markers which immediately flank the SDHD gene were common among normal control chromosomes: D11S5015 allele 162 had a frequency of ~35% and D11S5019 alleles 168 and 170 had frequencies of ~50% and 45%, respectively. Therefore, these alleles shared among families cannot be readily concluded to be identical by descent. Even under the conservative assumption that the observed alleles are identical by descent, five independent ancestral recombination events within an approximate 200 kb interval between D11S5017 and D11S5030 would be required to explain the recombination events occurring in the intervals indicated by the horizontal bars. This scenario would require the occurrence of five recombinations. Similarly, if family 11 and family 1 were identical by descent for the R38X mutation, two recombinations must have occurred.

**DISCUSSION**

To determine the prevalence of SDHB, SDHC, and SDHD germline mutations in the aetiology of head and neck paragangliomas, we tested a total of 18 subjects (10 families) with a family history and 37 subjects without a family history, all of whom were previously evaluated in two otolaryngology clinics. We identified mutations in SDHD in five of the 10 (50%) families and two of the 37 (~5%) subjects without a family history. These two subjects were among the five cases that showed multifocality without a family history, suggesting that tumour multifocality is predictive of hereditary PGL.

Mutations in SDHB were identified in two of the 10 (20%) PGL families and in one of the 33 (~3%) subjects without any family history. The one non-familial case had a single tumour. No mutations were identified in the SDHC gene in the remaining four PGL families and in 20 non-familial cases without a family history and without mutations in the SDHD and SDHB genes. Thus, mutations in SDHD are the leading causes of paraganglioma tumours among the three mitochondrial complex II genes tested in this study. These findings also indicate that mutations in SDHD and SDHB account for 70% of head and neck paragangliomas with a positive family history and for ~8% of those without a positive family history. The remaining familial cases could be the result of mutations that are not detectable by exon amplification and sequencing in the tested genes or there may be other genes involved in the aetiology.
Germline SDHD mutations were predicted to occur in non-familial paragangliomas because the familial nature of the tumours could be obscured by de novo mutations in the SDH genes. In fact, the frequent (~36%) de novo mutation rate suggests that SDH mutations may be transmitted in non-familial cases. In our study, we investigated 26 SDHD mutations and found that two founder mutations in the SDHD gene were associated with familial pheochromocytomas and paragangliomas. The mutations were detected in 100% of cases, strongly suggesting that SDHD mutations are operative in familial non-familial pheochromocytoma.

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**ECHO**

**ARC syndrome is not so rare**

AARC syndrome has a wider clinical spectrum than previously thought. Its clinical features cover abnormal morphology, abnormal platelets, recurrent fevers, diarrhoea, and failure to thrive, as well as the classic picture of arthrogryposis, renal tubular acidosis, and cholestasis.

ARC syndrome is associated with consanguinity and is generally supposed to be a rare autosomal disorder. Since the first description in 1973, 11 pedigrees have been reported. New observations are available from a review of six further cases from three paediatric centres in the UK over 10 years.

In common with previous findings, clinical features proved somewhat variable. However, typical findings of arthrogryposis, renal Fanconi syndrome, and cholestasis were present in all but one patient, who had nephrogenic diabetes insipidus. Unusually, despite severe cholestasis, serum γ-glutamyltransferase values were normal for all patients. Further features included abnormal morphology, such as lax skin, low set ears, arched palate; scaly skin; recurrent febrile illnesses or sepsis; diarrhoea; and failure to thrive—all of which have been reported sporadically for ARC syndrome. Abnormally large platelets were also a common feature.

These observations indicate that ARC syndrome has a broader clinical picture and is likely to be less rare than previously thought. Indeed, a syndrome showing these features and reported as new in 1990 is probably ARC syndrome. Two consistent features from these latest cases—normal serum γ-glutamyltransferase values and occurrence in Pakistani immigrant families, where marriages between blood relatives are common—may give clues to a way of identifying a candidate gene.

Please visit the Journal of Medical Genetics website [www.jmedgenet.com](http://www.jmedgenet.com) for link to this full article.

Since the study a multicentre project to locate the ARC gene locus has started in Birmingham and Utrecht. (Contact: Dr P J McKiernan, Pat.McKiernan@bhamchildrens.wmids.nhs.uk)
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