**The genetics of phaeochromocytoma: using clinical features to guide genetic testing**

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**Abstract**

Phaeochromocytoma is a rare, usually benign, tumour predominantly managed by endocrinologists. Over the last decade, major advances have been made in understanding the molecular genetic basis of adrenal and extra-adrenal phaeochromocytoma (also referred to as adrenal phaeochromocytoma (aPCA) and extra-adrenal functional paraganglioma (eFPGL)). In contrast to the previously held belief that only 10% of cases had a genetic component, currently about one-third of all aPCA/eFPGL cases are thought to be attributable to germline mutations in at least nine genes (NF1, RET, SDHA, SDHB, SDHC, SDHD, TMEM127, MAX and VHL). Recognition of inherited cases of aPCA/eFPGL is critical for optimal patient management. Thus, the identification of a germline mutation can predict risks of malignancy, recurrent disease, associated non-chromaffin tumours and risks to other family members. Mutation carriers should be offered specific surveillance programmes (according to the relevant gene).

In this review, we will describe the genetics of aPCA/eFPGL and strategies for genetic testing.

**Background**

Adrenal and extra-adrenal phaeochromocytoma (aPCA and extra-adrenal functional paraganglioma (eFPGL) respectively) has an incidence of 2–8 cases per million per year. The nomenclature of these tumours can be confusing. The World Health Organization tumour classification defines phaeochromocytoma as chromaffin tumours arising from the adrenal medulla, and extra-adrenal tumours as paraganglioma. However, in the published literature the term paraganglioma may be used to refer to tumours derived from sympathetic nervous system or parasympathetic tissue. (Sympathetic tissue is located within the adrenal medulla, prevertebral, paravertebral, thoracoabdominal and pelvic paraganglia or in the reproductive organs, prostate, bladder, liver and organ of Zuckerkandl. Parasympathetic paraganglia are located in the vicinity of the major arteries and nerves, e.g. carotid body, jugular, vagal, tympanic, pulmonary and aortic paraganglioma.) In this review, we refer to aPCA/eFPGL to cover both chromaffin tumours that arise from the adrenal medulla (aPCA) and those functional extra-adrenal tumours derived from sympathetic ganglia (eFPGL). aPCA/eFPGL are usually secretory and typically present with features of catecholamine excess. In contrast, paragangliomas arising from the parasympathetic nervous system (usually within the head and neck and herein referred to as head and neck parasympathetic paraganglioma (HNPGL)) are predominantly endocrinologically inactive.

About 10% of aPCA/eFPGL patients have a family history of phaeochromocytoma and up to 25% of apparently sporadic cases with no family history will harbour a germline mutation in an inherited aPCA/eFPGL gene (1). To date, nine genes have been described to be associated with a predisposition to the development of aPCA/eFPGL and/or HNPGL (see Table 1). Although clues to inherited syndromic causes of aPCA/eFPGL may be provided by a previous history or family history of relevant tumour (see Table 1), many aPCA/eFPGL genes are associated with incomplete penetrance and/or variable expression (e.g. some VHL gene mutations may only be associated with aPCA/eFPGL). In addition to a positive family history of aPCA/eFPGL or evidence of a specific syndrome, the presence of multiple primary tumours is an indicator of genetic predisposition. In sporadic cases with a single tumour, early age at diagnosis, malignancy or extra-adrenal location are all risk factors for the presence of a
germline mutation (though the relevance of these risk factors varies for different genes) but there is no absolute cut off for mutation-positive cases (i.e. a patient with a later onset aPCA will have a low risk of harbouring a detectable mutation but the possibility cannot be completely excluded on clinical grounds).

Clinical and molecular genetic features of specific phaeochromocytoma predisposing genes

VHL
von Hippel–Lindau (VHL) disease is an autosomal dominant familial cancer syndrome with variable expression and age-dependent penetrance (>90% by the age of 60 years) (2, 3). Germline VHL mutations are most commonly associated with retinal angioma, haemangioblastoma and clear cell renal cell carcinoma (average lifetime risk >70% by the age of 60 years) (2). In addition to aPCA/eFPGL, non-secreting pancreatic neuroectodermal tumours occur in ~10% of patients (4). Rarely, HNPGL may occur (<1%) (5). Though renal, pancreatic and epididymal cysts are frequent (and may provide useful clues to the underlying diagnosis) they rarely cause morbidity. Interestingly, the risk of individual tumours can vary according to the age of diagnosis (mean late 60 years) and increased frequency of bilateral or multiple tumours although the risks of malignancy are not elevated (4). As many VHL-associated aPCA/eFPGL are detected during routine imaging surveillance, they are more frequently asymptomatic at diagnosis than sporadic aPCA/eFPGL.

RET
Germline activating mutations in the RET proto-oncogene cause multiple endocrine neoplasia type 2 (MEN2) and inactivating mutations cause Hirschsprung disease (8). Interestingly, a case of familial phaeochromocytoma described in 1886 was shown to result from a germline RET mutation 121 years later (9). MEN2 is an autosomal dominantly inherited disorder and is divided into three clinical subtypes, two of which (MEN2A and MEN2B) are characterised by the development of medullary thyroid cancer (MTC) and

<table>
<thead>
<tr>
<th>Gene</th>
<th>Clinical phenotype</th>
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<tbody>
<tr>
<td>VHL</td>
<td>von Hippel–Lindau disease – autosomal dominantly inherited predisposition to: aPCA/eFPGL, retinal and cerebellar haemangioblastomas, clear renal cell carcinoma, non-secretory pancreatic neuroendocrine tumours, endolymphatic tumours and visceral cysts (renal, pancreatic and epididymal). Rarely HNPGL. Epididymal cystadenomas</td>
</tr>
<tr>
<td>RET</td>
<td>Multiple endocrine neoplasia type 2 (MEN2) – autosomal dominantly inherited predisposition to medullary thyroid carcinoma and aPCA. MEN2A is characterised by medullary thyroid carcinoma, aPCA and primary hyperparathyroidism. MEN2B is characterised by medullary thyroid carcinoma and aPCA and developmental anomalies (marfanoid habitus, mucosal neuromas, etc.). Phaeochromocytomas in MEN2 are characteristically adrenal and benign</td>
</tr>
<tr>
<td>NF1</td>
<td>Neurofibromatosis type 1 (von Recklinghausen disease) autosomal dominantly inherited predisposition to aPCA/eFPGL, peripheral nerve sheath tumours (cutaneous, nodular and plexiform neurofibromas), intestinal tumours, gastrointestinal stromal cell tumours, malignant gliomas and juvenile chronic leukaemias. Relatively late mean age of diagnosis (41 years)</td>
</tr>
<tr>
<td>SDHA</td>
<td>Autosomal dominantly inherited predisposition to aPCA, eFPGL, HNPGL (though infrequent cause of these tumours) (autosomal recessively inherited juvenile encephalopathy or neonatal cardiomyopathy)</td>
</tr>
<tr>
<td>SDHB</td>
<td>Autosomal dominantly inherited predisposition to aPCA, eFPGL, HNPGL and renal cell carcinoma. High frequency of extra-adrenal phaeochromocytoma and malignancy</td>
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<tr>
<td>SDHC</td>
<td>Autosomal dominantly inherited predisposition to HNPGL and, less frequently, aPCA and eFPGL</td>
</tr>
<tr>
<td>SDHD</td>
<td>Autosomal dominantly inherited predisposition to parasympathetic HNPGL and aPCA and eFPGL. Tumours generally only develop in individuals who have inherited the mutation from their father (i.e. parent-of-origin effects on disease phenotype)</td>
</tr>
<tr>
<td>TMEM127</td>
<td>Autosomal dominantly inherited predisposition to aPCA, eFPGL and HNPGL. Relatively late mean age of diagnosis (compared with VHL disease and SDHB mutations)</td>
</tr>
<tr>
<td>MAX</td>
<td>Autosomal dominant inheritance with parent-of-origin effects (see text). Increased incidence of bilateral and malignant phaeochromocytoma</td>
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aPCA, adrenal phaeochromocytoma; eFPGL, extra-adrenal functioning sympathetic paraganglioma; HNPGL, head and neck parasympathetic paraganglioma.
aPCA (and hyperparathyroidism in MEN2A and a marfanoid habitus and mucosal neuromas in MEN2B). A number of genotype–phenotype correlations have been described in MEN2. Missense mutations in the intracellular tyrosine kinase domain (e.g. p.Met918Thr) that are associated with a strong transforming activity are associated with MEN2B (MTC and aPCA have an earlier age at onset in MEN2B than MEN2A), whereas missense mutations (e.g. p.Cys654Arg or p.Cys654Tyr) in the extracellular domain that cause abnormal dimerization (and autoactivation) of the mutant proteins have a less transforming activity and cause MEN2A (10–12). Individuals with MEN2A have about a 50% risk of developing aPCA but the mean age at diagnosis of MTC is earlier than that of aPCA (~40 years) and so, compared with individuals with germline SDHB, SDHD or VHL mutations, individuals with MEN2 are less likely to present with sporadic non-syndromic phaeochromocytoma. Nevertheless, a germline RET mutation can be detected in about 5% of such cases (13). Individuals with MEN2A often develop aPCA (which may be synchronous or metachronous) but eFPGL and malignancy are rare (14).

**Neurofibromatosis 1 (von Recklinghausen disease)**

Neurofibromatosis 1 (NF1) has been associated with phaeochromocytoma since 1910. Cutaneous (localised and plexiform neurofibromas, neurofibrosarcoma) and internal tumours such as aPCA/eFPGL, central nervous system tumours (glioma, astrocytoma and optic gliomas), carcinoïd and leukaemia may occur (15). Although NF1 is relatively common (incidence one in 3000 persons), the prevalence of aPCA/eFPGL in NF1 is low (~1%). Therefore, NF1 is not a common diagnosis in aPCA/eFPGL patients (16). The median age at diagnosis of aPCA/eFPGL in NF1 is relatively late (~41 years) and so other features of the disease (e.g. cutaneous cafe-au-lait spots, axillary freckling, neurofibromas and Lisch nodules) are usually present (so making clinical diagnosis of NF1 straightforward) (17, 18). The NF1 gene is a large gene (57 exons) and NF1 is associated with a wide variety of, frequently de novo, inactivating mutations making genetic analysis difficult. Therefore, although molecular genetic analysis is becoming more available, it is usually not indicated for diagnosis because of the ease of clinical diagnosis (16, 19).

**The succinate dehydrogenase complex**

Succinate dehydrogenase (SDH) is a heterotetrameric enzyme comprising four subunits (A, B, C and D). An associated protein, SDHAF2, is a highly conserved cofactor of flavin adenine dinucleotide (FAD) that is implicated in flavination of SDHA and is essential for SDH function. The SDH complex is attached to the inner mitochondrial wall by SDHC and SDHD. SDH has two key roles in cellular energy production: as part of the mitochondrial tricarboxylic acid cycle (catalysing the oxidative dehydrogenation of succinate coupled to the reduction of ubiquinone) and as the complex II component of the electron transport chain.

Loss-of-function mutations in the four SDH complex subunits and SDHAF2 have been demonstrated to cause HNPGL and/or aPCA/eFPGL, though the relative mutation frequency and associated tumour types varies between individual genes.

SDHD mutations were initially associated with HNPGL and subsequently with aPCA/eFPGL (20, 21). Although early studies suggested that SDHD mutations were more strongly associated with HNPGL than aPCA/eFPGL (22), a recent report suggests that this is mainly attributable to a very low risk of aPCA/eFPGL with a common SDHD missense mutation (p.Pro81Leu) (23). Multifocal tumours are more common with SDHD mutations (22). It should be noted that the tumour risk with SDHD mutations is for mutations that have been paternally inherited (maternally transmitted mutations have only very rarely been linked with disease and routine surveillance is not indicated in such cases) (24, 25). Mean age at diagnosis of phaeochromocytoma in patients with SDHD mutations is 35 years (23).

**SDHB mutations are an important cause of HNPGL and aPCA/eFPGL (13, 23, 24, 26). In addition, SDHB mutations predispose to renal tumours and may present with familial renal cell carcinoma (RCC) (27). Important clinical features of SDHB aPCA/eFPGL are a high frequency of extra-adrenal location and malignancy (~20% of mutation carriers will develop malignant disease and up to 50% of patients with a malignant eFPGL harbour a germline SDHB mutation) (22, 23, 28–31). Compared with all patients with SDH mutations, the overall risk of HNPGL (e.g. carotid body tumours) is higher with SDHD mutations whereas the risk of aPCA/eFPGL is higher with SDHB mutations (22, 27). Initial reports had suggested a high clinical penetrance for SDHB mutations; however, as testing of apparently sporadic cases has become more widespread and asymptomatic relatives of mutation carriers undergo genetic testing, the observed penetrance of SDHB mutations has fallen (e.g. 25–40% in non-probands) (32). In addition to renal tumours, SDHB mutations may possibly predispose to thyroid tumours (though the absolute risk is small) and SDHB and SDHC mutations have been found in 12% of individuals with gastrointestinal stromal tumours without PDGFRA receptor mutations (33, 34).

**SDHC mutations may be present in ~4% of HNPGL patients and though aPCA/eFPGL can occur it is rare (35–38). In the French Paraganglioma cohort, 14 of the 16 patients with SDHC mutations had HNPGL and two had a thoracic eFPGL (39).**
SDHA mutations were initially described in a biallelic state (homozygous or compound heterozygous) in children with an autosomal recessively inherited juvenile encephalopathy (Leigh syndrome) (40) and mutations in SDHA were initially thought to be absent from patients with HNPGL or aPCA/eFPGL. However, recent reports have described heterozygous germline SDHA mutations in a small subset of patients with HNPGL and aPCA/eFPGL (41, 42).

TMEM127

Following genetic linkage studies that mapped a novel locus for familial phaeochromocytoma to chromosome 2q11, germline mutations were identified in TMEM127 (43). The presence of loss-of-function mutations and allele loss TMEM127 phaeochromocytoma was consistent with the TMEM127 acting as a tumour suppressor gene. TMEM127 encodes a three spanner transmembrane protein that is highly conserved and, although the function of TMEM127 is not well defined, it has been linked to mTOR signalling (43). In an extensive cohort analysis of 990 individuals with phaeochromocytoma or HNPGL, the frequency of TMEM127 mutations was ~2% (44). In contrast to VHL and SDHB mutations, patients with mutations in TMEM127 do not have an early age at diagnosis (median age of 41.5 years). Typically, patients have aPCAs (often bilateral) and malignancy is infrequent (43, 44). The role of genetic testing for TMEM127 mutations in clinical practice is not well defined as, though mutations are uncommon, they may present in patient groups that are frequently excluded from genetic testing (see later) (44). Furthermore, the recent description of HNPGL and eFPGL in association with TMEM127 mutations demonstrates how the phenotype associated with inherited phaeochromocytoma gene mutations can continue to evolve (45).

MAX

Recently, germline inactivating mutations in MAX were detected by exome sequencing (46). Loss of wild-type MAX was also noted in tumour DNA and immunostaining revealed loss of MAX expression consistent with a tumour suppressor function. MAX is a key component of the MYC-MAX-MXD1 network that regulates cell proliferation and differentiation and there is crosstalk between this network and the mTOR pathway (to which TMEM127 has been linked (see above)). Interestingly, evidence was found that paternal transmission of the gene was necessary for tumour development (as with SDHD and SDHAF2). Most cases of MAX-associated phaeochromocytoma were bilateral, confirming the association between genetic abnormalities and multiple tumours. An association with malignancy was also found.

Other genes

Two apparently very rare causes of phaeochromocytoma are mutations in KIF1B and PHD2. Mutations in these genes have been described in only a few or single families, respectively (47–50), and neither gene is routinely analysed in clinical practice. Mutations in SDHAF2/SDH5 have been described in association with HNPGL but, to date, not with phaeochromocytoma (51, 52). The SDHAF2 gene maps to chromosome 11q13 and, as with SDHD (11q23), there are parent-of-origin effects on expression such that tumour development only occurs after paternal inheritance (51, 52).

The molecular pathophysiology of inherited phaeochromocytoma

It is clear that many genes are implicated in the pathogenesis of inherited phaeochromocytoma. However, gene expression studies have highlighted potential shared mechanisms of pathophysiology. For example, aPCA/eFPGL associated with germline mutations in VHL, SDHD and SDHB are associated with activation of hypoxic gene response pathways, whereas tumours associated with NF1 and RET mutations do not show a hypoxic gene signature but do show dysregulation of genes implicated in adrenergic metabolism, protein synthesis and kinase signalling (53–55). It was estimated that about 30% of sporadic aPCA and eFPGL have a similar gene expression signature to VHL/SDHX tumours and the other 70% to NF1/RET (53). Interestingly, the differences in gene expression profiles can be correlated with the tendency of VHL and SDHX tumours to produced mainly noradrenaline, and RET/NF1 tumours to be adrenergic (56). Recently, Burnichon et al. (55) have demonstrated that the gene expression profiling can be used to identify which subset of sporadic tumours are likely to harbour somatic VHL or RET mutations and so provide a basis for potential personalised therapies.

Genetic testing in phaeochromocytoma patients

The identification of inherited phaeochromocytoma genes provided the opportunity to define the frequency of specific gene mutations in both familial cases and in apparently non-syndromic sporadic cases. Unexpectedly, mutation analysis of RET, SDHB, SDHD and VHL revealed mutations in up to 25% of sporadic cases suggesting that at least a third of patients with phaeochromocytoma had inherited disease (13). The implications of identifying a germline mutation are profound – not only may close relatives be at risk for familial disease but the proband is generally placed at
increased risk of developing further phaeochromocytoma tumours and, depending on the specific gene mutation, there may be a risk of developing other tumour types (see Table 1). Although it has been suggested that all patients with phaeochromocytoma should be offered genetic testing, such a policy is (with conventional mutation detection methodologies) expensive (e.g. a recent estimate for testing SDHB, SDHC, SDHD, VHL and RET was $4100 (57)), and so more targeted screening has been advocated. Clinical features that influence the odds of detecting a mutation in an individual case will include whether there is: i) a personal or family history to indicate a syndromic cause (e.g. MTC, HNPGL and haemangiblastoma); ii) a positive family history for phaeochromocytoma; iii) multiple primary phaeochromocytomas; iv) malignancy; v) extra-adrenal location; and vi) age at diagnosis (generally inherited tumours occur at an earlier age than non-inherited). In the seminal study by Neumann et al. (13), 70% of individuals with phaeochromocytoma aged <10 years had a detectable mutation (in SDHB, SDHD, RET or VHL) compared with 8% of those who presented after age 40 years.

If a targeted approach to mutation analysis is to be pursued then it is generally agreed that those with a personal or family history of a specific syndromic cause and, in apparently non-syndromic cases, those with a positive family history for phaeochromocytoma, or multiple or bilateral tumours, should be analysed. It is usual to test individual genes sequentially and the order of testing can be individualised according to the likelihood of a syndromic cause (e.g. RET if there is a family history/personal history of MTC, SDHB/D if a HNPGL) or, in apparently non-syndromic cases with familial or multicentric phaeochromocytoma, the presence of extra-adrenal or malignant tumours would favour starting with SDHB analysis (but VHL if an aPCA). There are no generally agreed criteria regarding which patients with apparently non-syndromic sporadic phaeochromocytoma should be offered genetic testing for most frequently mutated genes (SDHB, SDHD, VHL and RET) (NF1 genetic testing is only indicated if there is clinical evidence of the disorder). Clearly, the more targeted mutation analysis is designed to be, the more cost-effective (in terms of expenditure per mutation detected) but less sensitive it will be. We suggest that all patients with malignant phaeochromocytoma should be offered testing for SDHB, VHL and SDHD mutations as about 40–50% of such patients will have a detectable mutation (~35, 5 and 1% respectively). For patients with sporadic non-syndromic eFPGLs the mutation detection rate is also high and we would also suggest testing SDHB, VHL and SDHD in such cases. For patients with a single benign aPCA it is generally agreed that the age at diagnosis be used to prioritise testing – though the precise age cut off has varied between investigators. In the largest study yet reported, Erlic et al. (57) suggested that an age cut off at 45 years would appreciably reduce the costs of genetic testing at the expense of only missing a relatively small number of mutation-positive cases (<5%) and so, when budgets for genetic testing are limited, it seems reasonable to restrict genetic testing (VHL, RET, SDHB then SDHD) to these younger cases (see Fig. 1). As testing for TMEM127 and MAX becomes more widely available then the indications for including testing for these genes will need to be incorporated into genetic testing protocols.

Within 5 years a number of scientific research and technological advances are likely to make it entirely feasible to evaluate all phaeochromocytoma patients for the risk of inherited disease. Immunohistochemical analysis has been used to identify tumours with loss of SDHB and SDHA expression that can then be prioritised for mutation analysis. Thus, in a series of 220 phaeochromocytomas, van Nederveen et al. (58) found a 100% sensitivity and 84% specificity for the association of SDHB immunohistochemistry and the presence of a detectable mutation. Likewise, SDHA immunohistochemistry was used to identify cases for SDHA mutation analysis (42). Such immunohistochemical information (and we note that aPCA/eFPGL

![Figure 1](http://www.eje-online.org)
associated with MAX mutations demonstrated loss of MAX expression (46)) might be combined with other biomarkers (e.g. evidence of tumour pseudohypoxia) to develop more sensitive and specific strategies for targeting mutation analysis (53–55, 58, 59). In addition, advances in sequencing technologies (e.g. exon capture and targeted resequencing by second generation massively parallel sequencing) will reduce greatly the cost of genetic diagnosis and so allow comprehensive analysis for all (i.e. currently known and those yet to be identified) inherited phaeochromocytoma genes (60). Hence, clinicians should bear in mind that individuals who appear likely to have inherited disease but do not have a mutation in a currently known gene should have DNA banked to allow for future mutation analysis. However, it should be noted that whilst comprehensive genetic analysis will increase the identification of inherited predisposition cases, it will also identify rare genetic variants that might not be easily classified as a pathogenic mutation or a benign polymorphism and in such cases further investigations may be required. Thus, it is important to take into account all available clinical and laboratory information to advise on optimum management.

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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