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Phaeochromocytoma and paraganglioma: next-generation sequencing and evolving Mendelian syndromes

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The clinical and molecular investigation of familial neoplasia has provided important insight into the molecular mechanisms of neoplasia and enhanced the clinical management of affected individuals and their at-risk relatives across a broad range of human neoplasias. For most tumour types, only a small minority (typically up to 10%) of cases will occur in individuals with a high- or moderate-penetrance Mendelian disorder, but this proportion is much higher in patients with phaeochromocytoma and paraganglioma (PPGL). Thus, although the traditionally taught '10% rule' suggests that only 10% of cases of PPGL are familial, it is now recognised that at least 25% of apparently sporadic cases of PPGL have a genetic basis.[1,2]

Currently, germline mutations in 12 genes are known to be associated with inherited PPGL, and it is likely that this number will increase. The genes and associated clinical features are summarised in Table 1, which highlights that the precise type of PPGL and the nature of other associated tumours vary according to the specific gene involved. The three longest recognised genetic causes of predisposition to PPGL are neurofibromatosis type 1 (NF1, von Recklinghausen's disease), multiple endocrine neoplasia 2 syndrome types A and B (MEN2A and MEN2B) and von Hippel–Lindau (VHL) disease.

Patients with an underlying diagnosis of NF1 who present with phaeochromocytoma can usually be recognised clinically (mean age at diagnosis of phaeochromocytoma in NF1 is about 40 years, by which time the classic features of NF1 [café-au-lait spots, neurofibromas, axillary freckling, etc] are apparent). In MEN2A, phaeochromocytoma generally present in the context of a personal or family history of medullary thyroid carcinoma and in MEN2B patients will have other characteristic physical stigmata (eg mucosal neuromas, Marfanoid habitus).[3,4] However, in VHL disease PPGL may be the only feature at presentation, so the threshold for molecular genetic analysis for VHL should reflect this (a subgroup of *VHL* gene mutations may predispose to PPGL but not other VHL-type tumours).[4–6]

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Box 1. Case history illustrating pattern of inheritance seen with *SDHD*, *SDHAF2* and *MAX* mutations.

A 34-year-old lady presented with hypertension, occasional palpitations and chest pain. Her paternal grandmother had previously been diagnosed with non-functioning carotid body paraganglioma. A diagnosis of noradrenaline-secreting phaeochromocytoma was made by measuring plasma metanephrines in the granddaughter. The patient's blood pressure was controlled with inpatient-monitored combined α- and β-blockade in the first instance. Genetic testing identified a pathogenic mutation in the *SDHD* gene. The patient's father had not been affected because he had inherited the mutation from his mother (we can therefore assume that the grandmother had inherited the mutation from her father). Non-penetrance and parent-of-origin effects demonstrate the need to take at least a three-generation family history when assessing the likelihood of a Mendelian syndrome.

Succinate dehydrogenase (SDH) is a heterotetrameric enzyme (subunits A, B, C and D) that is attached to the inner mitochondrial wall and has key roles in cellular energy production by virtue of its roles in the tricarboxylic acid (TCA, Krebs) cycle and as the complex II component of the electron transport chain. Mutations in genes that encode SDH subunits (*SDHA*, *SDHB*, *SDHC* or *SDHD*) or an associated protein (*SDHAF2*/*SDH5*) have been associated with inherited PPGL or head and neck paraganglioma (HNPGL),[7–11] with most mutations occurring in *SDHB* and *SDHD*. Although germline mutations in *SDHA*, *SDHB*, *SDHC*, *SDHD* and *SDHAF2* all compromise SDH activity, there are genotype–phenotype correlations. Mutations in *SDHB* cause PPGL more frequently than HNPGL, while the reverse is true for mutations in *SDHD*.[12,13] Importantly, *SDHB* mutations are associated with an increased risk of malignant paraganglioma. Thus, although only about 10% of PPGLs are malignant, a germline *SDHB* mutation may be detected in up to 50% of individuals with a malignant paraganglioma.[14,15] These genotype–phenotype correlations can be utilised to prioritise gene testing for germline mutations, although the increasing trend is

Table 1. Clinical characteristics associated with mutations in 12 genes associated with development of phaeochromocytoma–paraganglioma (PPGL) or head and neck paraganglioma (HNPGL) (see text for further details).

Gene	Clinical phenotype	Frequency in patients with PPGL and penetrance in mutation-positive patients
FH	> *FH* mutations are typically associated with predisposition to leiomyomas (cutaneous and uterine) and papillary kidney cancer. Very recently *FH*-associated phaeochromocytomas have been described. To date insufficient information is available to predict the risk of other *FH*-related tumpours in individuals with *FH*-related phaeochromocytomas	> Very rare (≤1%) > Currently insufficient data to assess penetrance reliably
HIF2A	> *HIF2A* mutations initially described in patients with multiple paragangliomas and polycythaemia (although germline mutations were not detected in blood DNA, tumours contained identical activating *HIF2* mutations) > Subsequent, very recent reports have expanded phenotype to include presentations with multiple PPGL without polycythaemia and cases associated with germline *HIF2* mutations	> Rare > Currently insufficient data to assess penetrance reliably
MAX	> Inherited *MAX* mutations are rare cause of PPGL > Mutations are transmitted in an autosomal dominant manner but, like *SDHD* and *SDHAF2* mutations, tumour risk depends on whether mutation is paternally or maternally inherited > Tumours develop in individuals who inherited mutation from father	> Rare > Currently insufficient data to assess penetrance reliably
NF1	> NF1 (von Recklinghausen's disease) is an autosomal dominant disorder characterised by peripheral nervous system tumours (cutaneous, subcutaneous, nodular and plexiform neurofibromas), gastrointestinal stromal cell tumours, malignant gliomas and juvenile chronic myelogenous leukaemia > Small number of patients with NF1 develop phaeochromocytoma, with mean age of diagnosis of about 40 years (when other features of NF1 will be present) > Mutation analysis not usually undertaken in patients with PPGL, as diagnosis is clinically apparent	> About 1% of patients with PPGL > < 6% of patients with NF1 develop PPGL
RET	> MEN2 caused by germline-activating mutations in *RET* proto-oncogene > MEN2 is autosomal dominant-inherited disorder associated with predisposition to medullary thyroid carcinoma (MTC) and phaeochromocytoma > MEN2 is subdivided into three subtypes, two of which are associated with phaeochromocytomas – MEN2A characterised by MTC, phaeochromocytoma and primary hyperparathyroidism – MEN2B characterised by MTC, phaeochromocytoma and developmental anomalies (Marfanoid habitus, mucosal neuromas, Hirschsprung syndrome, etc) > Phaeochromocytomas in MEN2 characteristically adrenal and benign	> *RET* mutations found in about 5% of patients with PPGL > About 50% of patients with MEN2A and almost all patients with MEN2B develop PPGL > Germline *RET* mutations detected in up to 5% cases of sporadic phaeochromocytoma[13]
SDHA	> Homozygous *SDHA* mutations initially identified in children with autosomal recessive-inherited juvenile encephalopathy (Leigh syndrome) but now recognised that heterozygous germline *SDHA* mutations may predispose to PPGL (albeit rarely)	> Rare > Currently insufficient data to assess penetrance reliably
SDHB	> *SDHB* mutations associated with autosomal dominant-inherited predisposition to PPGL and HNPGL > *SDHB* mutations also predispose to renal tumours and may present with familial renal cell carcinoma > Important clinical features of *SDHB*-associated PPGL are high frequency of extra-adrenal and/or malignant tumours	> *SDHB* mutations found in 10–15% of patients with PPGL (up to 50% of those with malignant PGL) > Lifetime penetrance of *SDHB* mutations in at-risk relatives is < 50%
SDHC	> *SDHC* mutations typically cause autosomal dominant-inherited predisposition to HNPGL and may be detected in about 4% of patients with HNPGL > *SDHC* mutations seem to be rare cause of PPGL	> Rare > Currently insufficient data to assess penetrance reliably

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Table 1. ***(Continued)***

Gene	Clinical phenotype	Frequency in patients with PPGL and penetrance in mutation-positive patients
SDHD	> Although *SDHD* mutations are transmitted in an autosomal dominant manner, risk of tumour depends on whether mutation is paternally or maternally inherited – Tumours generally only develop in individuals who have inherited mutation from father (ie parent-of-origin effects on disease phenotype) > Most *SDHD* mutations predispose to both HNPGL and PPGL.	> *SDHD* mutations found in 5–10% of patients with PPGL; risk of HNPGL and PPGL depends on parental origin of mutation (PPGL may occur in up to 40% of patients with paternally inherited *SDHD* mutations)
SDHAF2	> Mutations in *SDHAF2/SDH5* have been described in association with HNPGL > *SDHAF2* gene maps to chromosome 11q13 and, as with *SDHD* (11q23), exhibits parent-of-origin effects on expression, such that tumour development occurs only after paternal inheritance	> Seems to be rare > Currently insufficient evidence to assess penetrance
TMEM127	> Mutations in *TMEM127* tumour suppressor gene associated with autosomal dominant-inherited predisposition to phaeochromocytoma, with relatively late age of diagnosis (about 40 years compared with *VHL*- and *SDHB*-associated PPGL) > *TMEM127* mutations are infrequent cause of inherited phaeochromocytoma and rare cause of paraganglioma and HNPGL	> Rare > Currently insufficient data to assess penetrance reliably
VHL	> von Hippel–Lindau disease is autosomal dominant-inherited familial cancer syndrome predisposing to retinal and cerebellar haemangioblastomas, clear renal cell carcinoma, PPGL, non-secretory pancreatic neuroendocrine tumours, endolymphatic tumours and visceral cysts (renal, pancreatic and epididymal); rarely HNPGL may occur > Risks of PPGL vary according to germline *VHL* mutation. Although PPGLs occur in about 20% of individuals with VHL, most common *VHL* mutations (deletions, truncating and splice site mutations) are associated with low risk of phaeochromocytoma, whereas missense mutations not predicted to impair stability of VHL protein have high risk of phaeochromocytoma > Rare *VHL* mutations may cause PPGL-only phenotype	> *VHL* mutations found in 5–10% of patients with PPGL > PPGL occurs in up to 20% of patients with VHL disease

HNPGL = head and neck paraganglioma; MEN2 = multiple endocrine neoplasia type 2; MTC = medullary thyroid carcinoma; NF1 = neurofibromatosis; PPGL = phaeochromocytoma/paraganglioma.

to test multiple genes simultaneously rather than individual genes sequentially (see below).[16–18] Rarer and more recently recognised genes implicated in inherited PPGL and HNPGL (*SDHA*, *SDHC*, *SDHAF2*, *TMEM127*, *MAX*, *FH* and *HIF2A*) are summarised in Table 1.[10,11,19–21]

To whom should genetic testing be offered?

Phaeochromocytoma is a rare tumour with an incidence of 2–8 cases per million per year. The personal and family history and clinical examination may reveal risk factors for inherited disease (see Table 2). The presence of two or more cases of PPGL or HNPGL in a family thus can usually be assumed to result from a familial mutation. In an individual with a phaeochromocytoma, paraganglioma or HNPGL, the presence of a family history of a tumour associated with a syndromic cause of PPGL should prompt genetic testing for the suspected syndrome. Familial PPGL, irrespective of the gene involved, is usually inherited as an autosomal dominant trait, so the children of a mutation carrier will have a 50% chance of having inherited the relevant PPGL gene mutation. However, for three genes (*SDHD*, *SDHAF2* and *MAX*), the risk of a mutation carrier developing a tumour is dependent the parent from which the gene has been inherited – for these genes, clinical disease is generally seen only when the mutation has been inherited from the father.[7,11,20] Hence, if a son were to inherit an *SDHD* mutation from his mother, his risk of developing a tumour is remote, but if any of his children were to inherit the *SDHD* mutation from him, they would be at significant risk of developing a tumour.

In addition to patients with syndromic causes of inherited PPGL recognisable by combinations of tumours as in VHL and MEN2A or by clinical phenotype as in NF1 and MEN2B, about 25% of individuals with apparently sporadic non-syndromic PPGL will harbour a clinically unsuspected disease gene mutation.[1,2,22] In such cases, the lack of family history may result from a variety of causes: the mutation may have arisen *de novo* in the affected individual (up to 20% of cases of VHL disease) or may be non-penetrant in other relatives (recent work suggests that <50% of individuals with an *SDHB* mutation will develop a tumour); alternatively, it may be a reflection of parent-of-origin effects on penetrance (*SDHD*, *SDHAF2* and *MAX* mutations). Among individuals with apparently sporadic non-syndromic PPGL or HNPGL, certain clinical features may point to those most likely to have a mutation. The presence of multiple tumours, extra-adrenal location, malignancy or early age at diagnosis thus all can indicate an increased likelihood of a germline mutation, and the presence or absence of such features has been used to stratify which individuals should be offered genetic testing.[16,17] Although

Table 2. Features that suggest underlying genetic susceptibility in patients with phaeochromocytoma or paraganglioma (PPGL).

Feature	Examples
Tumour characteristics	> Multiple tumours
	> Malignancy
	> Young age at diagnosis (eg under 45–50 years)
Previous medical history	> Previous history of related tumour:
	– Renal tumour (commonly-associated with VHL disease or *SDHB* mutation)
	– Head and neck paraganglioma
	– Medullary thyroid cancer (MEN2)
	> Brain tumour: haemangioblastoma (VHL), gliomas (NF1)
	> Eye: retinal angioma (VHL), optic nerve gliomas (NF1)
	> Pancreas (VHL)
	> Other conditions:
	– Renal, pancreatic or epididymal cysts (VHL)
	– Hypercalcaemia/hyperparathyroidism (MEN2A)
	– Polycythaemia (HIF2)
	– Hirschsprung disease (MEN2)
Family history	> Relative with inherited PPGL predisposing syndrome, PPGL or PPGL-related tumour (see above)
Examination	> Skin: café-au-lait spots, neurofibromas, Marfanoid habitus, mucosal neuromas (MEN2B)
	> Eyes: Lisch nodules, optic gliomas (NF1), retinal angioma (VHL)

it had been recommended that all individuals with PPGL should be offered testing for *SDHB*, *SDHD*, *VHL* and *RET* mutations,[2] such an approach is costly and most clinical centres have adopted a more focused approach to genetic testing based on clinical features. For example, testing is offered to all familial or syndromic cases and sporadic cases of PPGL with multiple tumours, malignancy, extra-adrenal location or age at onset <50 years.[16,17] Evaluation of a UK-based genetic testing programme demonstrated that such 'clinically targeted' testing programmes can be cost efficient, but some, albeit infrequent, 'low-risk' individuals (eg those older than 50 years with isolated phaeochromocytoma) will harbour a germline mutation.[22] Complete ascertainment of all mutation carriers thus requires universal testing (although the sensitivity of targeted testing programmes might be enhanced by the addition of tumour immunohistochemistry[23]).

How should genetic testing be performed?

Until recently, genetic testing for germline mutations in genes predisposing to PPGL and HNPGL involved sequentially testing single genes, prioritised according to clinical features, until a mutation was detected or until all genes (typically up to four – *SDHB*, *SDHD*, *VHL* and *RET*) tested negative. Such a testing protocol is expensive and might take longer than 6 months. Recently, advances in massive parallel sequencing technologies (next-generation sequencing/second-generation sequencing) have transformed the practice of DNA sequencing (initially in genome centres and research laboratories but more recently in diagnostic laboratories). Powerful but compact DNA sequencers thus allow simultaneous sequencing of multiple genes ('gene panels') in a single run at a much lower cost than conventional (Sanger) DNA-sequencing techniques. It has been estimated that a second-generation sequencing test for nine genes predisposing to phaeochromocytoma (*MAX*, *RET*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *TMEM127* and *VHL*) costs about £500 per sample, whereas sequencing of *SDHB*, *SDHD*, *VHL* and *RET* by conventional sequencing cost about £1,800.[18] Although second-generation sequencing approaches have many advantages, there can be issues with sensitivity. Nevertheless, technological advances are widely expected to improve sensitivity and specificity, and sequencing costs will fall. Although it seems likely that further genes predisposing to PPGL and HNPGL remain to be identified, the cost of testing for these genes is anticipated to continue to fall and all or most patients with PPGL or HNPGL might be offered genetic testing in the medium term.

However, it is very important that all patients are counselled appropriately about the limitations of genetic testing. The analysis of genes predisposing to PPGL and HNPGL often yields the discovery of missense variants ('variants of uncertain significance'), which cannot currently be interpreted reliably, thus producing an uninformative result. In addition, for patients with pathogenic mutations in newly recognised disease genes, information on long-term risks and optimum surveillance may be extremely limited, and this uncertainty can be difficult for patients and clinicians alike.

Conclusions

Both PPGL and HNPGL provide examples of how advances in molecular genetics can transform our knowledge of the genetic basis of a human disease. At least 12 genes are currently known to predispose to PPGL and HNPGL, with more likely to be

Key points

About one-third of all patients with phaeochromocytoma and paraganglioma (PPGL) have an underlying genetic cause

Most patients with heritable PPGL do not have a positive family history

Mutations in at least 12 genes may cause inherited PPGL

Identification of individuals with germline mutations in inherited PPGL allows them (and their mutation-positive relatives) to be screened for development of PPGL and other relevant tumours

Clinical features (eg family history, multiple tumours, location, malignancy, young age at onset, etc) can be used to prioritise genetic testing, but complete ascertainment of inherited cases would require all cases to be tested

Advances in DNA sequencing technologies are enabling the development of more comprehensive genetic testing at lower cost

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found, but it is technologically and economically feasible that advances in DNA sequencing will enable universal testing of all individuals with PPGL or HNPGL. Such testing would have value for predicting the prognosis of individual patients (eg high risk of malignancy in those with an *SDHB* mutation or associated tumours in those with a *VHL* mutation). However, it is important that implementation of genetic testing does not outstrip our ability to interpret the results of genetic testing and our knowledge of the optimum strategies for managing mutation-positive individuals. It is important, therefore, that as genetic testing expands, there is a concerted effort to gather molecular and clinical data in order to provide a strong evidence base to guide future recommendations on the care of individuals susceptible to PPGL and HNPGL. ■

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