Paraganglioma and phaeochromocytoma: from genetics to personalized medicine

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Abstract | Paragangliomas and phaeochromocytomas are neuroendocrine tumours whose pathogenesis and progression are very strongly influenced by genetics. A germline mutation in one of the susceptibility genes identified so far explains ~40% of all cases; the remaining 60% are thought to be sporadic cases. At least one-third of these sporadic tumours contain a somatic mutation in a predisposing gene. Genetic testing, which is indicated in every patient, is guided by the clinical presentation as well as by the secretory phenotype and the immunohistochemical characterization of the tumours. The diagnosis of an inherited form drives clinical management and tumour surveillance. Different 'omics' profiling methods have provided a neat classification of these tumours in accordance with their genetic background. Transcriptomic studies have identified two main molecular pathways that underlie development of these tumours, one in which the hypoxic pathway is activated (cluster 1) and another in which the MAPK and mTOR (mammalian target of rapamycin) signalling pathways are activated (cluster 2). DNA methylation profiling has uncovered a hypermethylator phenotype in tumours related to SDHx genes (a group of genes comprising *SDHA*, *SDHB*, *SDHC*, *SDHD* and *SDHAF2*) and revealed that succinate acts as an oncometabolite, inhibiting 2-oxoglutarate-dependent dioxygenases, such as hypoxia-inducible factor prolyl-hydroxylases and histone and DNA demethylases. 'Omics' data have suggested new therapeutic targets for patients with a malignant tumour. In the near future, new 'omics'-based tests are likely to be transferred into clinical practice with the goal of establishing personalized medical management for affected patients.

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Introduction

In the twentieth century, 'paraganglioma' was the term used to designate head and neck parasympathetic tumours, which were treated by ear, nose and throat surgeons. The term 'phaeochromocytoma' designated thoracic–abdominal or pelvic catecholamine-secreting adrenal or sympathetic tumours, which were usually managed by endocrinologists.

The description of the first mutations in the *SDHD* gene in patients with phaeochromocytoma and/or paraganglioma (PPGL) in 2000 constituted a turning point that led to a completely new way of viewing these two types of neuroendocrine tumours.[1–3] In 2004, the WHO defined phaeochromocytoma as an intra-adrenal paraganglioma, highlighting the common origin of phaeochromocytomas and sympathetic or parasympathetic paragangliomas, which are all derived from neuroectoderm and can all occur in patients with the same genetic predisposition.[4] In the past 15 years, germline mutations in a dozen PPGL susceptibility genes have been reported and research has shown that ~40% of patients carry a causal germline mutation (Figure 1). PPGL is thus now considered to be a neuroendocrine tumour that is strongly influenced by genetics.[5]

This Review focuses on research from the past 15 years, a period during which our understanding of the disease and the management of affected patients and their relatives have been revolutionized.

Genes and diseases

When faced with a patient with a PPGL, a clinician must consider whether the PPGL is the result of the patient having neurofibromatosis type 1 (caused by mutations in *NF1*), multiple endocrine neoplasia type 2 (MEN2; linked with mutations in *RET*), von Hippel–Lindau disease (associated with mutations in *VHL*), hereditary paraganglioma (caused by mutations in the SDHx group of genes [*SDHA*, *SDHB*, *SDHC*, *SDHD* and *SDHAF2*]), familial phaeochromocytoma (caused by mutations in *TMEM127* or *MAX*), polycythemia paraganglioma syndrome (associated with mutations in *EPAS1* [also known as *HIF2A*]) or Reed syndrome (linked with mutations in *FH*). However, of the PPGL susceptibility genes, the *VHL*, *SDHB*, *SDHD* and *SDHC* genes have the highest frequencies of germline mutations.[6] The main phenotypes associated with germline mutations in these genes are summarized in Table 1.

Familial or syndromic PPGL

A family history and/or a syndromic presentation are key elements for the diagnosis of inherited PPGL. If the family

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The authors declare no competing interests.

Key points

- Over half of all phaeochromocytoma and/or paraganglioma (PPGL) can be attributed to genetic alterations
- 80% of inherited PPGLs are caused by a germline mutation in *VHL* or the SDHx group of genes
- Genetic testing is indicated for all patients with PPGLs, as identification of the underlying mutation guides patient management and genetic counselling
- Gene expression profiling can be used to classify PPGLs, assigning them to either an angiogenic cluster or a kinase signalling cluster, each of which has specific treatment targets
- DNA methylation profiling has revealed a hypermethylated cluster that is specific to tumours related to the SDHx genes and *FH* that accounts for the malignant and noradrenergic phenotype of tumours related to mutations in these genes
- Genomic studies of PPGLs are generating important findings that should pave the way for personalized medicine for affected patients

history is not known, physicians should search for familial diseases by drawing the family pedigree and should check for the clinical signs corresponding to the specific hereditary syndromes described in the following sections.

Neurofibromatosis type 1

The *NF1* gene, located on chromosome 17q11.2, is one of the largest genes in humans, with 60 exons spanning >350 kb that encode 2,839 amino acids.[7] This gene encodes neurofibromin, which is a tumour suppressor and downregulates RAS proteins and the downstream RAS–RAF–MAPK signalling cascade. Neurofibromatosis type 1, which was previously known as von Recklinghausen disease, is a frequent autosomic disorder (with a prevalence of 1 in 3,000 to 1 in 4,000 people in the general population) with a high penetrance (~100% of carriers have clinical symptoms by the age of 5 years).[8] A NIH consensus development conference selected seven clinical features, at least two of which must be present for the establishment of a diagnosis of neurofibromatosis type 1: ≥6 café-au-lait spots with a longest diameter of at least 5 mm in prepubertal patients and a longest diameter of at least 15 mm in postpubertal patients; ≥2 neurofibromas of any type, or one plexiform neurofibroma; freckling in the axillary or inguinal regions; optic glioma (optic pathway glioma); two or more Lisch nodules (iris hamartomas); a distinctive bone lesion, such as sphenoid wing dysplasia or thinning of the cortex of long bones, with or without pseudoarthrosis; and a first-degree relative (parent, sibling or child) with neurofibromatosis type 1 according to the aforementioned criteria.[8]

PPGL is rare in patients with neurofibromatosis type 1; it is reported in 0.1–5.7% of these patients, and is generally diagnosed in patients when they are in their thirties (which is early compared with sporadic cases).[9–11] *NF1* genetic testing is generally not indicated in patients who present with PPGL, as neurofibromatosis type 1 can be diagnosed on the basis of clinical findings alone in 95% of patients aged ≥11 years.[12] Nevertheless, four patients with neurofibromatosis type 1 have been reported who had a single PPGL tumour and an apparently sporadic presentation.[11,13] All these patients presented with mild features of neurofibromatosis type 1 at clinical re-evaluation, highlighting the importance of very careful investigations of the typical features of neurofibromatosis type 1, including particular skin features (such as café-au-lait spots and neurofibromas) in all patients with a PPGL.[11,13]

Multiple endocrine neoplasia type 2

The *RET* proto-oncogene, which has 21 exons and encodes a 60 kb mRNA, is located on chromosome 10q11.2.[14]

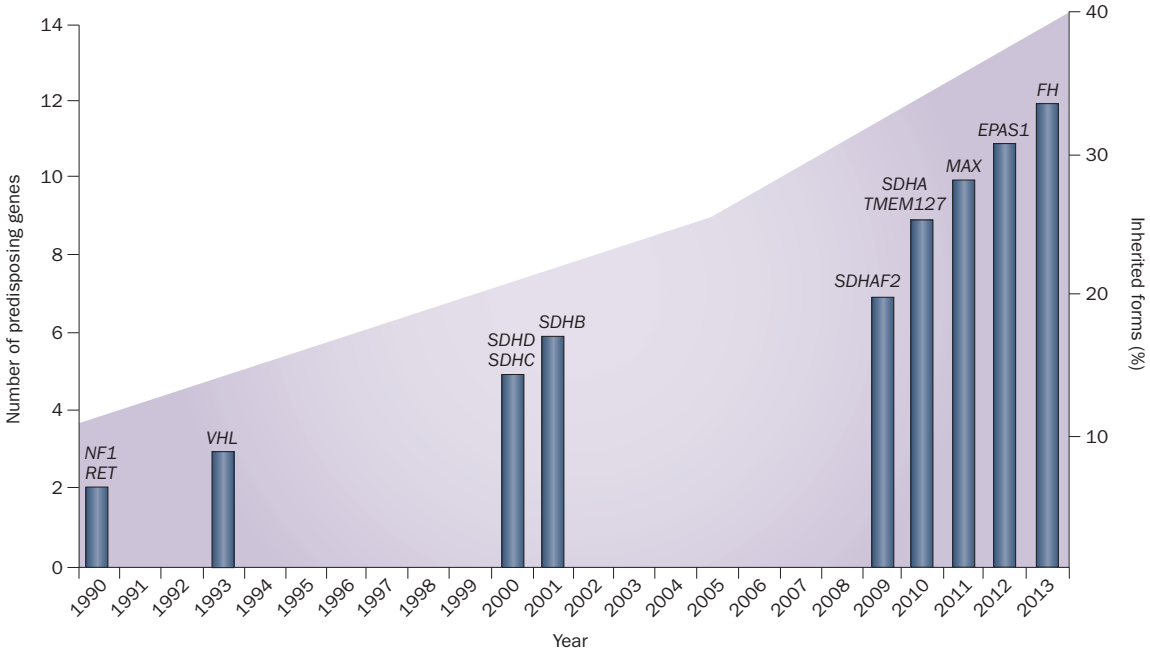

Figure 1 | Timescale of the discovery of PPGL susceptibility genes. The cumulative number of susceptibility genes and the year of identification for each of them are illustrated by the histograms. The curve highlights the increase in the percentage of known inherited forms of PPGL through time. Abbreviation: PPGL, phaeochromocytoma and/or paraganglioma.

Table 1 | Genes and diseases

Disease (phenotype MIM numbers)	Genes	Mutation rate (%)*	Main features
Neurofibromatosis type 1 (162200)	*NF1*	3	Café-au-lait spots, neurofibromas, axillary and inguinal freckling, Lisch nodules, osseous lesions, optic gliomas, mainly phaeochromocytomas
Multiple endocrine neoplasia type 2 (171400; 162300)	*RET*	6	2A: Medullary thyroid cancer, primary hyperparathyroidism, PPGL 2B: Medullary thyroid cancer, PPGL, Marfanoid habitus, mucocutaneous neuromas, gastrointestinal ganglioneuromatosis
von Hippel–Lindau disease (193300)	*VHL*	7	Central nervous system or retinal haemangioblastomas, renal cell carcinoma, PPGL, pancreatic neuroendocrine tumours and cysts, endolymphatic sac tumours, papillary cystadenoma of the epididymis and broad ligament
Hereditary paragangliomas (168000; 605373; 115310; 601650; 614165)	SDHx genes: *SDHB* *SDHD* *SDHC* *SDHA* *SDHAF2*	 10 9 1 <1 <0.1	 PPGL, rare renal cancers, GIST PPGL, rare renal cancers, GIST PPGL, rare renal cancers, GIST PPGL, GIST Head and neck paraganglioma
Familial phaeochromocytomas (173300; 613403; 154950)	*TMEM127* *MAX*	1 1	Mainly phaeochromocytomas, rare renal cancers Mainly PPGL
Polycythemia paraganglioma syndrome (603349)	*EPAS1*	1	Polycythemia, PPGL, somatostatinoma
Leiomyomatosis and renal cell cancer (150800)	*FH*	1	Cutaneous and uterine leiomyomas, type 2 papillary renal carcinoma, rare PPGL

*The mutation rate is the percentage of patients with PPGL with mutations in the gene concerned. Abbreviations: GIST, gastric stromal tumours; MIM, Mendelian Inheritance in Man; PPGL, paraganglioma and/or phaeochromocytoma.

The RET (re-arranged during transfection) tyrosine kinase receptor is activated by disulphide-bond-mediated dimerization of the receptor (the dimer binds its ligands and coreceptors). Phosphorylation of its tyrosine kinase domain also activates the receptor, which leads to activation of the PI3K–AKT and MAPK–ERK kinase signalling pathways.[15] RET can be constitutively activated by gain-of-function mutations in seven specific exons of the *RET* gene (exons 8, 10, 11, 13, 14, 15 or 16, all of which contain codons for specific cysteine or tyrosine residues), which causes MEN2. RET can also be inactivated by loss-of-function mutations in other exons, which results in Hirschsprung disease.

MEN2 is a rare autosomal dominant disorder that affects ~1 in 30,000 individuals.[16] MEN2A, which is also known as Sipple syndrome, is characterized by medullary thyroid carcinoma, PPGL and primary hyperparathyroidism; patients can also have cutaneous conditions (such as notalgia or lichen amyloidosis). MEN2B (previously known as Gorlin syndrome) is characterized by medullary thyroid carcinoma, PPGL, Marfanoid habitus, mucosal neuromas and ganglioneuromatosis of the gut and intestine. MEN2B accounts for only 5% of MEN2 cases, but it is the most aggressive form, as it is associated with the highest risk of early development (from the first year of life) of medullary thyroid carcinoma.[16] Approximately half of these patients develop PPGLs.[16] The various mutations in *RET* that can cause MEN2 have been classified into four levels (A–D) by the American Thyroid Association. Level D mutations confer the highest risk of medullary thyroid carcinoma (youngest age at onset, highest risk of metastasis and highest risk of death from the disease).[17]

von Hippel–Lindau disease

The *VHL* tumour suppressor gene contains three exons and is located on chromosome 3p25. *VHL* encodes two proteins—$pVHL_{30}$, which is present in the nucleus and cytoplasm, and $pVHL_{19}$, which is exclusively nuclear—that are responsible for driving the ubiquitylation and proteosomal degradation of hypoxia-inducible factor (HIF).[18] Loss-of-function mutations in *VHL* lead to the inappropriate activation of the hypoxic response, thus promoting angiogenesis, glycolysis and proliferation. Von Hippel–Lindau disease is a rare (incidence of 1:36,000 in the general population) autosomal dominant disease characterized by renal clear-cell carcinoma, PPGL and central nervous system or retinal haemangioblastomas associated with neuroendocrine pancreatic tumours or cysts, endolymphatic sac tumours, and epididymal and/or broad ligament cystadenomas.[19]

Leiomyomatosis and renal cell cancer

The *FH* gene is a tumour suppressor gene with eight exons that is located on chromosome 1q42.1 and encodes fumarate hydratase (also known as fumarase), which converts fumarate to malate in the tricarboxylic acid cycle. Reed syndrome, also known as multiple cutaneous and uterine leiomyomatosis, is an autosomal dominant disease that is characterized by smooth muscle tumours (leiomyomas) in the skin and uterus. Some affected patients are at risk of developing type 2 papillary renal carcinoma and, in exceptional cases, PPGL.[20]

Nonsyndromic and familial forms

Around 12% of patients with an apparently sporadic presentation (no family history of PPGL or syndromic

presentation) actually have hereditary PPGL, which is mostly due to germline mutations in the SDHx group of genes.[21,22]

Hereditary paragangliomas

The SDHx group of genes encode the subunits of succinate dehydrogenase (SDH), which assemble into mitochondrial complex II, a mitochondrial enzyme responsible for oxidizing succinate to fumarate in the Krebs cycle and for electron transport to the ubiquinone pool via the mitochondrial respiratory chain. Succinate dehydrogenase consists of two anchorage proteins (SDHC and SDHD) and two catalytic proteins (SDHA and SDHB). The SDHAF2 protein is responsible for the flavination of the SDHA protein, which is essential for the complex to form. These five proteins are encoded by five nuclear genes: *SDHC* (six exons, chromosome 1q23), *SDHD* (four exons, on chromosome 11q23), *SDHA* (15 exons, chromosome 5p15), *SDHB* (eight exons, chromosome 1p36) and *SDHAF2* (four exons, chromosome 11q13). Causal germline mutations in these five tumour suppressor genes have been identified in patients with PPGL.[1,23–26] Inactivation of succinate dehydrogenase results in the accumulation of succinate, which acts as a competitive inhibitor of the 2-oxoglutarate-dependent dioxygenases (such as HIF prolyl-hydroxylases and histone or DNA demethylases), which leads to the stabilization of HIF-α and the subsequent activation of hypoxic signalling (referred to as pseudohypoxia) and to epigenetic modifications.[27–29]

Hereditary paraganglioma related to the SDHx genes is an autosomal dominant disorder that is associated with genomic imprinting of *SDHD* or *SDHAF2* mutations.[30] Most patients with mutations in SDHx genes present with PPGL. Correlations between the genotype and phenotype in patients with mutations in these genes have been assessed in many large studies that used international registries.[31] Multiple head and neck tumours or a family history of PPGL in the paternal branch of the family are suggestive of *SDHD*-related paraganglioma. By contrast, *SDHB*-related PPGL is often diagnosed as a single extra-adrenal tumour in the absence of a family history of PPGL. A mutation in the gene that encodes SDHB is a biomarker of malignancy[32] and a poor prognosis.[33] Carriers of mutations in *SDHC* are rare, for unknown reasons, but might develop all the signs of the disease.[34] Mutations in *SDHA* and *SDHAF2* have been described in only a small number of patients.[22] In addition to PPGL, rare renal cancers in young patients (<40 years old), single gastric stromal tumours (GIST) or GIST associated with PPGL in the context of Carney–Stratakis syndrome are also described in rare cases.[35,36]

Familial phaeochromocytomas

In 2010 and 2012, integrative genomic approaches based on linkage or transcriptomic analyses combined with high-throughput sequencing methods in patients with familial phaeochromocytomas identified germline mutations in two new tumour suppressor genes—*TMEM127* and *MAX*.[37,38] The *TMEM127* gene (four exons, chromosome 2q11) encodes transmembrane protein 27, which has a role in the mTOR (mammalian target of rapamycin) signalling pathway. The loss of TMEM127 activates mTOR target phosphorylation by a mechanism that involves the Rab5-dependent endocytic pathway.[39] The *MAX* gene (five exons, located on chromosome 14q23) encodes MYC-associated protein X (MAX). MAX is a key partner of the MYC transcription factor, and it downregulates oncogenic MYC signalling.[40] Mutations in one or both of these genes should be sought in patients with familial, bilateral or apparently sporadic PPGL.[41,42] *TMEM127* gene mutations have also been reported in rare cases of renal cell carcinoma.[39]

Polycythemia paraganglioma syndrome

Germline mutations in *EPAS1,* which encodes endothelial PAS domain-containing protein 1 (also known as hypoxia-inducible factor 2α), were initially described in families with hereditary polycythemia. In 2012, gain-of-function somatic mutations in exons of the *EPAS1* gene that carry proline residues, the specific targets of the prolyl hydroxylase enzymes, were identified in patients with PPGLs.[43] These mutational events occur after the zygote is formed, affect different tissues depending on the degree of mosaicism and can lead to PPGL, polycythemia and/or somatostatinoma in the same patient.[44] *EPAS1* is the second most frequently implicated PPGL susceptibility oncogene, after *RET*. Its activation triggers the initiation of the hypoxia-inducible pathway in normoxic conditions.

Other PPGL susceptibility genes

Mutations in *KIF1B* (two germline and two somatic mutations), *EGLN1* (also known as *PHD2*; two germline mutations) and *IDH1* (one somatic mutation) have been reported in exceptional cases, and their contribution to the genetics of the disease remains unclear.[45–48] Somatic mutations in the *HRAS* proto-oncogene are present in ~5% of patients with sporadic benign phaeochromocytoma, but germline mutation in this gene has never been reported.[48,49]

PPGL genetic testing

Up to now, decision algorithms for PPGL genetic testing 'from the patient to the candidate genes' were used by specialist and accredited genetic laboratories to determine the order of tests to be carried out and to decrease the time required to obtain results and the cost of analysis. These genetic tests involved Sanger sequencing followed by multiplex ligation-dependent probe amplification to detect large chromosomal rearrangements.[22] The decision of which gene to test is made on the basis of three levels of analysis: clinical presentation; secretory phenotype; and immunohistochemical characterization (Figure 2a).

Clinical presentation

An accurate recording of clinical history, drawing the family pedigree and a physical examination checking for evidence of a specific genetic disease (for example,

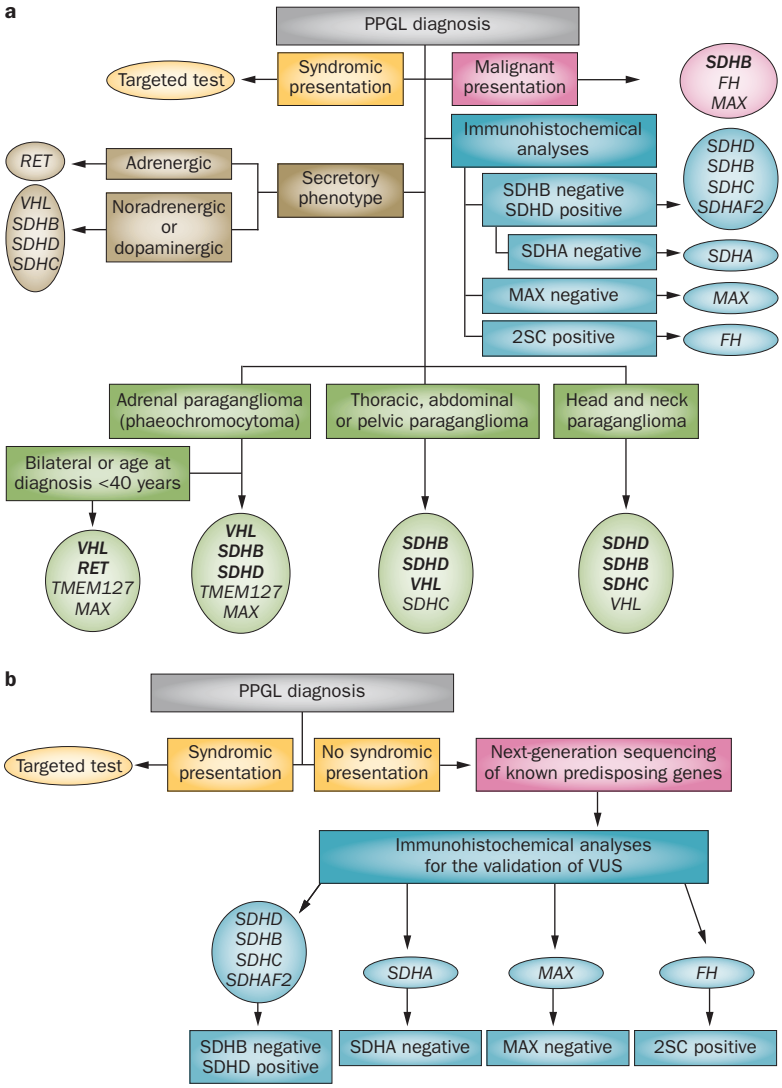

Figure 2 | Algorithms for PPGL genetic testing by Sanger or by next-generation sequencing. **a** | Proposed algorithm based on clinical presentation, secretory phenotype and immunohistochemical characterization to guide a sequential genetic testing (Sanger sequencing). **b** | Proposed algorithm to guide the biological validation of the variants identified by the analysis of all susceptibility genes by next-generation sequencing. Abbreviations: 2SC, S-(2-succinyl)cysteine; MAX, MYC-associated protein X; PPGL, phaeochromocytoma and/or paraganglioma; SDH, succinate dehydrogenase; VUS, variants of unknown significance.

café-au-lait spots for neurofibromatosis type 1, familial or personal history of renal cancer for von Hippel–Lindau disease or medullary thyroid carcinoma for MEN2) is the first step in the genetic diagnosis of PPGLs. This step enables a single gene to be identified for analysis, which can lead to the rapid identification of the causal mutation in almost all cases.[21] The WHO defines malignant phaeochromocytoma as the presence of distant metastases, not local invasion.[4] The presence of one or several metastases is an indication for *SDHB* genetic testing; if this test is negative it can be followed by *FH* screening. Depending on the patient population, 10–70% of patients with metastatic PPGL have been reported to carry a germline *SDHB* mutation.[31] A meta-analysis of studies that included only mutation carriers with manifest disease confirmed the high risk of malignancy in carriers of a *SDHB* mutation, as it reported a 23% prevalence of malignant PPGL for *SDHB* mutation carriers versus only 3% for *SDHD* mutation carriers.[50] A mutation in *SDHB* is also associated with low levels of metastasis-free survival in children carrying an *SDHB* mutation who develop PPGL and with a poor prognosis in adults with malignant PPGL.[33,51] The rapid establishment of *SDHB* mutation status therefore seems to be crucial for patient management and follow-up. *FH* mutations have also been reported to be associated with malignancy.[20]

Secretory phenotype

Dopamine, norepinephrine (also known as noradrenaline) and epinephrine (or adrenaline) are produced from tyrosine and are converted into 3-methoxytyramine, normetanephrine and metanephrine, respectively, the concentrations of which can be determined in plasma or urine.[52] Careful analysis of concentrations of plasma free or urinary fractionated metanephrine can be used to guide genetic testing for PPGL, as hereditary forms of PPGL are characterized by specific secretory phenotypes. A dopaminergic or noradrenergic phenotype is suggestive of mutations in the SDHx genes or in *VHL*, whereas an adrenergic phenotype implies the presence of a *RET* mutation.[53] The noradrenergic phenotype of SDHx-related PPGL results from an epigenetic mechanism. The massive accumulation of succinate in tumour tissue leads to hypermethylation of the promoter of the gene that encodes phenylethanolamine *N*-methyltransferase, which results in the downregulation of this enzyme (which is responsible for converting norepinephrine into epinephrine).[29] SDHx tumours are therefore unable to complete the processing of catecholamines and cannot produce epinephrine.

Immunohistochemical characterization

Before a patient has had surgery, hereditary PPGL can be suspected on the basis of clinical or secretory phenotypes. The biochemical phenotype might give an indication of which genes are involved. After surgery, immunohistochemistry can give information on the occurrence of a mutation in the SDHx genes or in *MAX*. Immunohistochemistry for SDHB detects SDHx mutations with a high sensitivity and specificity.[54] Tumours with mutations in *SDHB*, *SDHC* and *SDHD* are negative at immunohistochemistry for SDHB and positive for SDHA. Tumours with mutations in *SDHA* are negative at immunohistochemistry for both SDHA and SDHB. Immunohistochemical staining is negative for SDHB but positive for SDHD in tumour tissues carrying SDHx gene mutations. This combination of analyses is particularly useful in situations in which staining for SDHB is weak or difficult to interpret (M. Menara *et al.*, personal communication). SDHA immunohistochemistry, which gives negative results for tumour tissues with *SDHA* mutations, is an efficient screening test for detecting rare SDHA-related PPGLs and SDHA-related GISTs.[15,55] For MAX tumours, only tumours with truncating *MAX*

mutations can be identified on the basis of negative immunohistochemical staining for MAX.[42] Finally, *S*-(2-succinyl)cysteine (2SC) staining completes the panel of available immunohistochemical tests for the triage of PPGL genetic tests by revealing tumours deficient in fumarate hydratase (encoded by *FH*).[56] Indeed, these tumours accumulate large amounts of fumarate, which favours the covalent modification of cysteine residues to 2SC, and the positive immunohistochemical detection of 2SC can be used to detect rare *FH*-related PPGLs.[29]

The place of next-generation sequencing

The advent of next-generation sequencing (NGS) methods for PPGL genetic testing offers the tantalizing promise of a single assay for the screening of all PPGL susceptibility genes, which would result in the rapid identification of causal mutations. The use of custom-developed targeted NGS methods and whole-exome sequencing for PPGL genetic testing has already been reported.[57–59] However, the implementation of these new techniques in molecular genetic laboratories remains challenging for geneticists, as NGS generates large amounts of sequence data. This amount of data results in the detection of a larger number of variants of unknown significance than with Sanger sequencing, for which correct biological interpretation is required. A rigorous multistep interpretation pipeline is needed to distinguish the disease-causing variant from the many variants of unknown significance detected by high-throughput sequencing methods whilst avoiding false-positive results.[60] In this context, decision algorithms extending 'from the mutated genes to the affected patient' developed through close collaboration between geneticists and pathologists would be required to assess the causality of variants of unknown significance by immunohistochemical analyses of tumour tissues (Figure 2b). The misinterpretation of NGS data can lead to an incorrect genetic diagnosis, with potentially serious consequences for the outcomes of the patients and their relatives.[61]

PPGL management

The Endocrine Society clinical practice guidelines for phaeochromocytoma and paraganglioma that were published in 2014 recommend personalized management by a specialist referral centre with a multidisciplinary team for patients with hereditary PPGL.[22] Indeed, the expert central reading of imaging examinations (contrast head and neck MRI and thoracic–abdominal–pelvic CT scan with somatostatin receptor scintigraphy) for patients with mutations in SDHx genes substantially increases diagnostic sensitivity (99% versus 92% for local reading) for the detection of PPGL.[62] In addition, the choice of nuclear imaging and of the PET tracer should be tailored to genetic status of the patient and tumour localization. For instance, ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphy has low sensitivity for the diagnosis of paragangliomas in patients with malignant PPGL and PPGL related to von Hippel–Lindau disease or with SDHx-related hereditary paraganglioma.[62,63] The sensitivity of ^{18}F-FDG–PET and ^{18}F-fluoro-L-DOPA (^{18}F-FDOPA) PET are also influenced by genetic status. Joint guidelines from the European Association of Nuclear Medicine and the Society of Nuclear Medicine have proposed an algorithm based on genetic status that can be used to choose the nuclear imaging modality.[64] Patients with PPGL related to neurofibromatosis type 1 or MEN2 should undergo ^{18}F-FDOPA–PET or ^{123}I-MIBG as the first-line imaging modality, patients with von Hippel–Lindau disease should undergo either ^{18}F-FDG–PET or ^{18}F-FDOPA–PET and patients with SDHx-related malignant PPGL should undergo ^{18}F-FDG–PET. Finally, patients with SDHx-related nonmetastatic paraganglioma should undergo both ^{18}F-FDG–PET and ^{18}F-FDOPA–PET as some SDHB-related abdominal paraganglioma can poorly concentrate ^{18}F-FDOPA.[65] These guidelines will surely evolve with the arrival of gallium-labelled tracers and other new tracers.

The surgical approach should also be personalized. Adrenal-cortical-sparing surgery can be considered in patients with bilateral phaeochromocytoma who have a low risk of metastatic recurrence.[22] This type of surgery has mainly been performed in patients with von Hippel–Lindau disease or RET-related phaeochromocytoma. The procedure is safe and the recurrence rate is ~10%.[66] This procedure also avoids long-term dependence on corticosteroids in the majority of patients.[22] Adrenal-cortical-sparing surgery of at least one adrenal gland is frequently indicated for patients with von Hippel–Lindau disease or MEN2 who underwent an operation on a first unilateral phaeochromocytoma or bilateral phaeochromocytoma.[67,68] However, it is not recommended for patients at risk of metastatic recurrence, such as patients with a *SDHB* mutation.[22]

Syndromic PPGL

Patients with von Hippel–Lindau disease, MEN2 or neurofibromatosis type 1 require particular clinical management and tumour surveillance. Recommendations for the follow-up of each of these diseases are summarized in Table 2.[8,16,17,19,69,70]

SDHx-related PPGL

Paraganglioma can arise from any paraganglionic tissue, regardless of which SDHx gene is mutated, even if the gene concerned is rarely mutated, as is the case for *SDHC* or *SDHA*.[71] Physicians responsible for managing patients with mutations in the SDHx genes should be aware that tumours can occur in other rare locations: the tumours reported for such patients included renal cancer and GIST.[35,36] For the first screening, complete radiological imaging by contrast MRI or CT scan for the head and neck plus thoracic, abdominal and pelvic areas is recommended.[62] In a large, prospective, multicentre series of 256 patients with mutations in the SDHx genes, the use of Octreoscan® (Mallinckrodt LLC, Hazelwood, MO, USA) for radiological imaging led to the detection of 202 PPGLs in 238 patients, with a sensitivity of 99%. Interestingly, head and neck paragangliomas were diagnosed in 62.5% of the patients with mutations in

Table 2 | PPGL management according to genotype

Disease	First screening	Follow-up after a negative result for first screening
Neurofibromatosis type 1	Physical examination with blood pressure monitoring and skin examination Metanephrine level determination Ophthalmologic examination	Physical examination with blood pressure monitoring every year Metanephrine level determination every year Ophthalmologic examination every year Abdominal MRI or CT scan only if hypertension or abnormal levels of metanephrines found
Multiple endocrine neoplasia type 2	Physical examination with blood pressure monitoring Metanephrine level determination Abdominal MRI or CT scan Plasma calcitonin, calcium and parathyroid hormone level determinations Thyroid ultrasonography	Physical examination with blood pressure monitoring every year Metanephrine and calcium level determinations every year Plasma calcitonin level determination every year (in the absence of prophylactic thyroidectomy*) Abdominal MRI or CT scan only if hypertension or abnormal levels of metanephrines found
von Hippel–Lindau disease	Physical examination with blood pressure monitoring Metanephrine level determination Thoracic–abdominal–pelvic MRI Ophthalmologic examination Head and spine MRI scan	Physical examination with blood pressure monitoring every year Metanephrine level determination every year Ophthalmologic examination every year Thoracic–abdominal–pelvic MRI or ultrasonography every year MRI scan of head and spine every 2 years
SDHx-related hereditary paragangliomas	Physical examination with blood pressure monitoring Metanephrine level determination Head and neck plus thoracic–abdominal–pelvic contrasted MRI or CT ^{111}In-pentetreotide scintigraphy and/or ^{18}F-FDG–PET/CT (for *SDHB* mutation carriers) and/or ^{18}F-FDOPA–PET/CT (for *SDHD* mutation carriers)	Physical examination with blood pressure monitoring every year Metanephrine level determination every year Whole-body MRI every 2 or 3 years
PPGL related to *TMEM127* or *MAX* mutations	Physical examination with blood pressure monitoring Metanephrine level determination Head and neck plus thoracic–abdominal–pelvic contrast MRI or CT	Physical examination with blood pressure monitoring every year Metanephrine level determination every year Whole-body MRI every 2 or 3 years
PPGL related to *EPAS1* mutations	Physical examination with blood pressure monitoring Haemoglobin level determination Metanephrine level determination Head and neck plus thoracic–abdominal–pelvic contrast MRI or CT	Physical examination with blood pressure monitoring Metanephrine and haemoglobin level determinations every year Whole-body MRI every 2 or 3 years
PPGL related to *FH* mutations	Physical examination with blood pressure monitoring Metanephrine level determination Head and neck plus thoracic–abdominal–pelvic contrast MRI or CT ^{18}F-FDG–PET/CT	Physical examination with blood pressure monitoring every year Metanephrine level determination every year Kidney or whole-body MRI every year Gynaecological and dermatological examination every year

*The indication for prophylactic thyroidectomy depends on the American Thyroid Association classification of the RET mutation and calcitonin level.[17,69]
Abbreviation: PPGL, paraganglioma and/or phaeochromocytoma.

SDHB, highlighting the need to investigate all paraganglionic sites, regardless of which gene is mutated.[62] Large, multicentre, prospective studies are still required, but the promising performances of new tracers for PET or CT suggest that PET or CT scanning with ^{18}F-FDG or ^{18}F-FDOPA should be carried out in carriers of *SDHB* and *SDHD* mutations, respectively.[64]

Exposure to radiation should be limited during the follow-up of patients with syndromes known to be linked with PPGLs who have not yet developed tumours. Monitoring of plasma and/or urinary levels of metanephrines plus whole-body MRI every 2–3 years is indicated in the absence of a personal history of tumours.[72] The first diagnosis of PPGL in a patient with a mutation in *SDHB* should lead to ^{18}FDG-PET or CT to detect potential metastases, and the patient should be monitored closely, at least twice per year.[73]

Rare hereditary forms of PPGL

The *TMEM127*, *MAX*, *EPAS1* and *FH* genes have now been implicated in the genetics of PPGLs,[20,37,38,43] but no evidence-based recommendations have yet been published for the management of patients with mutations in these genes. However, a screening and surveillance program similar to that for SDHx-related PPGLs is advisable for affected patients and asymptomatic mutation carriers. In addition, specific surveillance of the kidneys of patients with *FH* and *TMEM127* mutations, of haemoglobin levels for those carrying *EPAS1* mutations (polycythemia might reveal the occurrence of a new PPGL) and of the skin and uterus for those carrying *FH* mutations should be carried out.[20] As *FH* mutations might be associated with a malignant phenotype, ^{18}F-FDG–PET should be performed during the initial screening.[20]

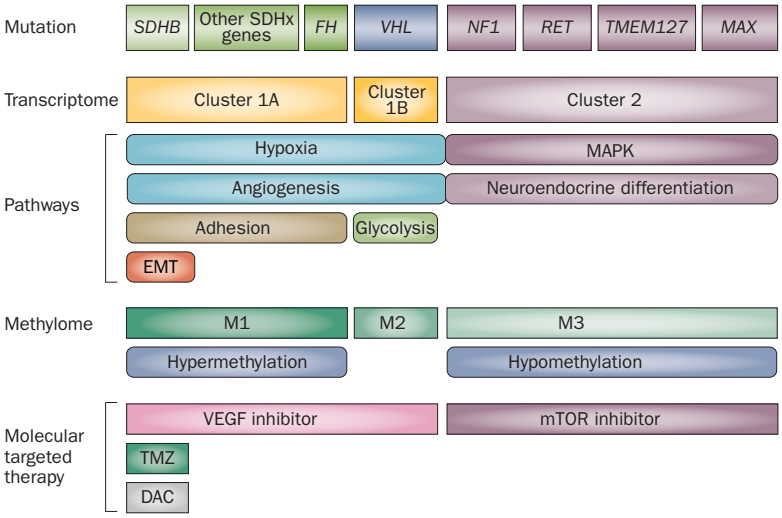

Figure 3 | From integrative genomics studies to molecular targeted therapies. The integration of the gene expression (clusters 1A, 1B and C2) and methylation (clusters M1, M2 and M3) profiling data is schematically represented. In the first line, the genetic components of the tumours are drawn in a given colour: green for tumours related to SDHx genes and *FH*, blue for tumours related to *VHL* and violet for tumours related to *RET*, *NF1*, *TMEM127* or *MAX* and sporadic tumours. Suggestions of targeted molecular therapies are proposed, according to the genotype of the patient. The combination of different molecules and the development of novel strategies based on new molecular findings will most probably be added to this scheme in the near future. Abbreviations: DAC, decitabine; EMT, epithelial-to-mesenchymal transition; mTOR, mammalian target of rapamycin; TMZ, temozolomide.

From 'omics' to targeted therapies

PPGL, like other cancers, has benefited from the advances in pangenomic analyses that have improved understanding of pathogenesis and will ultimately lead to advances in treatment. Gene expression and methylation profiling studies have demonstrated the major effect of genetics on the tumorigenesis of PPGL and have led to the identification of new treatment targets for patients with malignant PPGL (Figure 3).

The PPGL transcriptome

Unsupervised hierarchical classification of gene expression profiling data neatly classified PPGLs into two different clusters.[74–77] The first cluster (the angiogenic cluster) is characterized by the strong expression of genes involved in the hypoxic pathway. The second cluster (the kinase signalling cluster) is characterized by activation of the MAPK and PI3K–AKT–mTOR pathways.[5] Cluster 1 can be divided into two subclusters: subcluster 1A contains the PPGLs related to the SDHx genes and cluster 1B contains *VHL*-related PPGLs. Most sporadic tumours, as well as those related to *RET* and *NF1*, are classified together in cluster 2.[76] Several minor PPGL susceptibility genes were identified by combining transcriptomic data with whole-exome sequencing and/or copy number variation data for tumours without known mutations attributed to cluster 1 or 2, such as *FH* and *EPAS1* for cluster 1 and *TMEM127* and *MAX* for cluster 2.[29,37,38,78] Integrative genomics studies combining gene expression profiling with sequencing data also revealed the presence of somatic mutations in major PPGL-susceptibility genes, such as *VHL*, *RET* and *NF1*, in tumours classified as belonging to cluster 1 (*VHL)* or cluster 2 (*RET* and *NF1)*. About 20% of sporadic PPGLs have mutations in the *NF1* gene, which is the gene with the highest frequency of somatic mutations in PPGL.[13,79] About 5% of sporadic tumours have a somatic mutation in *HRAS*.[48,49] Overall, a germline and/or somatic mutation in a known PPGL susceptibility gene is present in ~60% of tumours.

PPGL is clearly the cancer for which the contribution of genetics is the strongest. All the susceptibility genes identified are major drivers of cancer, with mutations triggering particular tumorigenic pathways that could theoretically be targeted by specific treatments. For cluster 1, an antiangiogenic approach would seem to be appropriate, but with strict monitoring of cardiovascular function. A few clinical reports relating to antiangiogenic therapies in a small number of patients have been published.[80–82] The most comprehensive study reported so far is a retrospective review of medical records of 17 patients (eight with an *SDHB* mutation, one with a *VHL* mutation and eight with sporadic tumours) with metastatic phaeochromocytoma or paraganglioma who were treated with sunitinib.[83] Among the 14 evaluable patients (medication was stopped in three patients owing to toxicity early in the study), three had a partial response according to RECIST 1.1 criteria and five had stable disease, while the disease progressed in the other six patients. Interestingly, a partial response or stable disease were observed in the patient with a *VHL* mutation and five of the six evaluable patients with a *SDHB* mutation, which suggests that patients with cluster 1 disease might be better responders to antiangiogenic treatments than patients with cluster 2 tumours. These observations need to be confirmed in larger cohorts of patients and compared with appropriate placebo conditions. Several clinical trials are currently ongoing and will hopefully enable such validations. Examples of these clinical trials include the FIRSTMAPPP study, a randomized double-blind phase II international multicentre study that is evaluating the efficacy of sunitinib versus placebo in patients with progressive malignant PPGL,[84] and two nonrandomized phase II studies that are evaluating the response to sunitinib[85] and axitinib.[86]

As reported in some patients with PPGL and in those with other tumour types, such as renal, breast or brain cancer,[87] some patients are expected to develop resistance to antiangiogenic treatments. In these patients, the use of successive antiangiogenic therapies containing other VEGF inhibitors and/or a combination with mTOR inhibitors or other chemotherapies will have to be evaluated and might constitute a more efficient approach than a single antiangiogenic molecule.

For cluster 2 tumours, inhibitors of mTOR signalling might be indicated and the use of everolimus has been reported in a few patients, albeit with disappointing results.[88] In particular, a phase II study reported a modest efficacy in patients with PPGL, although five of seven patients achieved stable disease.[89] However, experimental studies with AZD8055, a selective ATP-competitive dual

mTORC1/2 small-molecular inhibitor, in a cellular model of metastatic *Nf1*-related phaeochromocytoma have shown that this treatment decreases the tumour burden in athymic nude mice.[90] The therapeutic response to mTOR inhibitors in patients with malignant PPGL remains to be tested in a clinical trial and to be tested against the genotype of patients. Sporadic malignant PPGL generally belong to cluster 2 and these tumours are molecularly very similar to *RET*-mutated and *NF1*-mutated tumours. For patients with sporadic malignant PPGL, it thus seems that targeting either the mTOR or the RAS–RAF pathway might be a pertinent therapeutic approach.

The PPGL methylome

DNA methylation is a critical process in the regulation of gene transcription and mostly involves the methylation of CpG islands located in promoter regions. Genome-wide DNA methylation profiling in human PPGL and GIST revealed that tumours related to the SDHx genes are characterized by a hypermethylator phenotype that results in a decrease in gene expression.[29,91] Mutations in the SDHx genes lead to a huge accumulation of succinate, which inactivates 2-oxoglutarate-dependent dioxygenases, the Jumonji histone demethylases that catalyse the demethylation of histones and the TET (ten eleven translocation) hydroxylases that hydroxylate 5-methylcytosine to generate 5-hydroxy methylcytosine. These two groups of enzymes both have a crucial role in regulating the epigenome. A pattern of global histone and DNA hypermethylation was also found in *FH*-related paraganglioma. In this case, fumarate, as with succinate for SDHx-related tumours, acts as an oncometabolite.[29]

This pattern of global DNA and histone hypermethylation accounts for several of the characteristics of SDHx-related PPGLs. The silencing or downregulation of genes involved in neuroendocrine cell differentiation, such as *PNMT,* underlies the noradrenergic secretory phenotype of SDHx tumours described in a previous section. Hypermethylation of the *KRT19* gene, which encodes keratin 19, contributes to the activation of epithelial-to-mesenchymal transition observed in SDHB-related metastatic PPGL.[92] In addition, hypermethylation of the O^6-methylguanine-DNA methyltransferase promoter, the hallmark of a good response to temozolomide, suggests that this alkylating agent might have increased efficacy in patients with *SDHB* mutations and metastatic PPGL. Indeed, a retrospective study published in 2014 that evaluated the response to temozolomide in 15 patients with malignant PPGL reported a partial response in four of 10 patients with SDHB mutations and in none of the five patients with sporadic PPGL.[93] Although these data are promising, the findings will need to be confirmed in a prospective clinical trial. Finally, an *in vitro* study that examined the effects of a DNA demethylating agent (decitabine) in a model of *Sdhb*-knockout chromaffin cells showed a statistically significant decrease in the migration of SDHB-deficient cells.[29] This finding suggests that drugs targeting epigenetic pathways might constitute useful alternative treatments for SDHx-related or FH-related malignant PPGLs in the future.[29]

Future directions

One of the main issues in the clinical management of patients with malignant PPGLs remains the treatment of those with mutations in *SDHB*, as these patients develop particularly aggressive and rapidly progressing PPGL. For these patients, the increased understanding of the molecular mechanisms involved in PPGL will certainly pave the way to original targeted therapies that will specifically target the activated oncogenic pathways. One of the main consequences of the inactivation of succinate dehydrogenase is apparently the accumulation of succinate, which acts as an oncometabolite, competitively inhibiting a large family of 2-oxoglutarate-dependent dioxygenases that comprises HIF hydroxylases and collagen prolyl-hydroxylases as well as histone and DNA demethylases. Theoretically, reactivation of these enzymes using 2-oxoglutarate should overcome the broad consequences of succinate dehydrogenase inactivation and thus seems to be a very tempting approach. 2-oxoglutarate enhances myocardial protection during heart surgery and has been tested for other applications including renal failure, stomach, intestinal and liver disorders, muscle wounds and infections, but never against cancer. Similarly, ascorbate (that is, vitamin C), which is necessary for the activity of these enzymes, might also be an interesting molecule, and has already shown promising efficacy at high doses for the management of patients with terminal colon, stomach, breast, bladder or renal cancers.[94] Obviously, these innovative therapeutic approaches will first need to be evaluated in preclinical experimental models before moving to testing in patients.

Conclusions

Over the past 15 years, the tremendous effect of genetics on the initiation and development of PPGL has been clearly demonstrated. Genetic and genomic studies have already provided important new insights into the management and treatment of patients with malignant forms of the disease. 'Omics'-based tests will probably be transferred from the research laboratory to clinical practice in the near future, facilitating personalized medicine, with the goal of improving patient care and outcomes for these cancers. Metastatic PPGL remains an orphan disease that is only curable by surgery, but hope now exists that it will become a multifaceted disease curable by a large panel of effective treatments in the near future.

Review criteria

The data and information reported in this Review were extracted from original articles selected by searching PubMed for papers published between February 2000 and June 2014 that focused on the genetics of paraganglioma and phaeochromocytoma. The key search terms used were "paraganglioma", "phaeochromocytoma", "*RET*", "*VHL*", "*SDHA*", "*SDHB*", "*SDHC*", "*SDHD*", "*SDHAF2*", "KIF1B", "*IDH1*", "*HIF2A*", "*PHD2*", "*TMEM127*", "*MAX*", "*NF1*", "*FH*". In addition, the reference lists of the publications identified in this way were used for the identification of additional references. All the articles identified were in English and were full-length papers.

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Author contributions

J.F. researched data for the article, contributed to discussion of the content, and reviewed and edited the manuscript before submission. L.A. researched data for the article and contributed to discussion of the content. A.-P.G.-R. researched data for the article, contributed to discussion of the content, wrote the article and reviewed and edited the article before submission.