Human Molecular Genetics, 2020, Vol. 29, No. R2 R128–R137

doi: 10.1093/hmg/ddaa201
Advance Access Publication Date: 15 October 2020
Invited Review Article

INVITED REVIEW ARTICLE

Genetic stratification of inherited and sporadic phaeochromocytoma and paraganglioma: implications for precision medicine

Ruth Casey[1,2,3], Hartmut P.H. Neumann[4] and Eamonn R. Maher[1,2,*,†]

[1]Department of Medical Genetics, University of Cambridge, Cambridge, CB2 0QQ, UK, [2]NIHR Cambridge Biomedical Research Centre, Cambridge, CB2 0QQ, UK, [3]Department of Endocrinology, Cambridge University Hospital Foundation Trust, Cambridge CB2 0QQ, UK and [4]Section for Preventive Medicine, Faculty of Medicine, Albert-Ludwigs-University, Freiburg, Germany

*To whom correspondence should be addressed. Email: erm1000@medschl.cam.ac.uk

Abstract

Over the past two decades advances in genomic technologies have transformed knowledge of the genetic basis of phaeochromocytoma and paraganglioma (PPGL). Though traditional teaching suggested that inherited cases accounted for only 10% of all phaeochromocytoma diagnosis, current estimates are at least three times this proportion. Inherited PPGL is a highly genetically heterogeneous disorder but the most frequently results from inactivating variants in genes encoding subunits of succinate dehydrogenase. Expanding knowledge of the genetics of PPGL has been translated into clinical practice by the provision of widespread testing for inherited PPGL. In this review, we explore how the molecular stratification of PPGL is being utilized to enable more personalized strategies for investigation, surveillance and management of affected individuals and their families. Translating recent genetic research advances into clinical service can not only bring benefits through more accurate diagnosis and risk prediction but also challenges when there is a suboptimal evidence base for the clinical consequences or significance of rare genotypes. In such cases, clinical, biochemical, pathological and functional imaging assessments can all contribute to more accurate interpretation and clinical management.

Introduction

Phaeochromocytomas and paragangliomas (PPGL) are well-vascularized tumours that arise from cells derived from the sympathetic (e.g. adrenal medulla or sympathetic trunk) or parasympathetic (e.g. carotid body, glomus tympanicum, glomus jugulare, glomus vagale, etc.) paraganglia. According to the World Health Organization classification (1), the term phaeochromocytoma is reserved exclusively for tumours of the adrenal medulla, whereas the term paraganglioma is recommended for tumours at all the other extra-adrenal sites [though paragangliomas derived from parasympathetic ganglia are commonly referred to as head and neck paraganglioma (HNPGL) and sympathetic paraganglioma as paraganglioma]. Both PPGL may contain elements of related neurogenic tumours such as ganglioneuroma, ganglioneuroblastoma, neuroblastoma, etc. Such tumours are referred to as composite phaeochromocytomas or composite paragangliomas, respectively. Over the last two decades, advances in the genetics of phaeochromocytoma has led to improved molecular diagnosis, effective predictive testing of asymptomatic relatives and informed gene-specific medical management.

PPGL has a very high heritability rate, and almost half of all cases (~40%) can be attributed to an inherited mutation. To date,

†Eamonn R. Maher, http://orcid.org/0000-0002-6226-6918
Received: August 25, 2020. **Revised:** August 30, 2020. **Accepted:** September 3, 2020

more than 15 PPGL predisposition genes (PCGs) (including *NF1, RET, VHL, SDHA, SDHB, SDHC, SDHD, SDHAF2, FH, MAX, EPAS1, TMEM127, DLST, MDH2, GOT2, SLC25A11, DNMT3A*) have been implicated in hereditary PPGL, and this number increases every year with the increasing uptake of large-scale genomic sequencing (2,3) and references within. Traditionally, hereditary PPGL was considered to account for approximately 10% of cases and to occur predominantly as part of three familial syndromes: neurofibromatosis type 1 (NF1) caused by germline mutations in the neurofibromin 1 gene (*NF1*), multiple endocrine neoplasia type 2 (MEN2) caused by germline mutations in the *RET* proto-oncogene and von Hippel-Lindau disease (VHL) caused by germline mutations in the *VHL* tumour suppressor gene (4–6). Each of these syndromes are associated with other characteristic phenotypic features, and although each predisposes to phaeochromocytoma (including bilateral tumours), paragangliomas are unusual (2).

At the start of this century, the seminal findings that inherited HNPGL and PPGL could be caused by germline mutations in genes encoding three subunits (*SDHB, SDHC, SDHD*) of succinate dehydrogenase (SDH) were reported (7–10). It quickly became apparent that the frequency of germline mutations in individuals with PPGL was much higher than 10% (11) and that many cases of apparently sporadic non-syndromic PPGL were inherited. Furthermore, these findings kick-started an era of PPGL gene discovery, and additional PPGL predisposition genes were then identified. The genetic landscape of inherited PPGL is complex and heterogeneous (see below), but the ability to identify individuals with germline mutations has changed clinical practice around surveillance in patients and their relatives (12,13). Though the impact on therapy is currently much more limited, gene-stratified functional studies are providing important insights into the molecular pathogenesis of PPGL. For example, transcriptomic analysis has facilitated a better understanding of the major pathways perturbed and suggested that inherited PPGL can be subclassified into two broad transcriptomic categories, either an angiogenic cluster (14) or a kinase signalling cluster (15). Furthermore, epigenetic and metabolomic profiling, immunohistochemistry (IHC) and *in vivo* functional imaging can all be applied to further subcharacterize PPGL (16). In this review, we describe the molecular basis and genotype–phenotype correlations of inherited PPGL and outline how progress in omic technologies could lead to a new age of precision management and targeted therapies for PPGL.

Genomic Landscape of PPGL

PPGL predisposition genes

More than 15 different genes have been implicated in autosomal dominant familial PPGL to date. The SDH genes (*SDHA, SDHB*, SDHC and *SDHD*) are the most common inherited PPGL predisposition genes, followed by mutations in genes associated with syndromic presentations as described above (*VHL*, *RET* and *NF1* genes). Mutations in any of the four *SDHx* genes or the *SDHAF2* gene, which encodes its namesake protein responsible for the flavination of the SDHA protein, lead to disruption of the SDH enzyme in the citric acid cycle and accumulation of the oncometabolite succinate, which drives tumorigenesis by inhibiting alpha-ketoglutarate-dependent dioxygenase enzymes leading to hypermethylation and pseudohypoxia (17) (Fig. 1). Mutations in these genes predispose to multi-focal and synchronous PPGL, which can be parasympathetic arising in the head and neck or mediastinum or sympathetic and develop in the abdomen and pelvis. *SDHx* mutations also predispose to non-PPGL tumours including gastrointestinal stromal tumours, renal cell carcinoma and rarely pituitary tumours (12).

Mutations in further citric acid cycle genes have been implicated in hereditary PPGL including germline mutations in the fumarate hydratase (*FH*) gene (associated with hereditary leiomyomatosis, renal cell carcinoma and rarely phaeochromocytoma) (18,19). Malate dehydrogenase (*MDH2*) (implicated in rare cases of familial PPGL) (20) and more recently germline mutations in the gene encoding the mitochondrial 2-oxoglutarate/malate carrier (*SLC25A11*) (21) and in a gene encoding a component of the oxoglutarate dehydrogenase complex, dihydrolipoamide S-succinyltransferase (*DLST*), have also been implicated in rare cases of familial PPGL (22). The mechanisms of tumorigenesis provoked by citric acid cycle mutations (namely hypermethylation and pseudohypoxia) have also recently led to the discovery that a gain of function mutations in a DNA methyltransferase gene (*DNMT3A*) is also rarely be implicated in familial PPGL (23).

Beyond citric acid cycle predisposition genes and syndromic causes of familial PPGL, mutations in genes involved in the regulation of kinase pathways including *TMEM127*, a gene that encodes a transmembrane protein involved in modulation of the mTOR pathway (24) and mutations in the MYC-associated factor X (*MAX*) gene (25), the hypoxia-inducible factor-2 alpha subunit gene (*HIF2A/EPAS1*) (26) and *EGLN1/PHD2* (27) complete the list of the genes currently proposed to be implicated in familial PPGL.

Somatic events and tumourigenic pathways in PPGL

The somatic genetic and epigenetic events in both inherited and sporadic PPGL tumorigenesis have been delineated by targeted and genome-wide sequencing studies and epigenetic and metabolomic investigations. A large number of genes have been reported to harbour germline (see above) and/or somatic mutations in PPGL but PPGL are noteworthy because each tumour typically has a low mutation load and in many cases only a single driver mutation (germline or somatic) is detected (28). For inherited PPGL, the germline mutation usually inactivates a tumour suppressor gene (e.g. *NF1, VHL, SDHX, MAX, FH, TMEM127*, etc.) and the PPGL contains a somatic event (giving 'two hits') such as a large chromosomal deletion, somatic mutation or promoter methylation with transcriptional silencing that inactivates the wild-type allele (28,29). In sporadic PPGL, the most common copy number abnormalities are loss at chromosome 1p, 3p, 3q 11p, 17, 21q and 22q loss (28), which include the *VHL* (3p25) and *NF1* (17q11.2) gene locations. Overall, somatic inactivating mutations in *NF1* and *VHL* or somatic activating mutations in *RET* and *EPAS1* occur in ~25% of sporadic PPGL, but somatic mutations in other inherited PPGL such as *SDHx* or *FH* are rare (28,30). A number of genes that have not been implicated in inherited PPGL have been reported to be somatically mutated in PPGL including *HRAS, BRAF, SETD2*, *FGFR1*, *TP53*, *ATRX*, *ARNT*, *IDH1*, *H3F3A*, *MET, CSDE1*. In addition, *MAML3* fusion genes and structural rearrangements in telomerase reverse transcriptase (*TERT*) have been described (28,31). Interestingly, as often each PPGL includes a single driver mutation and somatic *HRAS* mutations occur in ~10% of sporadic PPGL but not inherited PPGL, it has been suggested that if a *HRAS* mutation is detected by somatic mutation profiling, the risk of inherited disease will be low (32).

Though a large number of genes have been implicated in the molecular pathogenesis of inherited and sporadic PPGL, many can be linked to a number of key signalling pathways. A decade ago, transcriptomic analysis of PPGL suggested two distinct subcategories comprising Cluster 1 that was

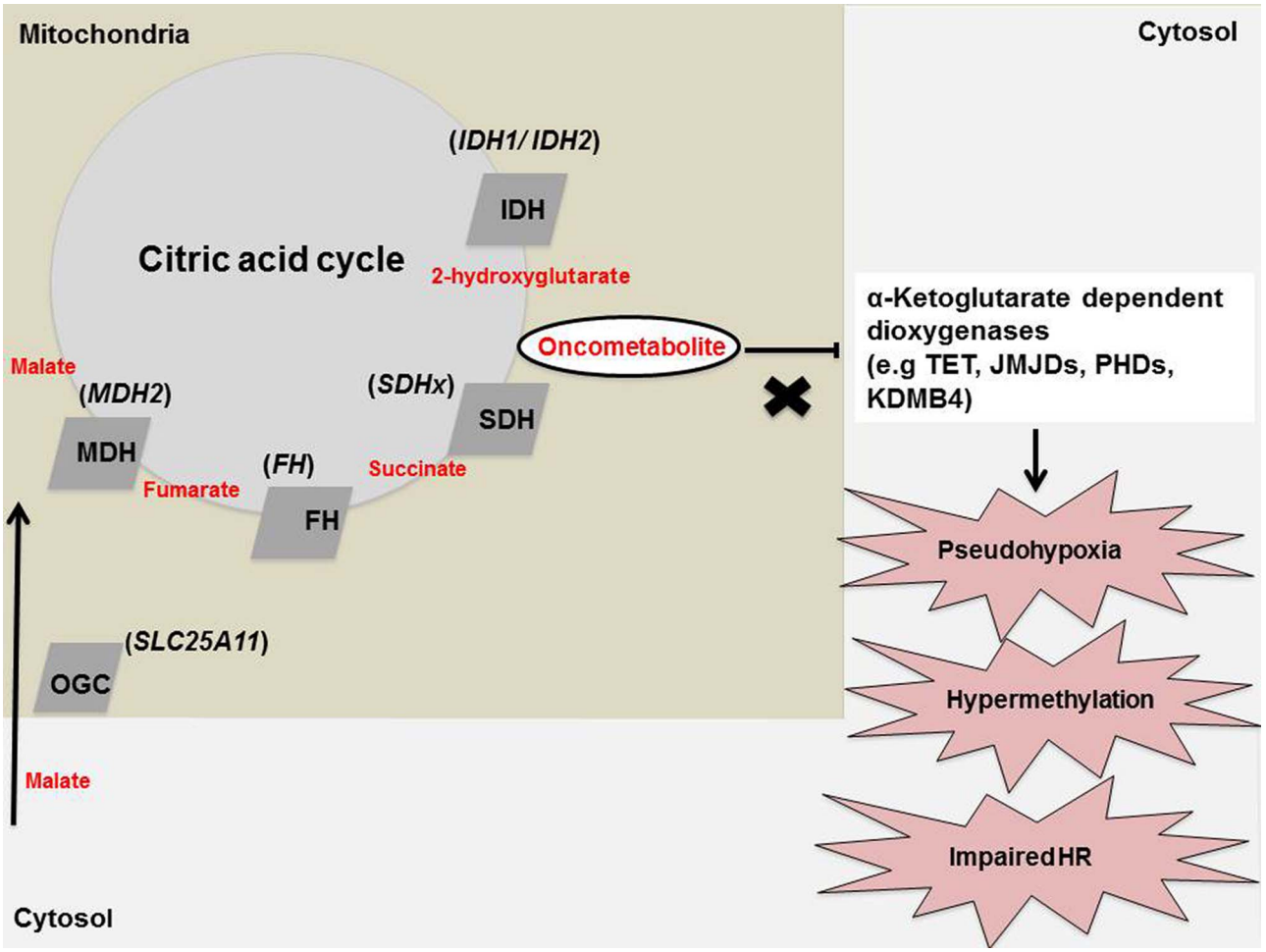

Figure 1. Illustration of how pathogenic variants in citric acid cycle genes result in enzyme dysfunction in the mitochondria resulting in accumulation of oncometabolites (shown in red) (e.g. succinate with SDH subunit gene mutations). The oncometabolites inhibit 2-oxyglutarate-dependant enzymes (including demethylase enzymes and prolyl hydroxylase enzymes) resulting in pseudohypoxia and DNA hypermethylation phenotypes and impair homology-dependent DNA repair, promoting tumour development.

characterized by upregulation of hypoxia signalling pathways and Cluster 2 in which there was no hypoxic signal but kinase signalling pathways were upregulated (14). Unsurprisingly, tumours with mutations in *VHL* and *HIF2A/EPAS1* (pVHL is a negative regulator of the hypoxia-induced transcription factors HIF-1 and HIF2) map to Cluster 1. In addition, *SDHX-*, *FH-*, *MDH-* and *SLC25A11*-mutated tumours fall into Cluster 1 (30). In these cases, the intracellular accumulation of the relevant oncometabolite (succinate, fumarate, etc.) inhibits hydroxylation of key HIF1A/HIF2A proline residues that are required for pVHL to bind and initiate proteasomal degradation of the HIF-alpha subunits [PPGL-associated mutations in *HIF2A* usually affect binding of pVHL to this proline residue (P531)] (33–35). The pVHL protein has a key role in targeting the HIF2A protein for proteasomal degradation and somatic inactivating mutations in *VHL* and activating mutations in *HIF2A* will both result in stabilization of HIF2A and activation of hypoxic gene response pathway (26,36). The oncometabolites also inhibit other alpha-ketoglutarate-dependent enzymes including the ten-eleven translocation proteins that actively demethylate DNA demethylation and *SDHX-*, *FH-*, *MDH-* and *SLC25A11*-related tumours are characterized by genome methylation (16,21). Recently, it has been reported that these oncometabolites also inhibit homology-dependent DNA repair (HDR) pathways by causing aberrant hypermethylation of histone 3 lysine 9 at DNA breaks resulting in impaired HDR (37,38) (see Fig. 1). Thus, though Cluster 1 tumours are characterized by activation of a pseudohypoxic gene response, there is heterogeneity for other pathways including DNA methylation and DNA repair (Table 1).

Within Cluster 2, there is also genetic and pathway heterogeneity. Mutations in *RET*, *NF1*, *TMEM127*, *MAX* and *HRAS* deregulate to varying degrees kinase pathways including PI3K/AKT, RAS/RAF/ERK amf mTORC1 pathways (REFS). *Wnt* pathway alterations have been associated with somatic *CSDE1* mutations and *MAML3* fusion events (28) (Table 1). Other somatic events include TERT promoter mutations and mutations in *ATRX*, an epigenetic regulator (39,40).

Demographic and phenotypic correlations in inherited PPGL

The presence of non-neoplastic syndromic features and non-PPGL tumour types can lead to suspicion of a syndromic diagnosis (e.g. medullary thyroid cancer in MEN2, haemangioblastoma and VHL disease, etc.), which can then be confirmed by diagnostic testing. Similarly the presence of a family history of PPGL

Table 1. Characteristics and functional consequences of mutations in genes that are frequently mutated (germline and/or somatic) in phaeochromocytoma and paraganglioma (PPGL). HDR = homology dependent repair

Gene	Germline or somatic	Germline mutation frequency in inherited PPGL[a]	Somatic mutation frequency in sporadic PPGL[a]	Hypoxic pathways activated	DNA hypermethylation	Impaired HDR	Kinase pathway dysregulation	Wnt pathway dysregulation
NF1	Both	3%	9%	–	–	–	+	–
RET	Both	6%	9%	–	–	–	+	–
VHL	Both	4%	3%	+	–	–	–	–
SDHA	Germline	1%	Rare	+	+	+	–	–
SDHB	Germline	9%	Rare	+	+	+	–	–
SDHC	Germline	1%	Rare	+	+	+	–	–
SDHD	Germline	2%	Rare	+	+	+	–	–
FH	Germline	1%	Rare	+	+	+	–	–
HIF2A	Both	Rare	5%	+	–	–	–	–
MAX	Germline	1%	Rare	–	–	–	+	–
TMEM127	Germline	0.6%	Rare	–	–	–	+	–
HRAS	Somatic	–	10%	–	–	–	+	–
CSDE1	Somatic	–	2%	–	–	–	–	+

[a]Estimates mainly taken from Fishbein *et al.* (28).

or HNPGL or of bilateral or multiple PPGL will invariably suggest the presence of an underlying genetic predisposition and trigger genetic testing. However, a range of other clinical, biochemical, pathological and imaging features can also be used to inform predictions about the likelihood of a genetic cause:

Age. A young age at presentation is associated with a higher risk of a germline pathogenic variant in a PPGL gene. Diagnostic yields as high as 80% have been reported in paediatric populations with PPGL, compared with 30–40% in adult populations (41).

Tumour location. Extra-adrenal location is major phenotypic predictor of germline *SDHx* genes mutations (42). The diagnostic yield for pathogenic variants in inherited PPGL genes in individuals with a paraganglioma was six times higher than in those with an isolated adrenal phaeochromocytoma (43).

Tumour secretory phenotype. Biochemical testing is an essential step in the diagnostic pathway for PPGL and current guidelines recommend urinary or plasma metanephrines and plasma 3-methoxytyramine (3MT) as the first-line biochemical tests in the diagnosis of PPGL (44). The pattern of catecholamine secretion from a PPGL is determined by paraganglial cell differentiation, and therefore, biochemistry can be used to predict genotype and/or malignant potential. Pseudohypoxic or 'Cluster 1' PPGL are characterized by poor differentiation of paraganglia cells and reduced expression of a catecholamine conversion enzyme called phenylethanolamine *N*-methyltransferase (PNMT). Reduced expression of this enzyme affects the conversion of noradrenaline to adrenaline, resulting in a predominant noradrenergic secretory pattern in tumours harbouring mutations in the Cluster 1 genes (45). In addition to reduced expression of PNMT, *SDHx*-mutated tumours also have reduced activity of the enzyme dopamine-β-hydroxylase, responsible for the conversion of dopamine to norepinephrine in the catecholamine synthesis pathway. Therefore, elevated levels of dopamine or its metabolite 3-methoxytyramine is also a characteristic biochemical signature of SDH-deficient PPGL (46). Elevated dopamine can be viewed as a surrogate marker for poor paraganglia cell differentiation and elevated levels of 3-methoxytyramine have been validated as an independent predictor of malignant disease (45). Finally, SDHx mutations can also affect the expression and/or activity of the rate-limiting enzyme in catecholamine synthesis, tyrosine hydroxylase, explaining why non-secretory PPGL are also more commonly associated with *SDHx* gene mutations (46). In contrast, 'Cluster 2' tumours are predominantly driven by mutations in kinase signalling genes, have a more mature phenotype associated with increased expression of PNMT and have a mixed or predominately adrenergic secretory pattern (28,45).

Malignancy. About 10% of PPGL are malignant (higher in paraganglioma than in phaeochromocytoma). Germline *SDHx*, particularly *SDHB* mutations, is associated with a higher risk of malignancy, and a recent meta-analysis has suggested a rate of metastatic PPGL of 48.9% in SDHB mutation carriers compared with a rate of 8.9% in non-SDHB mutation carriers (30). Two rarer PPGs linked to malignant PPGL predisposition genes (PPGL) are *FH* and *SLC25A11* (21,47). An increased risk of aggressive and metastatic disease has been associated with somatic *ATRX* mutations, *MAML3* fusions and *TERT* activation (28).

Immunohistochemistry. Histopathological examination is not a reliable predictor of malignancy in PPGL, and the diagnosis of malignancy is dependent on the presence of distant metastases (48). However, IHC is an important tool for detecting or confirming inherited PPGL. Biallelic inactivation (i.e. a germline mutation and somatic 'second hit') of any of the *SDHx* genes will typically destabilize the SDH enzyme complex resulting in proteolytic degradation of the anchor SDHB protein, which can be detected by loss of staining for the SDHB protein by IHC (49). Thus, SDHB IHC can be used to identify PPGL harbouring an *SDHx* mutation and as a functional tool for assessing the pathogenicity of uncharacterized or novel *SDHx* variants. IHC for SDHA expression can predict the presence of pathogenic *SDHA* variants specifically in the *SDHA* gene and can be utilized in clinical practice (50). The interpretation of variants in *FH* is facilitated by IHC to detect loss of expression of the fumarate hydratase protein (by FH IHC) or by the detection of protein succinylation

Table 2. Aspects of PPGL management that are influenced by results of genetic testing

	Germline pathogenic variant (PV) detected	Germline VUS in PSG or significant risk factors[a]	No genetic variant and no risk factors
Proband follow-up for recurrence/metastatic disease	Lifelong	Assess after 10 years	10 years
Surveillance for non-PPGL tumours	Yes—specific surveillance dependent on relevant gene	Occasionally applicable if strong suspicion of a syndromic cause	No[a]
Genetic testing of relatives	Offered	Usually not applicable	Not applicable
Surveillance of relatives for PPGL	Screening offered to PV-positive individuals; tailored to specific gene	Potentially applicable, e.g. if strong family history	Not applicable[a]
Treatment of metastatic disease	First-line therapies generally as per standard protocols Second-line treatment options should include genotype-driven clinical trials (see text)	Usually as per standard protocols	As per standard protocols

[a]Assuming no clinical or pathological features that suggest a genetic cause is likely.

(a post-translational modification resulting from the reaction of excess fumarate with cysteine residues) shown by positive staining to *S*-(2-succinyl)-cysteine (2SC) (51). IHC may also be utilized for other hereditary causes of PPGL including assessment of MAX expression and IHC for the membranous expression of carbonic anhydrase 9 in the assessment of germline or somatic *VHL* gene mutations (52). In some cases, loss of SDHB expression may not result from an *SDHX* mutation but from a germline or somatic *VHL* gene mutation (53).

Personalized Medicine Approaches in PPGL

The molecular stratification of patients with PPGL through germline and somatic testing opens up the possibility of genotype-driven personalized therapy. The concept of genotype-driven personalised management might be considered from a variety of perspectives (see below) and according to whether an individual does or does not have a pathogenic variant or a variant of uncertain significance (VUS) in an inherited PPGL gene (Table 2). As described above, there are a number of strategies available to enable precision medicine, including genetic testing, IHC and functional imaging. What is the current and future role of these in clinical practice?

The who and how of genetic testing

In view of the high diagnostic yield of germline testing in individuals with PPGL, it has been argued that a universal genetic testing strategy should be employed. However, currently (though this is likely to change as genetic testing becomes less expensive) most centres practice some form of selective testing. Patients with features of an inherited syndrome, family history of PPGL (or a relevant tumour, e.g. RCC or GIST) or multiple tumours (e.g. two PPGL or a PPGL and a related tumour such as HNPGL, wtGIST, RCC, etc.) should be routinely offered testing. Based on the genotype–phenotype correlations discussed above, those with an extra-adrenal location (sympathetic paraganglioma) or metastatic disease also qualify for testing. For patients with an isolated phaeochromocytoma, the decision to test is usually based on a younger age at diagnosis (e.g. $\leq$60 years but some centres may have lower age limits) but incorporation of additional factors such as biochemical profile or IHC (see below) may influence the decision to test older patients. Most centres will offer testing with a large panel of PPGL susceptibility genes (either a custom gene panel or exome sequencing with 'virtual gene panel') that will typically include major PPGL genes (*NF1, RET, VHL, SDHA, SDHB, SDHC, SDHD, FH, MAX, EPAS1, TMEM127*) but not necessarily rarer susceptibility genes. Hence, if first-line testing is negative, then inclusion of additional genes or further analysis for cryptic mutations that may not be detected by routine testing (e.g. for *VHL* (54)), IHC or tumour testing may be considered. Combined germline and tumour mutation analysis has resulted in diagnostic yields for germline or somatic driver mutations of 80% (30) though not all drivers are genetic events as somatic epimutations in the promoter region of the *SDHC* gene have been reported wtGIST and occasionally in PPGL (55,56).

The detection of a germline or genetic variant may not provide an unequivocal diagnosis. Resolving the pathogenicity of rare VUS, particularly in less frequently tested genes, can be challenging but may be facilitated by IHC (see above), segregation analysis in familial cases, somatic testing (e.g. by finding LOH or somatic variant that is not usually detected in familial disease) or functional imaging (see below).

In most centres, germline testing is performed in the first instance as, though tumour testing can have some advantages, in most cases only formalin-fixed material is available for analysis.

Role of immunohistochemistry

In addition to its utility in variant interpretation (see above), it can be used to screen for PPGL that require germline testing but have not been selected. Thus, in some centres, SDHB IHC is performed in older patients with isolated phaeochromocytoma. Though such an approach could be extended to screen for *MAX*- and *FH*-related phaeochromocytoma, these are much rarer.

Role of functional imaging for PPGL precision medicine

Nuclear imaging techniques can be utilized as adjuncts to morphological cross-sectional imaging studies and have diagnostic and theranostic utility in the management of PPGL. Nuclear imaging tracers specific for PPGL can be subclassified based on their target ligand into three groups: (i) catecholamine storage and synthesis [123I-metaiodobenzylguanidine, 18F-fluorodopamine (18F-FDA) and 18F-fluorodihydroxyphenylalanine (18F-FDOPA)], (ii) somatostatin receptor [^{111}indium–pentetreotide

and gallium-68 DOTA-conjugated peptide (^{68}Ga-DOTATATE)] and (iii) glucose metabolism [18F-fluorodeoxyglucose (18F-FDG)]. The selection of the most appropriate tracer for surveillance or diagnosis of PPGL is influenced by the patient genotype and the associated interplay with tumour biology, tumour location and tumour secretory pattern, all of which influence the expression of receptors targeted by functional imaging tracers, giving rise to a so-called functional imaging phenotype (57).

The tracer $^{123/131}$I-metaiodobenzylguanidine (MIBG) is taken up by the noradrenaline transporter (NET); however, the sensitivity of $^{123/131}$I-MIBG scintigraphy is affected by tumour de-differentiation resulting in loss of NET expression, therefore increasing the risk of false-negative results using $^{123/131}$I-MIBG scintigraphy. Furthermore, mutations in *SDHx* are also associated with downregulation of the NET transporter, affecting the sensitivity of $^{123/131}$I-MIBG scintigraphy in SDH-deficient tumours (58). Therefore, current recommendations advice that $^{123/131}$I-MIBG scintigraphy should be reserved for those cases being investigated for suitability of treatment with $^{123/131}$I-MIBG radionuclide therapy rather than for surveillance, diagnosis or the detection of occult metastases particularly in those patients with suspected *SDHx* mutations (59). Similar issues with sensitivity are seen with the tracer 18F-FDA, also taken up by the NET transporter. The imaging tracer 18F-FDOPA is taken up via neutral amino acid transporter system L, and the sensitivity of 18F-FDOPA PET-CT is notably reduced in patients with *SDHx* mutations, and although the exact mechanism for this is not fully understood, it is thought to relate to the truncated citric acid cycle and the impaired secretory status of *SDHx*-mutated tumours (57).

The sensitivity of 18F-FDG PET-CT also differs depending on the driver genetic mutation, as Cluster 1 tumours exhibit attenuated glycolysis and demonstrate increased standard uptake values of 18F-FDG due to increased expression of glucose transporters and glycolytic enzymes (60).

The tracer ^{68}Ga-DOTATATE can be used to identify tumours expressing somatostatin receptor subtype 2, and a recent meta-analysis of eight studies reviewing the sensitivity of functional imaging modalities for the detection of PPGL of unknown genotype demonstrated that ^{68}Ga-DOTATATE PET-CT had a pooled sensitivity of 93% and was superior to 18F-FDG PET-CT, 18F-FDOPA and $^{123/131}$I-MIBG scintigraphy (61). An earlier meta-analysis also reported a superior sensitivity for the detection of SDHx-mutated PPGL using ^{68}Ga-DOTATATE PET-CT compared with 18F-FDG PET-CT (62).

Therefore, ^{68}Ga-DOTATATE PET-CT is now recommended as the imaging modality of choice for staging and surveillance in patients with *SDHx*-mutated PPGL or sporadic or metastatic PPGL (63). In addition to the diagnostic role, ^{68}Ga-DOTATATE PET-CT can also predict the efficacy of peptide receptor radionuclide therapy with ^{177}Lu-DOTATATE for patients with metastatic PPGL. In centres where ^{68}Ga-DOTATATE PET-CT is not available, 18F-FDG PET-CT would be a reasonable alternative tracer to consider for staging and surveillance in patients with *SDHx*-mutated PPGL. On the contrary, ^{68}Ga-DOTATATE PET-CT has demonstrated poor sensitivity in patients with *EPAS1* mutations, and therefore, 18F-DOPA PET-CT is recommended as the first-line functional imaging modality for surveillance in patients with *EPAS1* mutations or patients with Cluster 2 gene mutations (*RET*, *MAX*, *NF1*) who are at higher risk of phaeochromocytoma owing to the high tumour to background normal adrenal uptake of this tracer compared with ^{68}Ga-DOTATATE (63,64).

Surgical management

The primary treatment of a single localized PPGL will generally be surgical, but a clinical or molecular diagnosis of inherited PPGL prior to surgery may influence the surgical strategy. For example, in individuals with MEN-2A/B or VHL disease with phaeochromocytoma, who are at risk of a further tumour in the other adrenal gland, an adrenal cortical sparing approach can be preferable (65). When a PPG mutation has been detected, the risks of further primary tumours and of malignant disease will need to be considered and is informed by established genotype–phenotype correlations.

Post-surgical follow-up

Individuals with PPGL and a PPG mutation should be designated for lifelong follow to enable early detection of further primary PPGL and non-PPGL tumours and metastatic disease; the specific risks of these events is dependent on the PPG implicated. For example, risk of metastatic disease is highest with *SDHB* mutations (42) but metastatic disease developed in an individual with a germline *SDHA* mutation more than two decades after the initial paraganglioma (66). Surveillance protocols for non-PPGL tumours in inherited multisystem inherited cancer syndromes such as VHL disease, MEN2, HLRCC and NF1 have been described elsewhere (67–70). For individuals with germline mutations in non-syndromic genes (*SDHX*, *MAX*, *TMEM127* and rarer genes), there is a recent trend towards moving to a more gene-specific approach to surveillance up of affected individuals and asymptomatic gene carriers (Table 3).

Management of metastatic disease

Widely used first-line treatments for metastatic PPGL include cytotoxic chemotherapeutic regimes (cyclophosphamide, vincristine and dacarbazine) targeted therapies such as sunitinib and temozolomide (71) or radiopharmaceutical options such as ^{131}I-MIBG, ^{90}Y- and ^{177}Lu-DOTATATE (71,72). In general, these have been applied irrespective of the genetic background but increasing evidence for genotype-specific differences in the cellular pathways dysregulation in inherited PPGL [e.g. hypoxia gene response pathways in *VHL*- and *SDHx*-mutated PPGL and DNA methylation and chromatin regulation in TCA gene mutations (see above)] is paving the way for molecularly stratified clinical trials targeting specific mechanisms of tumorigenesis. Angiogenic inhibition by tyrosine kinase inhibitors such as sunitinib and sorafenib is widely used for the treatment of metastatic RCC in *VHL* tumour suppressor gene inactivation is frequent (73). More precise targeting of hypoxic gene response pathways is promised by the development of HIFa antagonists such as PT2977 (74), which is currently being evaluated in VHL disease patients with RCC and might prove to be an option for metastatic Cluster 1 PPGL. The demonstration of genome hypermethylation and impaired HDR pathways in *SDHx*- and *FH*-related tumorigenesis suggests a potential role for demethylating agents and PARP inhibitors (37). If such approaches prove to be successful, then we would expect that genotype-driven treatment for metastatic PPGL will become an established part of clinical care.

Cascade testing and surveillance of at risk relatives

The identification of a pathogenic PPG variant in an affected individual enables genetic testing of their relatives to determine tumour risks and need for tumour surveillance. As for affected individuals (see above), there is an increasing trend towards

Table 3. Examples of genotype-specific surveillance for asymptomatic mutation carriers of non-syndromic PPGL. For SDHB clinical and biochemical testing is from 5 years and radiological surveillance from 10 years whereas for SDHA, SDHC, SDHD, TMEM127, MAX, SDHAF mutation carriers clinical and biochemical testing starts at 10 years and radiological surveillance from 15 years (for asymptomatic patients)

Gene	Recommended surveillance
SDHB	Annual clinical review and biochemistry Abdominal imaging at baseline and if normal every 12–24 months MRI/CT of neck, thorax at baseline and if normal every 3 years
SDHD	Screening should only be offered to patients who have a paternally inherited SDHD variant Annual clinical review and biochemistry Abdominal imaging and MRI/CT of neck, thorax at baseline and if normal every 3 years
SDHC	Annual clinical review and biochemistry Abdominal imaging and MRI/CT of neck, thorax at baseline and if normal every 3 years
SDHA	Annual clinical review and biochemistry Abdominal imaging and MRI/CT of neck, thorax at baseline and if normal every 3–5 years Recommended surveillance
MAX	Screening should only be offered to patients who have a paternally inherited MAX variant Annual clinical review and biochemistry Abdominal imaging at baseline and if normal every 3 years MRI of neck, thorax at baseline and if normal every 5 years
TMEM127	Annual clinical review and biochemistry Abdominal imaging at baseline and if normal every 3 years MRI of neck, thorax at baseline and if normal every 5 years
SDHAF2	Screening should only be offered to patients who have a paternally inherited *SDHAF2* variant Annual clinical review and biochemistry Abdominal imaging at baseline and if normal every 3 years MRI of neck, thorax at baseline and if normal every 5 years

genotype-specific surveillance of asymptomatic gene carriers identified through familial testing. Though all the major causes of inherited PPGL are caused by monoallelic pathogenic variants, for germline mutations in *SDHD*, *SDHAF1* and *MAX*, there are important parent-of-origin effects on tumour risks, which mean that maternal transmission of a pathogenic variant is associated with a low risk of clinical disease and this is reflected in the gene-specific surveillance programmes (Table 3).

Conclusion

Over the past two decades, our knowledge of the genetic basis of PPGL has been transformed and aspects of the clinical management PPGL are increasingly being influenced by the results of genetic testing. With falling costs of genomic technologies, we anticipate that genetic testing for PPGL will become eventually universal and more comprehensive (e.g. by application of germline whole genome sequencing and tumour testing for somatic mutations). However, in order for PPGL to become an exemplar of personalized medicine, important challenges remain in particular (i) improving variant interpretation to reduce the number of VUSs, (ii) accurate tumour risk prediction for each PPGL gene in various clinical settings, (iii) establishing the optimal genotype-specific surveillance protocols that enable both accurate early tumour diagnosis without undue health care costs or iatrogenic risks and (iv) elucidating what the optimal targeted therapies for metastatic disease are based on the specific driver PPGL gene.

ACKNOWLEDGEMENTS

We apologise to all the authors whose work we were unable to cite because of space constraints. We thank Dr Birke Bausch, University of Freiburg, Germany, and Anna Roslyakowa, Department of Surgery, Endocrinology Research Center, Moscow, Russia, for their expert input. *Conflict of Interest statement*. None declared.

FUNDING

R.C. acknowledges support from AMEND and GIST Support UK. E.R.M. acknowledges support from European Research Council (Advanced Researcher Award), NIHR (Senior Investigator Award and Cambridge NIHR Biomedical Research Centre) and Cancer Research UK Cambridge Cancer Centre. The views expressed are those of the authors and not necessarily those of the NHS or NIHR. The University of Cambridge has received salary support in respect of E.R.M. from the NHS in the East of England through the Clinical Academic Reserve.

References

1. Lloyd, R.V., Osamura, R.Y., Kloppel, G. and Rosai, J. (2017) *WHO Classification of Tumours: Pathology and Genetics of Tumours of Endocrine Organs*, 4th edn, Lyon. https://publicatio

ns.iarc.fr/Book-And-Report-Series/Who-Classification-Of-Tumours/WHO-Classification-Of-Tumours-Of-Endocrine-Organs-2017.
2. Neumann, H.P.H., Young, W.F., Jr. and Eng, C. (2019) Pheochromocytoma and paraganglioma. *N. Engl. J. Med.*, **381**, 552–565.
3. Buffet, A., Burnichon, N., Favier, J. and Gimenez-Roqueplo, A.P. (2020) An overview of 20 years of genetic studies in pheochromocytoma and paraganglioma. *Best Pract. Res. Clin. Endocrinol. Metab.*, **34**, 101416.
4. Wallace, M.R., Marchuk, D.A., Andersen, L.B., Letcher, R., Odeh, H.M., Saulino, A.M., Fountain, J.W., Brereton, A., Nicholson, J., Mitchell, A.L. *et al.* (1990) Type-1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. *Science*, **249**, 181–186.
5. Mulligan, L.M., Kwok, J.B., Healey, C.S., Elsdon, M.J., Eng, C., Gardner, E., Love, D.R., Mole, S.E., Moore, J.K., Papi, L. *et al.* (1993) Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. *Nature*, **363**, 458–460.
6. Latif, F., Tory, K., Gnarra, J., Yao, M., Duh, F.M., Orcutt, M.L., Stackhouse, T., Kuzmin, I., Modi, W., Geil, L. *et al.* (1993) Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science*, **260**, 1317–1320.
7. Baysal, B.E., Ferrell, R.E., Willett-Brozick, J.E., Lawrence, E.C., Myssiorek, D., Bosch, A., van der Mey, A., Taschner, P.E., Rubinstein, W.S., Myers, E.N. *et al.* (2000) Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science*, **287**, 848–851.
8. Astuti, D., Douglas, F., Lennard, T.W., Aligianis, I.A., Woodward, E.R., Evans, D.G., Eng, C., Latif, F. and Maher, E.R. (2001) Germline SDHD mutation in familial phaeochromocytoma. *Lancet*, **4**357, 1181–1182.
9. Astuti, D., Latif, F., Dallol, A., Dahia, P.L., Douglas, F., George, E., Sköldberg, F., Husebye, E.S., Eng, C. and Maher, E.R. (2001) Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. *Am. J. Hum. Genet.*, **69**, 49–54.
10. Müller, U., Troidl, C. and Niemann, S. (2005) SDHC mutations in hereditary paraganglioma/pheochromocytoma. *Familial Cancer*, **4**, 9–12.
11. Neumann, H.P., Bausch, B., McWhinney, S.R., Bender, B.U., Gimm, O., Franke, G., Schipper, J., Klisch, J., Altehoefer, C., Zerres, K. *et al.* (2002) Germ-line mutations in nonsyndromic pheochromocytoma. *N. Engl. J. Med.*, **346**, 1459–1466.
12. MacFarlane, J., Seong, K.C., Bisambar, C., Madhu, B., Allinson, K., Marker, A., Warren, A., Park, S.M., Giger, O., Challis, B.G. *et al.* (2020) A review of the tumour spectrum of germline succinate dehydrogenase gene mutations: beyond phaeochromocytoma and paraganglioma. *Clin. Endocrinol.*. doi: 10.1111/cen.14289 Online ahead of print.
13. Buffet, A., Ben Aim, L., Leboulleux, S., Drui, D., Vezzosi, D., Libé, R., Ajzenberg, C., Bernardeschi, D., Cariou, B., Chabolle, F. *et al.* (2019) Positive impact of genetic test on the management and outcome of patients with paraganglioma and/or pheochromocytoma. *J. Clin. Endocrinol. Metab.*, **104**, 1109–1118.
14. Dahia, P.L., Ross, K.N., Wright, M.E., Hayashida, C.Y., Santagata, S., Barontini, M., Kung, A.L., Sanso, G., Powers, J.F., Tischler, A.S. *et al.* (2005) A HIF1alpha regulatory loop links hypoxia and mitochondrial signals in pheochromocytomas. *PLoS Genet.*, **1**, 72–80.
15. Dahia, P.L. (2014) Pheochromocytoma and paraganglioma pathogenesis: learning from genetic heterogeneity. *Nat. Rev. Cancer*, **14**, 108–119.
16. Letouzé, E., Martinelli, C., Loriot, C., Burnichon, N., Abermil, N., Ottolenghi, C., Janin, M., Menara, M., Nguyen, A.T., Benit, P. *et al.* (2013) SDH mutations establish a hypermethylator phenotype in paraganglioma. *Cancer Cell*, **23**, 739–752.
17. Eijkelenkamp, K., Osinga, T.E., Links, T.P. and van der Horst-Schrivers, A.N.A. (2020) Clinical implications of the oncometabolite succinate in SDHx-mutation carriers. *Clin. Genet.*, **97**, 39–53.
18. Alam, N.A., Olpin, S. and Leigh, I.M. (2005) Fumarate hydratase mutations and predisposition to cutaneous leiomyomas, uterine leiomyomas and renal cancer. *Br. J. Dermatol.*, **153**, 11–17.
19. Clark, G.R., Sciacovelli, M., Gaude, E., Walsh, D.M., Kirby, G., Simpson, M.A., Trembath, R.C., Berg, J.N., Woodward, E.R., Kinning, E. *et al.* (2014) Germline FH mutations presenting with pheochromocytoma. *J. Clin. Endocrinol. Metab.*, **99**, E2046–E2050.
20. Calsina, B., Currás-Freixes, M., Buffet, A., Pons, T., Contreras, L., Letón, R., Comino-Méndez, I., Remacha, L., Calatayud, M., Obispo, B. *et al.* (2018) Role of MDH2 pathogenic variant in pheochromocytoma and paraganglioma patients. *Genet Med.*, **20**, 1652–1662.
21. Buffet, A., Morin, A., Castro-Vega, L.J., Habarou, F., Lussey-Lepoutre, C., Letouzé, E., Lefebvre, H., Guilhem, I., Haissaguerre, M., Raingeard, I. *et al.* (2018) Germline mutations in the mitochondrial 2-Oxoglutarate/malate carrier *SLC25A11* gene confer a predisposition to metastatic paragangliomas. *Cancer Res.*, **78**, 1914–1922.
22. Remacha, L., Pirman, D., Mahoney, C.E., Coloma, J., Calsina, B., Currás-Freixes, M., Letón, R., Torres-Pérez, R., Richter, S., Pita, G. *et al.* (2019) Recurrent germline DLST mutations in individuals with multiple pheochromocytomas and paragangliomas. *Am. J. Hum. Genet.*, **104**, 651–664.
23. Remacha, L., Currás-Freixes, M., Torres-Ruiz, R., Schiavi, F., Torres-Pérez, R., Calsina, B., Letón, R., Comino-Méndez, I., Roldán-Romero, J.M., Montero-Conde, C. *et al.* (2018) Gain-of-function mutations in DNMT3A in patients with paraganglioma. *Genet Med.*, **20**, 1644–1651.
24. Qin, Y., Yao, L., King, E.E., Buddavarapu, K., Lenci, R.E., Chocron, E.S., Lechleiter, J.D., Sass, M., Aronin, N., Schiavi, F. *et al.* (2010) Germline mutations in TMEM127 confer susceptibility to pheochromocytoma. *Nat. Genet.*, **42**, 229–233.
25. Comino-Méndez, I., Gracia-Aznárez, F.J., Schiavi, F., Landa, I., Leandro-García, L.J., Letón, R., Honrado, E., Ramos-Medina, R., Caronia, D., Pita, G. *et al.* (2011) Exome sequencing identifies MAX mutations as a cause of hereditary pheochromocytoma. *Nat. Genet.*, **19**43, 663–667.
26. Pacak, K., Jochmanova, I., Prodanov, T., Yang, C., Merino, M.J., Fojo, T., Prchal, J.T., Tischler, A.S., Lechan, R.M. and Zhuang, Z. (2013) New syndrome of paraganglioma and somatostatinoma associated with polycythemia. *J. Clin. Oncol.*, **31**, 1690–1698.
27. Ladroue C, Carcenac R, Leporrier M, Gad S, Le Hello C, Galateau-Salle F, Feunteun J, Pouysségur J, Richard S, Gardie B. (2008) PHD2 mutation and congenital erythrocytosis with paraganglioma. *N. Engl. J. Med.* **359**:2685–2692
28. Fishbein, L., Leshchiner, I., Walter, V., Danilova, L., Robertson, A.G., Johnson, A.R., Lichtenberg, T.M., Murray, B.A., Ghayee, H.K., Else, T. *et al.* (2017) Comprehensive molecular characterization of pheochromocytoma and paraganglioma. *Cancer Cell*, **31**, 181–193.
29. Prowse, A.H., Webster, A.R., Richards, F.M., Richard, S., Olschwang, S., Resche, F., Affara, N.A. and Maher, E.R. (1997)

Somatic inactivation of the VHL gene in Von Hippel-Lindau disease tumors. *Am. J. Hum. Genet.*, **60**, 765–771.
30. Crona, J., Lamarca, A., Ghosal, S., Welin, S., Skogseid, B. and Pacak, K. (2019) Genotype-phenotype correlations in pheochromocytoma and paraganglioma: a systematic review and individual patient meta-analysis. *Endocr. Relat. Cancer*, **26**, 539–550.
31. Dwight, T., Flynn, A., Amarasinghe, K., Benn, D.E., Lupat, R., Li, J., Cameron, D.L., Hogg, A., Balachander, S., Candiloro, I.L.M. *et al.* (2018) *TERT* structural rearrangements in metastatic pheochromocytomas. *Endocr. Relat. Cancer*, **25**, 1–9.
32. Luchetti, A., Walsh, D., Rodger, F., Clark, G., Martin, T., Irving, R., Sanna, M., Yao, M., Robledo, M., Neumann, H.P. *et al.* (2015) Profiling of somatic mutations in phaeochromocytoma and paraganglioma by targeted next generation sequencing analysis. *Int. J. Endocrinol.*, **2015**, 138573.
33. Kaelin, W.G., Jr. and Ratcliffe, P.J. (2008) Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol. Cell*, **30**, 393–402.
34. Gossage, L., Eisen, T. and Maher, E.R. (2015) VHL, the story of a tumour suppressor gene. *Nat. Rev. Cancer*, **15**, 55–64.
35. Selak, M.A., Armour, S.M., MacKenzie, E.D., Boulahbel, H., Watson, D.G., Mansfield, K.D., Pan, Y., Simon, M.C., Thompson, C.B. and Gottlieb, E. (2005) Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. *Cancer Cell*, **7**, 77–85.
36. Toledo, R.A., Qin, Y., Srikantan, S., Morales, N.P., Li, Q., Deng, Y., Kim, S.W., Pereira, M.A., Toledo, S.P., Su, X. *et al.* (2013) In vivo and in vitro oncogenic effects of HIF2A mutations in pheochromocytomas and paragangliomas. *Endocr. Relat. Cancer*, **20**, 349–359.
37. Sulkowski, P.L., Sundaram, R.K., Oeck, S., Corso, C.D., Liu, Y., Noorbakhsh, S., Niger, M., Boeke, M., Ueno, D., Kalathil, A.N. *et al.* (2018) Krebs-cycle-deficient hereditary cancer syndromes are defined by defects in homologous-recombination DNA repair. *Nat. Genet.*, **50**, 1086–1092.
38. Sulkowski, P.L., Oeck, S., Dow, J., Economos, N.G., Mirfakhraie, L., Liu, Y., Noronha, K., Bao, X., Li, J., Shuch, B.M. *et al.* (2020) Oncometabolites suppress DNA repair by disrupting local chromatin signalling. *Nature*, **582**, 586–591.
39. Papathomas, T.G., Oudijk, L., Zwarthoff, E.C., Post, E., Duijkers, F.A., van Noesel, M.M., Hofland, L.J., Pollard, P.J., Maher, E.R., Restuccia, D.F. *et al.* (2014) Telomerase reverse transcriptase promoter mutations in tumors originating from the adrenal gland and extra-adrenal paraganglia. *Endocr. Relat. Cancer*, **21**, 653–661.
40. Fishbein, L., Khare, S., Wubbenhorst, B., DeSloover, D., D'Andrea, K., Merrill, S., Cho, N.W., Greenberg, R.A., Else, T., Montone, K. *et al.* (2015) Whole-exome sequencing identifies somatic ATRX mutations in pheochromocytomas and paragangliomas. *Nat. Commun.*, **6**, 6140.
41. de Tersant, M., Généré, L., Freyçon, C., Villebasse, S., Abbas, R., Barlier, A., Bodet, D., Corradini, N., Defachelles, A.S., Entz-Werle, N. *et al.* (2020) Pheochromocytoma and paraganglioma in children and adolescents: experience of the French Society of Pediatric Oncology (SFCE). *J. Endocr. Soc.*, **4**, bvaa039.
42. Gimenez-Roqueplo, A.-P., Favier, J., Rustin, P., Rieubland, C., Crespin, M., Nau, V. *et al.* (2003) Mutations in the SDHB gene are associated with extra-adrenal and/or malignant phaeochromocytomas. *Cancer Res.*, **1**63, 5615–5621.
43. Currás-Freixes, M., Inglada-Pérez, L., Mancikova, V., Montero-Conde, C., Letón, R., Comino-Méndez, I., Apellániz-Ruiz, M., Sánchez-Barroso, L., Aguirre Sánchez-Covisa, M., Alcázar, V. *et al.* (2015) Recommendations for somatic and germline genetic testing of single pheochromocytoma and paraganglioma based on findings from a series of 329 patients. *J. Med. Genet.*, **52**, 647–656.
44. Bausch, B., Wellner, U., Bausch, D., Schiavi, F., Barontini, M., Sanso, G., Walz, M.K., Peczkowska, M., Weryha, G., Dall'igna, P. *et al.* (2014) Long-term prognosis of patients with pediatric pheochromocytoma. *Endocr. Relat. Cancer*, **21**, 17–25.
45. Eisenhofer, G., Klink, B., Richter, S., Lenders, J.W. and Robledo, M. (2017) Metabologenomics of phaeochromocytoma and paraganglioma: an integrated approach for personalised biochemical and genetic testing. *Clin. Biochem. Rev.*, **38**, 69–100.
46. Eisenhofer, G., Pacak, K., Huynh, T.T., Qin, N., Bratslavsky, G., Linehan, W.M., Mannelli, M., Friberg, P., Grebe, S.K., Timmers, H.J. *et al.* (2011) Catecholamine metabolomic and secretory phenotypes in phaeochromocytoma. *Endocr. Relat. Cancer*, **18**, 97–111.
47. Castro-Vega, L.J., Buffet, A., De Cubas, A.A., Cascón, A., Menara, M., Khalifa, E., Amar, L., Azriel, S., Bourdeau, I., Chabre, O. *et al.* (2014) Germline mutations in FH confer predisposition to malignant pheochromocytomas and paragangliomas. *Hum. Mol. Genet.*, **1**23, 2440–2446.
48. Papathomas, T.G., de Krijger, R.R. and Tischler, A.S. (2003) Paragangliomas: update on differential diagnostic considerations, composite tumors, and recent genetic developments. *Semin. Diagn. Pathol.*, **30**, 207–223.
49. van Nederveen, F.H., Gaal, J., Favier, J., Korpershoek, E., Oldenburg, R.A., de Bruyn, E.M., Sleddens, H.F., Derkx, P., Rivière, J., Dannenberg, H. *et al.* (2009) An immunohistochemical procedure to detect patients with paraganglioma and phaeochromocytoma with germline SDHB, SDHC, or SDHD gene mutations: a retrospective and prospective analysis. *Lancet Oncol.*, **10**, 764–771.
50. Papathomas, T.G., Oudijk, L., Persu, A., Gill, A.J., van Nederveen, F., Tischler, A.S., Tissier, F., Volante, M., Matias-Guiu, X., Smid, M. *et al.* (2015) SDHB/SDHA immunohistochemistry in pheochromocytomas and paragangliomas: a multicenter interobserver variation analysis using virtual microscopy: a multinational study of the European network for the study of adrenal Tumors (ENS@T). *Mod. Pathol.*, **28**, 807–821.
51. Bardella, C., El-Bahrawy, M., Frizzell, N., Adam, J., Ternette, N., Hatipoglu, E., Howarth, K., O'Flaherty, L., Roberts, I., Turner, G. *et al.* (2011) Aberrant succination of proteins in fumarate hydratase-deficient mice and HLRCC patients is a robust biomarker of mutation status. *J. Pathol.*, **225**, 4–11.
52. Favier, J., Meatchi, T., Robidel, E., Badoual, C., Sibony, M., Nguyen, A.T., Gimenez-Roqueplo, A.P. and Burnichon, N. (2020) Carbonic anhydrase 9 immunohistochemistry as a tool to predict or validate germline and somatic VHL mutations in pheochromocytoma and paraganglioma-a retrospective and prospective study. *Mod. Pathol.*, **33**, 57–64.
53. Casey, R.T., Warren, A.Y., Martin, J.E., Challis, B.G., Rattenberry, E., Whitworth, J., Andrews, K.A., Roberts, T., Clark, G.R., West, H. *et al.* (2017) Clinical and molecular features of renal and pheochromocytoma/paraganglioma tumor association syndrome (RAPTAS): case series and literature review. *J. Clin. Endocrinol. Metab.*, **1**102, 4013–4022.
54. Buffet, A., Calsina, B., Flores, S., Giraud, S., Lenglet, M., Romanet, P., Deflorenne, E., Aller, J., Bourdeau, I., Bressac-de Paillerets, B. *et al.* (2020) Germline mutations in the new E1' cryptic exon of the *VHL* gene in patients with tumours of von Hippel-Lindau disease spectrum or with paraganglioma. *J. Med. Genet.*, jmedgenet-2019-106519.

55. Haller, F., Moskalev, E.A., Faucz, F.R., Barthelmeß, S., Wiemann, S., Bieg, M., Assie, G., Bertherat, J., Schaefer, I.M., Otto, C. *et al.* (2014) Aberrant DNA hypermethylation of SDHC: a novel mechanism of tumor development in carney triad. *Endocr. Relat. Cancer*, **21**, 567–577.
56. Casey, R.T., Ten Hoopen, R., Ochoa, E., Challis, B.G., Whitworth, J., Smith, P.S., Martin, J.E., Clark, G.R., Rodger, F., Maranian, M. *et al.* (2019) SDHC epi-mutation testing in gastrointestinal stromal tumours and related tumours in clinical practice. *Sci. Rep.*, **9**, 10244.
57. Taïeb, D. and Pacak, K. (2017) New insights into the nuclear imaging phenotypes of cluster 1 pheochromocytoma and paraganglioma. *Trends Endocrinol. Metab.*, **28**, 807–817.
58. Fonte, J.S., Robles, J.F., Chen, C.C., Reynolds, J., Whatley, M., Ling, A., Mercado-Asis, L.B., Adams, K.T., Martucci, V., Fojo, T. *et al.* (2012) False-negative 123I-MIBG SPECT is most commonly found in SDHB-related pheochromocytoma or paraganglioma with high frequency to develop metastatic disease. *Endocr. Relat. Cancer*, **19**, 83–93.
59. Plouin, P.F., Amar, L., Dekkers, O.M., Fassnacht, M., Gimenez-Roqueplo, A.P., Lenders, J.W., Lussey-Lepoutre, C., Steichen, O. and Guideline Working Group (2016) European Society of Endocrinology Clinical Practice Guideline for long-term follow-up of patients operated on for a phaeochromocytoma or a paraganglioma. *Eur. J. Endocrinol.*, **174**, G1–G10.
60. van Berkel, A., Rao, J.U., Kusters, B., Demir, T., Visser, E., Mensenkamp, A.R., van der Laak, J.A., Oosterwijk, E., Lenders, J.W., Sweep, F.C. *et al.* (2014) Correlation between in vivo 18F-FDG PET and immunohistochemical markers of glucose uptake and metabolism in pheochromocytoma and paraganglioma. *J. Nucl. Med.*, **55**, 1253–1259.
61. Han, S., Suh, C.H., Woo, S., Kim, Y.J. and Lee, J.J. (2019) Performance of ^{68}Ga-DOTA-conjugated somatostatin receptor-targeting peptide PET in detection of pheochromocytoma and paraganglioma: a systematic review and Metaanalysis. *J. Nucl. Med.*, **60**, 369–376.
62. Kan, Y., Zhang, S., Wang, W., Liu, J., Yang, J. and Wang, Z. (2018) ^{68}Ga-somatostatin receptor analogs and ^{18}F-FDG PET/CT in the localization of metastatic pheochromocytomas and paragangliomas with germline mutations: a meta-analysis. *Acta Radiol.*, **59**, 1466–1474.
63. Taïeb, D. and Pacak, K. (2019) Current experts' views on precision nuclear medicine imaging of phaeochromocytoma and paraganglioma. *Eur. J. Nucl. Med. Mol. Imaging*, **46**, 2223–2224.
64. Taïeb, D. and Pacak, K. (2018) Molecular imaging and theranostic approaches in pheochromocytoma and paraganglioma. *Cell Tissue Res.*, **372**, 393–401.
65. Neumann, H.P.H., Tsoy, U., Bancos, I., Amodru, V., Walz, M.K., Tirosh, A., Kaur, R.J., McKenzie, T., Qi, X., Bandgar, T. *et al.* (2019) Comparison of pheochromocytoma-specific morbidity and mortality among adults with bilateral Pheochromocytomas undergoing total adrenalectomy vs cortical-sparing adrenalectomy. *JAMA Netw. Open*, **e198898**, 2.
66. Casey, R.T., Challis, B.G., Marker, A., Pitfield, D., Cheow, H.K., Shaw, A., Park, S.M., Simpson, H.L. and Maher, E.R. (2017) A case of a metastatic *SDHA* mutated paraganglioma representing twenty-three years after initial surgery. *Endocr. Relat. Cancer*, **24**, L69–L71.
67. Maher, E.R., Neumann, H.P. and Richard, S. (2011) von Hippel-Lindau disease: a clinical and scientific review. *Eur. J. Hum. Genet.*, **19**, 617–623.
68. Menko, F.H., Maher, E.R., Schmidt, L.S., Middelton, L.A., Aittomäki, K., Tomlinson, I., Richard, S. and Linehan, W.M. (2014) Hereditary leiomyomatosis and renal cell cancer (HLRCC): renal cancer risk, surveillance and treatment. *Familial Cancer*, **13**, 637–644.
69. Wells, S.A., Jr., Asa, S.L., Dralle, H., Elisei, R., Evans, D.B., Gagel, R.F., Lee, N., Machens, A., Moley, J.F., Pacini, F. *et al.* (2015) Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid*, **25**, 567–610.
70. Stewart, D.R., Korf, B.R., Nathanson, K.L., Stevenson, D.A. and Yohay, K. (2018) Care of adults with neurofibromatosis type 1: a clinical practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genet Med.*, **20**, 671–682.
71. Nölting, S., Grossman, A. and Pacak, K. (2019) Metastatic phaeochromocytoma: spinning towards more promising treatment options. *Exp. Clin. Endocrinol. Diabetes*, **127**, 117–128.
72. Mak, I.Y.F., Hayes, A.R., Khoo, B. and Grossman, A. (2019) Peptide receptor radionuclide therapy as a novel treatment for metastatic and invasive phaeochromocytoma and paraganglioma. *Neuroendocrinology*, **109**, 287–298.
73. Larkin, J., Paine, A., Foley, G., Mitchell, S. and Chen, C. (2015) First-line treatment in the management of advanced renal cell carcinoma: systematic review and network meta-analysis. *Expert. Opin. Pharmacother.*, **16**, 1915–1927.
74. Yu, Y., Yu, Q. and Zhang, X. (2019) Allosteric inhibition of HIF-2α as a novel therapy for clear cell renal cell carcinoma. *Drug Discov. Today*, **24**, 2332–2340.