Genetic Stratification of Inherited and Sporadic Phaeochromocytoma and Paraganglioma: Implications for Precision Medicine

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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 utilised to enable more personalised 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-vascularised tumors 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 (WHO) classification (1), the term phaeochromocytoma is reserved exclusively for tumors of the adrenal medulla, whereas the term paraganglioma is recommended for tumors 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 phaeochromocytomas and paragangliomas (PPGL) may contain elements of related neurogenic tumors such as ganglioneuroma, ganglioneuroblastoma, neuroblastoma etc. Such tumors 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, 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 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, 5, 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 were reported (7, 8, 9, 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 and *in vivo* functional imaging can all be applied to further sub-characterise 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 fifteen different genes have been implicated in autosomal dominant familial PPGL to date. The succinate dehydrogenase 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, leads to disruption of the succinate dehydrogenase 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) (see Figure 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 which 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 (29, 28). 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 characterised 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, 34, 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 stabilisation of HIF2A and activation of hypoxic gene response pathway (26, 36). The oncometabolites also inhibit other alpha-ketoglutarate-dependent enzymes including the TET (ten-eleven translocation) proteins that actively demethylate DNA demethylation and *SDHX*, *FH, MDH*, and *SLC25A11*-related tumours

are characterised 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 Figure 1). Thus though Cluster 1 tumours are characterised by activation of a pseudohypoxic gene response, there is heterogeneity for other pathways including DNA methylation and DNA repair (see 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) (see 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 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:

i) *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 to 30-40% in adult populations (41).

ii) *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 can be six times higher than in those with an isolated adrenal phaeochromocytoma (43).

iii) *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 characterised 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 has 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 a mixed or predominately adrenergic secretory pattern (45)(28).

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

v) *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 immunohistochemistry (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 destabilise 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 uncharacterised or novel *SDHx* variants. IHC for SDHA expression can predict the presence of pathogenic *SDHA* variants specifically in the *SDHA* gene and can be utilised 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 (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 utilised for other hereditary causes of PPGL including assessment of MAX expression and IHC for the membranous expression of carbonic anhydrase 9 (CA-9) in the assessment of germline or somatic *VHL* gene mutations (52). In some cases loss of SDHB expression may not result from a *SDHX* mutation but from a germline or somatic *VHL* gene mutation (53).

5. Personalised Medicine Approaches in Pheochromocytoma and Paraganglioma

The molecular stratification of patients with PPGL through germline and somatic testing opens up the possibility of genotype-driven personalised therapy. This might be considered from a variety of perspectives (see below) and for individuals who do or do not have a pathogenic variant in a PPG but also, the less definitive situation in which a VUS is detected or inherited PPGL is suspected but molecular confirmation is not available (see Table 2). As described above, there are a number of strategies available to enable precision medicine. including genetic testing, immunohistochemistry and functional imaging. What is the current and future role of these in clinical practice?

1. *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 pheochromocytoma, 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 immunohistochemistry (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), immunohistochemistry or tumour testing may be considered. Combined germline and tumour mutation analysis has resulted provided a diagnostic yield for germline/somatic driver mutation of ~80% (30) but not all driver events are genetic, 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 variants of uncertain significance (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.

2. *Role of Immunohistochemistry*: in addition to its utility in variant interpretation (see above), 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.

3. *Role of functional imaging for PPGL Precision Medicine:* Nuclear imaging techniques can be utilised 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 sub-classified 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 (111Indium-pentetreotide and Gallium-68 DOTA-conjugated peptide (^{68}Ga DOTATATE), 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-metaiodobenzylguanadine (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 123/131I-MIBG scintigraphy in SDH-deficient tumours (58). Therefore, current recommendations advice that 123/131I-MIBG scintigraphy should be reserved for those cases being investigated for suitability of treatment with 123/131I-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 ^{18}F-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 (SUV) 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 8 studies reviewing the sensitivity of functional imaging modalities for the detection of PPGL of unknown genotype, demonstrated that 68-Gallium 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-Gallium DOTATATE PET CT compared to 18F FDG PET CT. (62)

Therefore 68-Gallium 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, 68Ga-DOTATATE PET/CT can also predict the efficacy of peptide receptor radionuclide therapy (PRRT) with 177Lu-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 tumor to background normal adrenal uptake of this tracer compared to 68-Ga-DOTATATE (63, 64)

3. *Surgical management:* the primary treatment of a single localised 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.

4. *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, 68, 69, 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 (see Table 3).

5. *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 (72, 73). 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 are widely used for the treatment of metastatic RCC in VHL inactivation is frequent (74). More precise targeting of hypoxic gene response pathways is promised by the development of HIFa-antagonists such as PT2977 (75) 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 inpaired 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.

6. *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 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 (see 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 personalised 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, (iv) elucidating what the optimal targeted therapies for metastatic disease are based on the specific driver PPGL gene.

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Table 1:

Gene	Germline or somatic	Germline mutation frequency in inherited PPGL*	Somatic mutation frequency in sporadic PPGL*	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%	-	-	-	-	+

*Estimates mainly taken from Fishbein et al (2017)

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*	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*
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*

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

*assuming no clinical or pathological features that suggest a genetic cause is likely

Table 3: Examples of genotype-specific surveillance for asymptomatic mutation carriers of non-syndromic PPGL

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 <u>paternally</u> 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 3-5years

	Recommended surveillance
MAX**	·Screening should only be offered to patients who have a <u>paternally</u> 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 <u>paternally</u> 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

Figure Legends

Figure1: 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 succinate dehydrogenase subunit gene mutations). The oncometabolites and 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.

IDH- isocitrate dehydrogenase

SDH-Succinate dehydrogenase

FH- Fumarate hydratase

MDH-Malate dehydrogenase

OGC-oxoglutarate carrier

TET- Ten-eleven translocation enzyme

PHD- prolyl hydroxylase enzymes

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