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# Precision medicine in pheochromocytoma and paraganglioma: current and future concepts

**P. Björklund**[1], **K. Pacak**[2], **J. Crona**[1]

[1]Department of Surgical Sciences, Uppsala University, Uppsala, Sweden

[2]Section on Medical Neuroendocrinology, Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Bethesda, MD, USA

## Abstract

Björklund P, Pacak K, Crona J (Uppsala University, Uppsala, Sweden; Section on Medical Neuroendocrinology, Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Bethesda, MD, USA). Precision medicine in pheochromocytoma and paraganglioma: current and future concepts.

Pheochromocytoma and paraganglioma (PPGL) are rare diseases but are also amongst the most characterized tumour types. Hence, patients with PPGL have greatly benefited from precision medicine for more than two decades. According to current molecular biology and genetics-based taxonomy, PPGL can be divided into three different clusters characterized by: Krebs cycle reprogramming with oncometabolite accumulation or depletion (group 1a); activation of the (pseudo) hypoxia signalling pathway with increased tumour cell proliferation, invasiveness and migration (group 1b); and aberrant kinase signalling causing a pro-mitogenic and anti-apoptotic state (group 2). Categorization into these clusters is highly dependent on mutation subtypes. At least 12 different syndromes with distinct genetic causes, phenotypes and outcomes have been described. Genetic screening tests have a documented benefit, as different PPGL syndromes require specific approaches for optimal diagnosis and localization of various syndrome-related tumours. Genotype-tailored treatment options, follow-up and preventive care are being investigated. Future new developments in precision medicine for PPGL will mainly focus on further identification of driver mechanisms behind both disease initiation and malignant progression. Identification of novel druggable targets and prospective validation of treatment options are eagerly awaited. To achieve these goals, we predict that collaborative large-scale studies will be needed: Pheochromocytoma may provide an example for developing precision medicine in orphan diseases that could ultimately aid in similar efforts for other rare conditions.

*Correspondence:* Joakim Crona, Experimental Surgery Group, Uppsala University, Akademiska Sjukhuset, ing 70, FOA2, 75185 Uppsala, Sweden. (fax: +46185533943; Joakim.crona@surgsci.uu.se).

Conflict of interest statement

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## Management of pheochromocytoma and paraganglioma: current state of the art

Pheochromocytoma (PCC) and paraganglioma [(PGL) together referred to herein as PPGL] arise from neuroendocrine cells in the adrenal medulla as well as autonomous sympathetic and parasympathetic ganglia. According to the 2004 World Health Organization classification, PPGLs arising from the adrenal medulla should be termed PCCs, whereas tumours originating from autonomic ganglia are PGLs [1].

### Diagnosis

The gold standard for diagnosing PPGL is through the detection of excess catecholamine and/or dopamine production. Analysis of free metanephrines and metoxytyramine in plasma is currently the method of choice to both detect and exclude the disease [2–4]. In addition, 24-h urine collections may be used. To obtain optimal specificity of plasma free metanephrine analysis, the patient should rest in the supine position for 20–30 min before blood test from a peripheral vein [5]. The most common causes of false-positive results are medications, such as tricyclic antidepressants, that give rise to an increase in catecholamine release [5, 6]. There are no definitive pathological or molecular criteria for malignant PPGL. Instead, malignant cases are classified by the presence of distant metastasis that is defined by the presence of PPGL tumour cells in nonchromaffin organs [7]. Metastatic disease occurs in 10–20% of PCCs and in 15–35% of abdominal PGLs [8–10].

### Staging

Recent guidelines recommend the use of computed tomography (CT) for the localization of PCCs that occur in patients without a known genetic cause [11–14]. Magnetic resonance imaging (MRI) should be used in patients with metastatic PPGL or for localizing head and neck PGL [15]. Molecular imaging may allow for increased sensitivity in the detection of metastatic lesions [14]. Metaiodobenzylguanidine (MIBG)-based scintigraphy has long been used in the management of these patients and was recently found to have diagnostic value. Positron emission tomography (PET)/CT with $^{18}$F-dihydroxyphenylalanine, $^{18}$F-fluorodeoxyglucose (FDG) and 68 Ga-DOTATATE tracers have a role in selected patients [16, 17]. However, the sensitivity of PET/CT is suboptimal, especially for metastatic PPGL. Currently, the highest sensitivity for staging of PCC and PGL tumours can be achieved through a combination of anatomical and functional imaging [15].

### Treatment

Cure of localized PPGL can be achieved through complete surgical resection [18, 19]. Adequate preoperative preparation is required to optimize the patient for surgical intervention, and to reduce the risk of potentially life-threatening intra and postoperative

complications as a result of haemodynamic instability [20, 21]. The choice of surgical technique is dependent on both tumour location and size. In most cases, adrenal PCCs may be resected laparoscopically with our without adrenal-sparing technique [22, 23]. The larger abdominal PGLs and PCCs can be resected using open approaches.

According to a recent study, the 1-year progression-free survival of those with metastatic PPGL was 46% at diagnosis for therapy-naive patients with metastases [24]. Taking into account the relatively long survival of these patients, a ‘wait and see’ approach may therefore sometimes be advocated. The rationale for surgery would be to reduce the cumulative toxic side effects of both medical and radiation treatment. Surgical resection and/or locoregional therapy can also be considered in patients with metastatic PPGL in order to provide palliate relief or to allow long-term remission [25]. Combination chemotherapy with CVD regimen (cyclophosphamide, dacarbazine and vincristine) as well as $^{131}$I-labelled MIBG are the most validated systemic strategies for patients with unresectable tumours [26]. Positive effects of radiolabelled somatostatin compounds ($^{177}$Lu-DOTATATE) have also been described [27, 28].

### Precision medicine in PPGL

The concept of precision medicine, where the care of the individual patient is tailored by taking personal variability into account, has a long tradition in the management of PPGL. The individual’s genomic sequence is the most important factor for patient stratification. A total of 12 different syndromes with an increased risk of PPGL have been described to date (Table 1) [9, 18]. These syndromes have been linked to specific loci: *SDHA* [29], *SDHB* [30], *SDHC* [31], SDHD [32], *SDHAF2* [33], FH [34], *VHL* [35], *EPAS1* [36], *RET* [37], *NF1* [38], *TMEM127* [39] and *MAX* (Fig. 1) [40]. Genetic screening is currently performed in patients with a high probability of an underlying genetic disorder, i.e. those presenting at a young age, and/or with multifocal and/or metastatic tumours. The diagnostic procedure utilizes a stepwise approach with prioritization of the genes to be analysed. If a genetic syndrome can be established by genetic diagnosis, patient management can be individually tailored based on the specific characteristics of the syndrome.

This may provide not only information on the future risk for disease, but also empirical knowledge of the clinical and molecular characteristics of the particular PPGL (Fig. 2) [41]. These phenotype differences for PPGLs manifest as different hormonal profiles, anatomical localizations and risks of metastatic disease. Genotype-tailored selection of molecular imaging tracers has been shown to increase the sensitivity of such investigations; $^{18}$F-FDG is preferred in patients with *SDHx (SDHA-D and SDHAF2)* -related disease to detect primary and metastatic lesions [12, 42]. It has been hypothesized that, among PPGL patients with metastatic disease, some could respond differently to systemic treatment; however, this remains to be confirmed in prospective randomized trials.

## Krebs cycle cluster and familial PGLs

Truncating mutations in proteins constituting the Krebs cycle cause familial PGL syndromes types 1–5, as well as fumarate hydratase-associated PPGL (Fig. 3a). These diseases are transmitted in an autosomal dominant fashion. PGL types 1–3 (*SDHD, SDHAF2* and

*SDHC*) are mainly characterized by localized parasympathetic PGLs. By contrast, familial PGL type 4 *(SDHB)* is associated with significant morbidity and increased mortality due to increased risk of metastatic PPGL, as well as other cancers such as renal cell carcinoma. Familial PGL type 5 (*SDHA*) is associated with PPGL but a clear phenotype remains to be discovered. It was previously shown that inactivating mutations in the *FH* gene cause dominant hereditary leiomyomatosis and renal cell cancer. Such mutations were recently associated with PPGL with an increased frequency of metastatic disease. It was recently suggested that constitutional mutations in *MDH2*, which encodes a third enzyme involved in the Krebs cycle, may be involved in PPGL tumourigenesis [43].

### Familial PGL type 1

Familial PGL type 1 is caused by loss of function mutations in the *SDHD* gene and shows near complete penetrance for parasympathetic PGL tumours that are most commonly located in the head and neck region [44–46]. Unilateral PCC is reported to occur in about 50% of patients [46, 47]. Metastatic tumours are rare. *SDHD*-related PPGLs are heterogeneous with regard to hormone production; head and neck PGLs are often biochemically silent or produce either dopamine or methoxytyramine, whereas PCCs show norepinephrine secretion [48]. Additional pathologies have been reported, including renal cell carcinoma, gastrointestinal stromal cell tumours (GIST) and pituitary adenomas [49, 50]. It was recently suggested that mortality is not increased in carriers of *SDHD* mutations compared to a normal population [51].

### Familial PGL type 2

Familial PGL type 2 is caused by loss of function mutations in the *SDHAF2* gene and has so far been detected in a limited number of families [33, 52–54]. All reported cases have presented with parasympathetic head and neck PGL without signs of metastatic disease.

### Familial PGL type 3

Familial PGL type 3 is caused by loss of function mutations in the *SDHC* gene and is mainly characterized by parasympathetic PGLs located in the head and neck region with a low risk of metastases [55–57].

### Familial PGL type 4

Familial PGL type 4 is caused by loss of function mutations in *SDHB* and is associated with sympathetic PGL with an increased risk of malignancy. The high frequency of malignant PPGLs results in increased patient morbidity and mortality [47, 58, 59]. PCC and head and neck PGL are less commonly reported. Tumours secrete norepinephrine and/or dopamine. Patients with PGL type 4 show an increased risk of developing renal cell carcinoma as well as GIST [44]. Mutations in *SDHx* genes have also been associated with pituitary adenomas [49, 60].

### Familial PGL type 5

Familial PGL type 5 is caused by truncating mutations in the *SDHA* gene. These mutations have been associated with both PCC and PGL as well as GIST. It is thought that carriers of

*SDHA* mutations have a low penetrance for the development of PPGL tumours, and concomitant presentation with GIST is infrequently observed [29, 61, 62].

### Fumarate hydratase

Constitutional loss of function mutations in the fumarate hydratase (*FH*) gene were recently described in patients with PPGL [34, 63, 64]. The phenotype is characterized by multiple primary tumours as well as metastatic disease [64]. It was previously demonstrated that germline *FH* mutations cause autosomal dominant hereditary leiomyomatosis and renal cell cancer [65].

### Molecular genetics of familial PGLs

The *SDHx* genes encode different subunits of the succinate dehydrogenase complex that catalyses reactions in the tricarboxylic acid cycle (oxidation of succinate to fumarate) and in the respiratory electron transfer chain (complex II). Loss of function mutations in these genes causes an accumulation of succinate that inhibits EGLN1-3 enzyme activity (Fig. 2) [66]. Inhibition of this enzyme family causes decreased hydroxylation and subsequent ubiqutination of hypoxia-inducible factor (HIF) transcription factors, resulting in a pseudohypoxic state similar to that seen in *VHL*- and *EPAS1-mutated* tumours. It has recently been suggested that accumulation of succinate in *SDHx*-mutated tumours results in a hypermethylator phenotype through inhibition of histone and DNA methylases [34]. Tumours with *FH* mutations have increased levels of fumarate, and similar molecular consequences to those of *SDHx*-mutated tumours [63, 64].

## The pseudohypoxic cluster: von Hippel–Lindau and PGL–polycythemia syndromes

Mutations in genes associated with regulation of cellular oxygen sensation and response to hypoxia have been shown to give rise to PPGL (Fig. 3b). The von Hippel–Lindau (VHL) disease confers susceptibility to multiple tumours including PPGL. Gain of function mutations in the *EPAS1* gene causes PGL–somatostatinoma–polycythemia syndrome [67].

### VHL syndrome

VHL syndrome is caused by loss of function mutations in the tumour suppressor gene *VHL* with an incidence of 1/36 000 [68]. It is characterized by an increased risk of retinal haemangioma, central nervous system haemangioblastoma and PPGL. Pancreatic neuroendocrine tumours, renal clear cell carcinoma and lymphatic sac tumours occur but at lower frequencies. Patients with C598T mutation present with Chuvash polycythemia [69]. The penetrance of PPGL is 10–25% and about 50% of patients with PCC have bilateral disease [18, 70–72]. *VHL*-related PPGLs predominantly produce norepinephrine and less than 5% of patients develop metastatic disease [70–73].

### PGL–polycythemia syndrome

Gain of function mutations within the *EPAS1* gene that encodes the hypoxia-inducible factor-2alpha (HIF2A) protein is associated with autosomal dominant polycythemia [74].

Activating amino acid transitions at HIF2A hydroxylation sites was recently described as a cause of PPGL, polycythemia and somatostatinoma. Ocular manifestations have also been reported [75]. Germline mutations at these codons have been considered embryologically lethal and the mutations are absent in constitutional DNA in syndrome carriers [76]. The presence of the somatic mutations in different tissues indicates mosaic carrier status in these patients [34]. To determine the tissue distribution of somatic *EPAS1* mutations, deep sequencing of peripheral blood and/or buccal swabs should be performed [77]. The gender distribution for PGL–polycythemia syndrome shows a strong female bias; the cause of this trend remains to be determined [67].

### Molecular genetics of VHL and PGL–polycythemia syndromes

Mutations in *VHL* and *EPAS1* cause a cellular pseudohypoxic state through the stabilization of HIF transcription factor proteins (Fig. 2) [78]. Activated HIF alters transcription of target genes that results in increased angiogenesis as well as cellular proliferation and reduced apoptosis. Loss of function mutations in the *VHL* gene results in reduced ubiqutination of HIF transcription factors with subsequent reduction of degradation by the proteasome. This causes HIF accumulation and nuclear translocation [79]. Gain of function mutations at *EPAS1* hydroxylation sites causes reduced VHL protein binding that diminishes HIF2A ubiqutination enabling HIF escape from degradation [36, 67, 80, 81]. *EPAS1*-mutated tumours have been shown to have similar transcriptomic and metabolomic signatures compared to PPGL tumours with *VHL* mutations [36].

Mutations in *EGLN1* and *EGLN2*, genes that encode the proteins responsible for prolyl hydroxylation targeting HIF for degradation, may also confer susceptibility to PPGL [82, 83].

These tumours predominantly secrete norepinephrine, due to low expression of phenylethanolamine N-methyltransferase (PNMT), and have been linked to hypermethylation of the *PNMT* gene promoter [84]. Or: These tumours predominantly secrete norepinephrine due to low expression of phenylethanolamine N-methyltransferase (PNMT), which has been linked to hypermethylation of the *PNMT* gene promoter [84].

## Kinase signalling cluster

Mutated genes classified as cluster 2 PPGL are tightly linked to regulation of signalling in either RAS/RAF/MAPK or (mTOR) pathways (Fig. 3c). This cluster comprises the majority of PCCs, and shows a wide molecular spectrum. The associated syndromes show a different range of diseases involving the nervous system [neurofibromatosis type 1 (NF1)] and endocrine glands [multiple endocrine neoplasia type 2 (MEN2)], as well as exclusive association with PPGL in those with *TMEM127* and *MAX* mutations. Somatic *HRAS* mutations are common in PPGL, but no cases with constitutional *HRAS* mutation and PPGL have been presented.

### NF1

NF1, also known as von Recklinghausen's disease [85], is caused by loss of function mutations in the neurofibromin 1 (*NF1*) gene with an incidence of 1 : 2500–3000. The

clinical phenotype is characterized by fibromatous skin lesions, lichen eye nodules, optic gliomas and café au lait spots [86]. NF1 syndrome has a relatively low penetrance for PCC of about 5% and is mainly associated with unilateral PCC with mixed epinephrine/norepinephrine production [87].

### MEN2

MEN2 is caused by gain of function mutations within the *RET* (rearranged during transfection) gene that encodes a tyrosine kinase receptor [37, 88, 89]. MEN2 is characterized by susceptibility to multiple endocrine neoplasms: medullary thyroid carcinoma, PCC and parathyroid adenomas. Generally, bilateral PCCs occur in about 50% of probands [90]. Mutations in the cysteine-rich extracellular domains located in exons 10–11 underlie a majority of MEN2 cases. Disease-causing variants within noncysteine regions located in exons 13–16 are less common and characterized by pronounced interpatient phenotypic heterogeneity [91]. Among carriers of noncysteine mutations, only a minority develops PCC [91]. MEN2-associated PCCs typically produce a mixture of epinephrine/norepinephrine and rarely metastasize [9, 10, 18, 92–94].

### TMEM127

Loss of function mutations in the *TMEM127* (transmembrane protein 127) gene causes susceptibility to PCC, and less frequently abdominal PGL [39, 95, 96]. Association with renal cell carcinoma has been described but remains to be validated [97]. Results from a large Brazilian family showed 30% penetrance for PPGL with median onset at 43 years of age [98]. Bilateral PCCs are common. Mixed epinephrine/norepinephrine secretion is observed in *TMEM127*-associated PPGLs.

### MAX

Loss of function mutations in the *MAX* (myc-associated factor X) gene has been shown to cause hereditary PCC and less commonly PGL [40, 99]. PCCs are commonly bilateral and show an intermediate biochemical profile with moderately elevated levels of epinephrine [99]. No additional phenotypic associations have been reported.

## Molecular genetics of PPGL within the kinase signalling cluster

Cluster 2 tumours are characterized by an increased activity in the mitogenic signalling pathways ERK/MAPK and PI3K/AKT/mTOR (Fig. 2). Both these pathways are frequently altered in a wide variety of human cancers. However, heterogeneity within this cluster occurs and the tumours can be further subclassified according to their transcriptome and miRNAome profiles [84, 100].

It has been suggested that tumours with mutations in *NF1, RET* and *HRAS* overlap with regard to their mechanisms of tumourigenesis, that diverge on RAS-mediated signalling [84]. Mutations in *NF1* GTPas domain result in reduced inhibition of RAS intrinsic activity, whilst ligand or mutant-dependent activation of *RET* results in activation of RAS through downstream signalling [101–103]. Somatic mutations in the *NF1* gene have been described as frequent events in patients with sporadic PCC and were associated with activation of the ERK/MAPK signalling pathway [73, 99, 104, 105]. Somatic gain of function mutations in

*H-RAS* has been described in a significant proportion of PPGLs [84, 106–109]. Activating mutations in *RET* occurs at phosphorylation sites causing intrinsic activation resulting in downstream activation of RAS/RAF/MAPK and PI3K/AKT signalling pathways [110, 111].

Loss of function mutations in *TMEM127* results in reduced inhibition of the mTOR pathway in an RAS/RAF/MAPK- and PI3K/AKT-independent manner [39]. The definitive role of the TMEM127 protein in PPGL tumourigenesis remains to be identified.

Mutated *MAX* causes deregulation of the MYC–MAX–MXD1 pathway that leads to altered transcription and signalling in the NRAS–PIK3CA–AKT1–mTOR pathway [40]. It has been suggested that *MAX*-mutated tumours have a unique transcriptomic signature [100]. The fact that *MAX*-mutated tumours are distinct from those with *NF1/RET/HRAS* mutations is also supported by their intermediate expression of *PNMT*, and subsequent lower production of epinephrine.

## The genetic landscape of PPGL

Genome-wide characterization is a valuable tool not only for identification of genetic factors driving tumourigenesis, but also to provide information about the degree and spectrum of genetic instability. It has consistently been found that PPGLs are characterized by a relatively low degree of genetic instability both at the nucleotide and chromosomal level [84, 100, 112–114]. Furthermore, the observed mutational spectrum was consistent with ageing, and there was limited evidence of previous or ongoing mutagenic processes such as smoking or defects in cellular chromatin maintenance.

Somatic mutations may also provide ancestral information through chronological and spatial classification. Such characterization may allow for better understanding of the dynamic life of tumours from initiation, through acquisition of malignant capabilities, to metastatic dissemination [115]. In order to transform cells in the adrenal medulla or autonomous ganglia to initiate a tumour, it is likely that both nucleotide mutations and copy number alterations are needed (Fig. 4) [114, 116]. This hypothesis is supported by the classification of somatic mutations in driver genes and specific somatic copy number alterations as both obligate and early events [114]. *VHL*-mutated PPGLs show loss of 3p and 11p, whereas those with *NF1* mutations show loss of 17q. Cluster 2 tumours display loss of 1p and 3q with high frequencies [84, 116]. The pathogenic mechanisms of 11p inactivation in *VHL* tumours and losses of 1p and 3q in cluster 2 PPGL remain to be determined [117, 118].

Identifying the molecular mechanisms involved in malignant transformation of benign tumours can be regarded as the ‘holy grail’ of PPGL translational research. Novel findings indicate that somatic *ATRX* mutations occur in a subset of PPGLs that display a high frequency of metastatic disease [84, 100, 112]. Extrapolating findings from other neuroendocrine tumours, inactivation of *ATRX* is most probably a secondary event that promotes malignancy through establishing the alternative lengthening of telomeres phenotype [119, 120]. Somatic mutations in the *TERT* promoter (*SDHx*-deficient PPGL), as well as in the chromatin modifier *KMT2D,* have also been identified but remain to be validated by independent observers [113, 121]. It has also been demonstrated that metastases

accumulate chromosomal aberrations compared to the paired primary tumours [114]. Elucidating whether these somatic changes are drivers of malignant transformation remains a priority.

## Future concepts for precision medicine

### Molecular characterization

In this review, we describe 13 loci *(SDHA-D, SDHAF2, VHL, EPAS1, NF1, RET, HRAS, MAX and TMEM127)* that have been suggested to cause PPGL. *ATRX, KMT2D and TERT* promoter have also been identified with recurrent mutations but their contribution to the disease remains to be identified. Despite investigation with exome sequencing, there is still no identified disease-causing mechanism for a large proportion of PPGLs [84, 100]. Recurrent copy number alterations as well as miRNA and methylation patterns have been identified, but the particular mechanisms in which these events are involved in PPGL tumourigenesis remain to be clarified [84].

It is known from previous studies that the miRNAome of PPGL clusters in a similar manner to the transcriptome [122]. Further investigation of the PPGL miRNAome revealed a distinct subset of PPGLs with copy number neutral LOH of chromosome 14, that was subsequently linked to deregulation of the DLK1-MEG3 miRNA [84]. These cases were all characterized by aberrant kinase signalling, but no disease-causing mutations were detected by exome sequencing.

Deregulation of epigenetic maintenance was recently found to occur as a consequence of succinate accumulation [34, 123]. Whether specific epimutations can be directly linked to tumour formation or malignancy remains to be determined. Of particular interest for PPGLs was the recent identification of epimutated *SDHC* as a cause of GIST [124].

Up to 10% of patients with nonsyndromic presentation are carriers of germline mutations. Hence, a prioritization approach in diagnostic genetic screening may miss patients with an underlying genetic cause [125–127]. The limited use of genetic screening in the clinical setting is mainly due to the shortcomings of current methodologies. This includes poor cost effectiveness and long analysis times especially for extensive analyses such as genes of interest in PPGL. The advent of next-generation sequencing methods has the potential to decrease the cost of sequencing and enable all patients with PPGLs to be screened for all relevant loci [128]. Much work is needed to design such a comprehensive PPGL screening test: there are at least 20 relevant genes that span more than 200 exons and the assay should be able to detect nucleotide substations as well as gross deletions [128, 129].

### Phenotypic characterization of known syndromes

A correct genetic diagnosis may predict PPGL tumour characteristics, as well as the risk of other diseases associated with the specific syndrome. Pioneering work was performed with the traditional tumour susceptibility syndromes VHL, MEN and NF1, where appropriate surveillance may facilitate early diagnosis and treatment. A similar approach might soon be used for familial PGL syndromes where patients with *SDHB* mutations have been most commonly studied. *SDHB* carriers have a substantially increased risk of malignant tumours

as well as higher mortality, which motivates extensive follow-up [47]. Recently, another group has been recognized with polycythemia PGL syndrome, where patients have an increased risk of developing duodenal somatostatinoma in addition to PPGL [67]. Improved guidelines for the management of patients with familial PPGL diseases are eagerly awaited.

## Diagnostics and localization

The detection of excess hormone production underlies diagnosis in PPGL, and the hormone profile of individual tumours depends on mutation subgroup (Table 2). Tumours with mutations in *FH, VHL, EPAS1* and succinate dehydrogenase complex genes predominantly produce norepinephrine with low levels of epinephrine. Tumours with *RET, NF1, TMEM127, MAX* and *HRAS* mutations show increased levels of both epinephrine and norepinephrine [130, 131]. The molecular rationale for this phenomenon may be promoter methylation, and subsequent decreased transcription of PNMT, which is responsible for conversion of norepinephrine to epinephrine [84]. Cluster 1a tumours may secrete dopamine or may be biochemically silent [132].

Gene mutation status can also guide in the selection of appropriate imaging tests with *SDHx* tumours having high uptake of FDG as well with somatostatin-labelled PET tracers [16, 42]. Introduction of tumour- and/or genotype-specific radiopharmaceuticals will help to assess the degree of, for example, hypoxia, apoptosis, angiogenesis and invasiveness. Utilization of H-HRMAS NMR may help to identify specific mutations even without genetic testing [133–135]. Similarly MRI spectroscopy with hyperpolarization has the potential for *in vivo* molecular metabolomics profiling [136].

## Treatment

With the discovery of different mechanisms driving PPGL formation in the three clusters, it was hypothesized that mutation subtypes may be used as biomarkers to predict sensitivity to systemic therapy. Patients with pro-angiogenic PPGLs could benefit from antiangiogenic therapy, whereas those with PPGLs in which the kinase signalling cascades are activated could respond to inhibitors of these particular pathways [137, 138]. Within the ongoing Phase II studies of systemic therapy in metastatic PPGL, these hypotheses have a potential to be tested.

Genetic instability could be a potential druggable target in PPGL patients despite the low overall degree of genetic fragmentation [84, 100, 112]. The identification of deregulated telomere maintenance, especially in the alternative lengthening of telomeres (ALT) context, may have therapeutic implications as such tumours have been shown to respond to ATR inhibition [112, 121, 139, 140]. In the rare cases that have increased genetic instability, it is probable that the number of immunoreactive neo-antigens will be increased, thus conferring susceptibility to immunotherapy [84, 141, 142].

It has been demonstrated that succinate dehydrogenase-deficient PPGLs exhibit a hypermethylator phenotype, and related tumours (GIST) have shown epimutations in *SDHC* [124]. This implies that agents modulating the epigenome could be effective in the treatment of these tumours.

It was recently suggested that *SDHx* tumours had a relatively high response to temozolomide [138]. The explanation for the increased sensitivity to this alkalyting agent was the observed hypermethylation of the *MGMT* promoter [143]. In addition, *SDHx*-mutated tumours show increased expression of somatostatin receptors that could enable the use of somatostatin receptor-directed diagnostics and treatment [144]. The skewed metabolism in SDHx-deficient PPGLs may also render these tumours vulnerable to antimetabolite therapy.

### Tumour heterogeneity

A complication in the development of genetic biomarkers is genetic heterogeneity within and between PPGL lesions [84, 100, 114, 145]. Preliminary findings indicate that genetic divergence is considerable between clones observed within primary and metastatic lesions of the same patient (Fig. 4). Therefore, analyses from primary tumours should be extrapolated to metastases with caution. Before the introduction of genetic biomarkers, their chronological position in PPGL tumour evolution needs to be determined. Technologies such as liquid biopsies and/or circulating tumour cells may provide a way to circumvent the problem of spatial heterogeneity but remain to be evaluated in PPGLs [146, 147].

## Concluding remarks

Despite the orphan status of PPGL, considerable progress has been made in our understanding of the molecular biology underlying the diseases. This has been translated into improved diagnostics and allowed for identification of an increasing number of syndrome carriers. By contrast, the treatment of PPGL has changed marginally during the last decades and particularly the care of patients with metastatic disease has an urgent need for improvement. It remains to be proven whether translation of experimental data can provide new treatment options or serve as predictive factors for existing therapies. Developing and introducing new treatments may be the biggest challenge for the PPGL community, and the ultimate success will rely on strong cross-cultural collaboration between physicians, researchers and patients worldwide.

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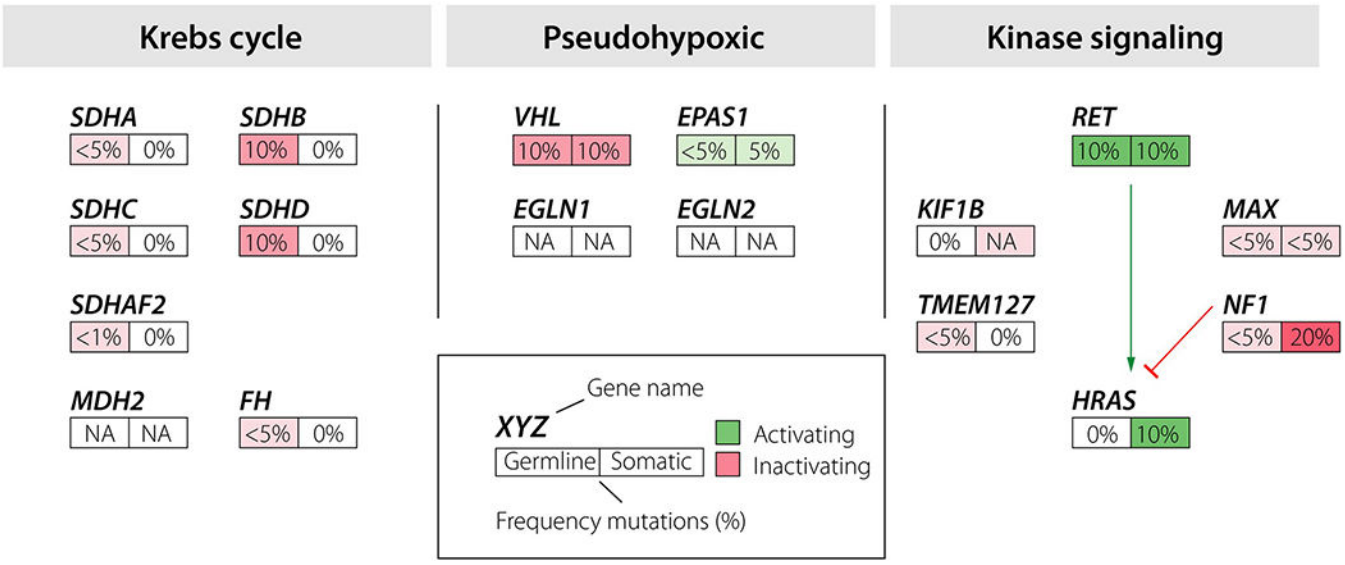


**Fig. 1.**

Description of genes involved in pheochromocytoma and paraganglioma tumourigenesis grouped in molecular clusters, and the estimated overall frequency of pathogenic mutations in these diseases.

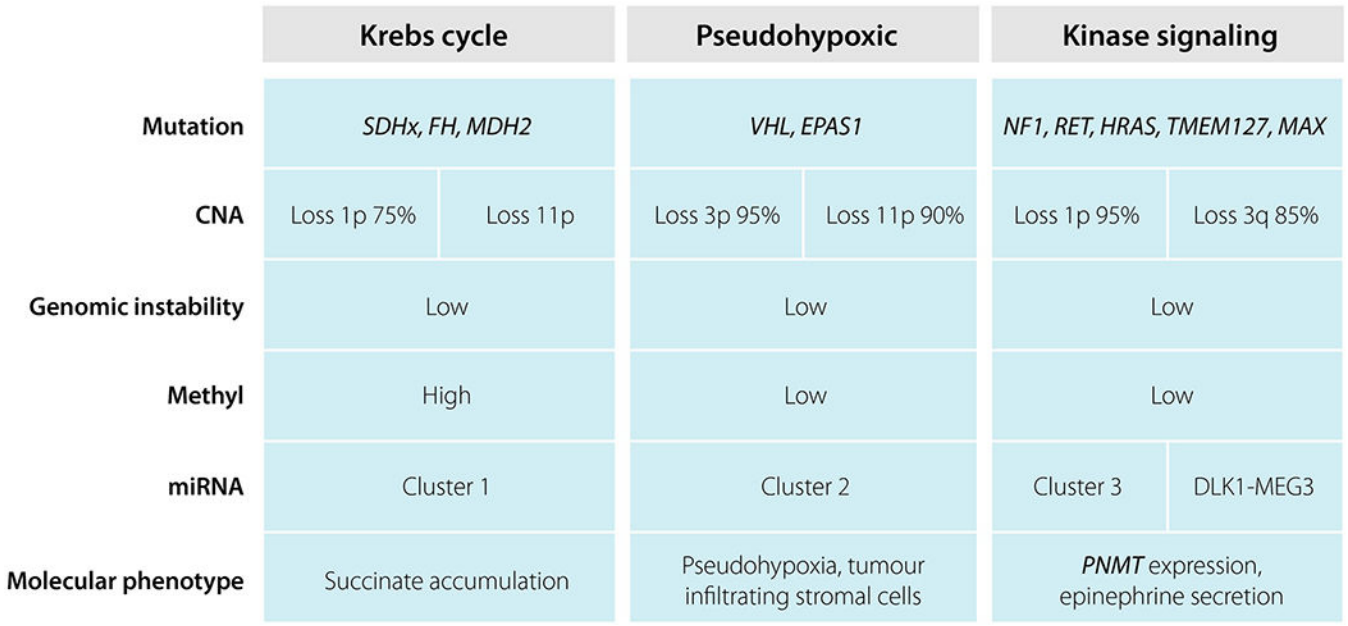


**Fig. 2.**

Summary of the genetic and molecular characteristics of pheochromocytoma and paraganglioma in the three different clusters.

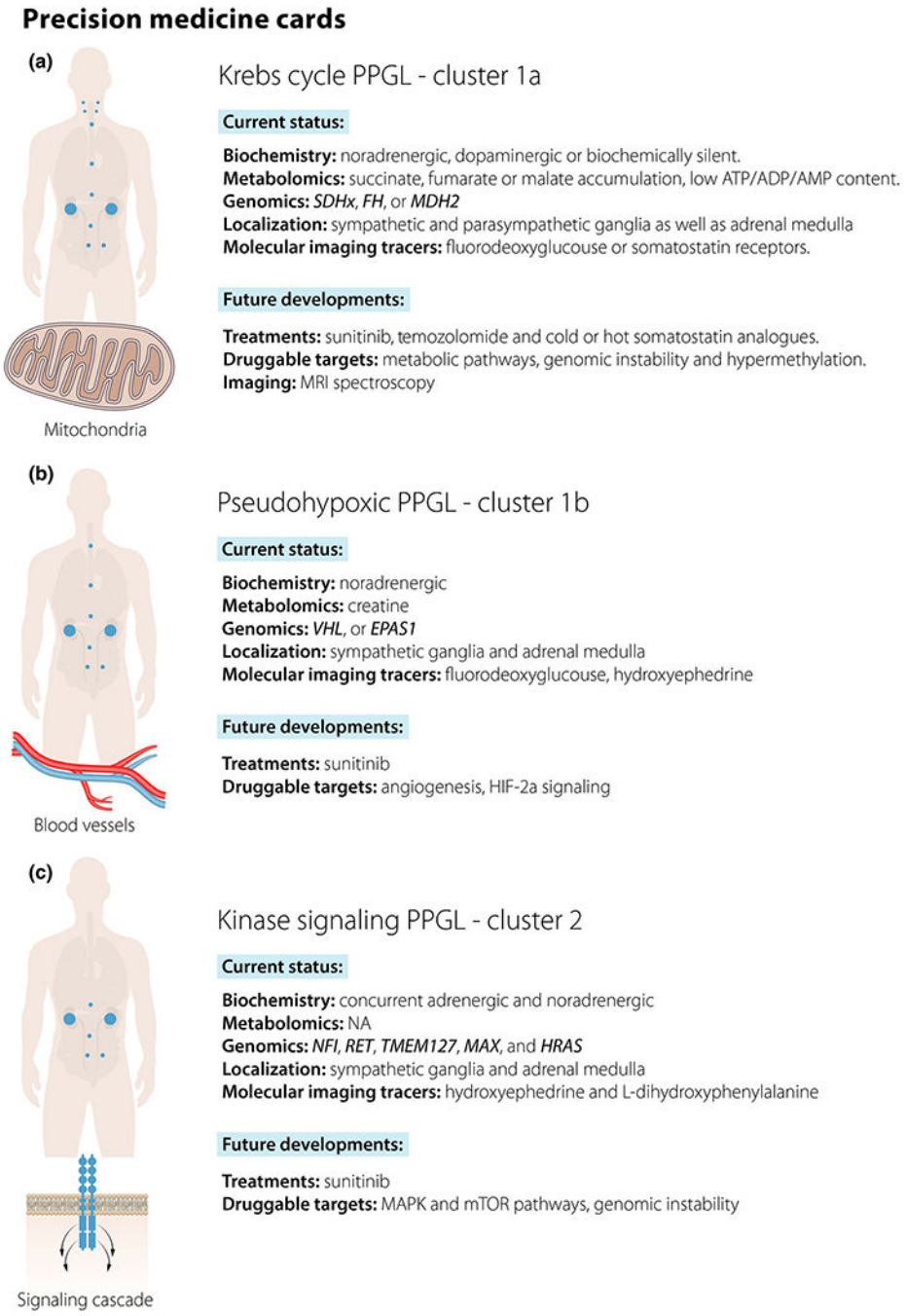


**Fig. 3.**

(a–c) Current and future perspectives of precision medicine in pheochromocytoma and paraganglioma stratified by molecular subgroup.

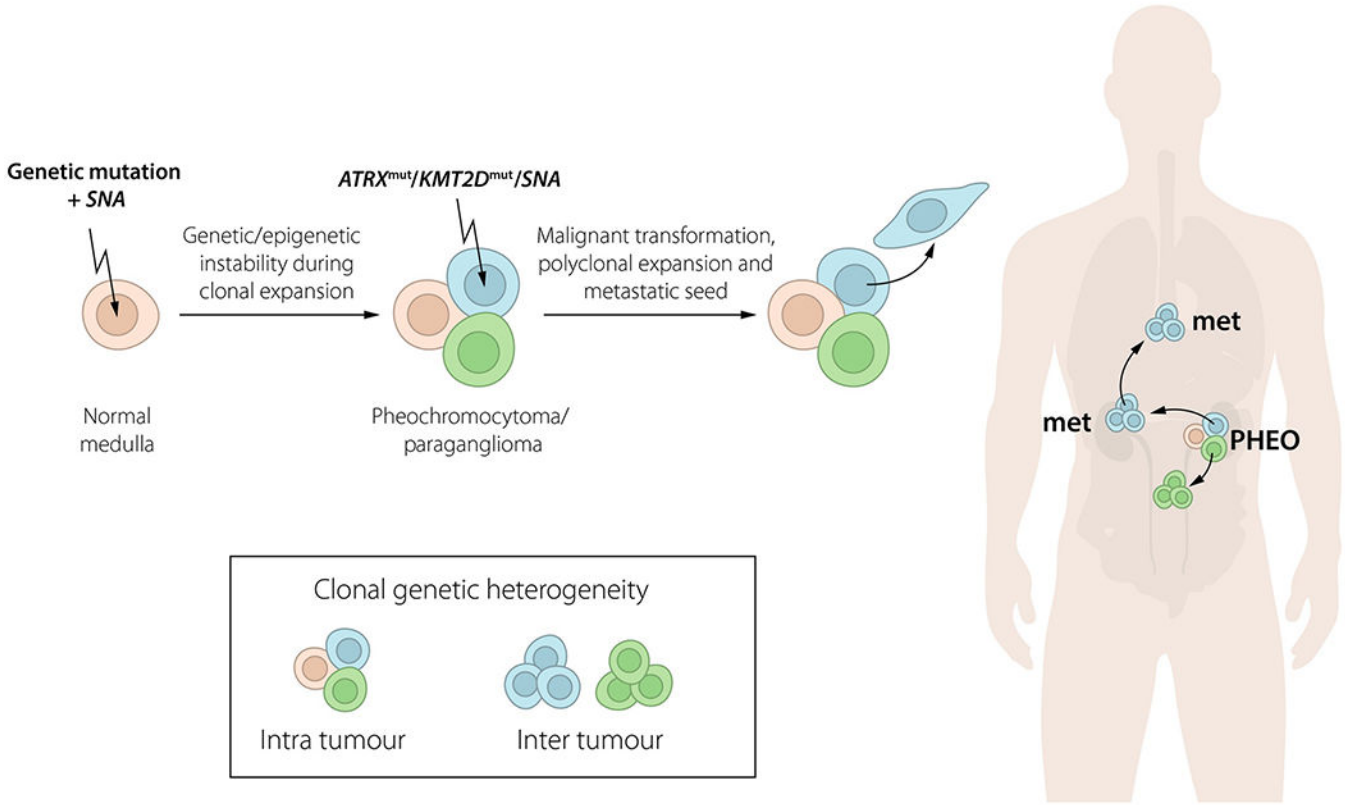


**Fig. 4.**

Proposed evolution of pheochromocytoma and paraganglioma from tumour initiation to metastatic seed. Genetic heterogeneity may occur within tumours as well as between different tumour lesions. SNA, somatic copy number alterations.

**Table 1**

Syndromes with increased risk of PPGL

| Syndrome | Gene | Location | Transmission | Original reference |
|---|---|---|---|---|
| PGL1 | *SDHD* | 11q23 | AD, paternal | Baysal *et al.* (32) |
| PGL2 | *SDHAF2* | 11q12 | AD, paternal | Hao *et al.* (33) |
| PGL3 | *SDHC* | 1q23.3 | AD | Niemann *et al.* (31) |
| PGL4 | *SDHB* | 1p36.13 | AD | Astuti *et al.* (30) |
| PGL5 | *SDHA* | 5p15 | NA | Burnichon *et al.* (29) |
| FH | *FH* | 1q42.1 | NA | Letouze *et al.* (34) |
| Von Hippel Lindau | *VHL* | 3p25.3 | AD | Latif *et al.* (35) |
| Paraganglioma-Polycytemia disease | *EPAS1* | 2p21 | NA | Zhuang *et al.* (36) |
| Neurofibromatosis type 1 | *NF1* | 17q11.2 | AD | Wallace *et al.* (38) |
| MEN2 | *RET* | 10q11.21 | AD | Mulligan *et al.* (37) |
| TMEM127 | *TMEM127* | 2q11.2 | AD | Qin *et al.* (39) |
| MAX | *MAX* | 14q23.3 | AD, paternal | Comino-Mendez *et al.* (40) |

PGL, paraganglioma; PPGL, pheochromocytoma and paraganglioma; FH, fumarate hydratase; MEN2, multiple endocrine neoplasia type 2; TMEM127, transmembrane protein 127; MAX, myc-associated factor X; AD, autosomal dominant; VHL, Von Hippel Lindau syndrome; NF1, Neurofibromatosis type 1; NA, not available.

**Table 2**

Clinical characteristics of mutation subtypes.

| Genes | Biochemistry | Radiology | Molecular imaging |
|---|---|---|---|
| *SDHx, FH* | NE, MT, dopamine | MRI | $^{18}$F-FDG or $^{68}$Ga-Som PET |
| *VHL, EPAS1* | NE | CT | $^{18}$F-DOPA or $^{11}$C-HED PET |
| *RET* | NE, E | CT | $^{18}$F-DOPA or $^{11}$C-HED PET |

NE, norepinephrine; E, epinephrine; FDG, fluorodeoxyglucose; Som, somatostatin receptor; DOPA, L-dihydroxyphenylalanine; HED, hydroxyephedrine; CT, computed tomography; MRI, magnetic resonance imaging; PET, positron emission tomographyMT, methoxytyramine.