# Genetics of Pheochromocytoma and Paraganglioma

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## Introduction

Pheochromocytomas (PCC) and paragangliomas (PGL) are rare tumors of neural crest origin with an incidence of 2–5 cases per million per year [1]. PCC arise from chromaffin cells of the adrenal medulla, while PGL arise from either sympathetic or parasympathetic paraganglia. PCC and PGL from sympathetic paraganglia are functioning tumors that secrete catecholamines.

These tumors can be of sporadic or hereditary origin. Initial description of the hereditary nature of these tumors cited heritability of about 10%. To date, approximately 40% of patients with PCC/PGL have germline mutations in at least one of 14 susceptibility genes [2]. The rate of germline mutation in the pediatric population with PCC/PGL is higher, reaching up to 70% [3, 4]. This higher rate is attributable to increased recognition resulting from discovery of novel susceptibility genes, awareness of disease inheritance, and better access to genetic testing. The autosomal dominant hereditary syndromes associated with PCC/PGL include von Hippel–Lindau disease (VHL), multiple endocrine neoplasia type 2 (MEN2), neurofibromatosis type 1 (NF1), and hereditary/familial paraganglioma disorders (PGLs 1-4). The genes involved in these syndromes are *VHL*, *RET, NF1,* and succinate dehydrogenase (*SDH)*, respectively. Other susceptibility genes with germline mutations linked to PCC/PGL include *MAX* and *TMEM127*. Currently, the latter genes do not have specific syndromes associated with mutation.

As the understanding of the genotype–phenotype relationship for these hereditary syndromes has improved, clinical management in patients with PCC/PGL can be tailored specifically to the causal gene(s). This is not only important for diagnosis, but genetic information can be used to identify risk of malignancy or recurrent disease, and to optimize treatments and surveillance for both the patient and family members. In addition, knowledge of the molecular mechanisms that lead to tumor initiation and progression can inform treatment strategy in patients with sporadic and hereditary PCC/PGL.

This chapter summarizes the current body of literature on known PCC/PGL susceptibility genes. Those associated with a hereditary syndrome will be described first, followed by a description of recently discovered germline mutations that as of yet have not been associated with a specific hereditary syndrome. The management of individuals with PCC/PGL based on the precise germline mutation is discussed.

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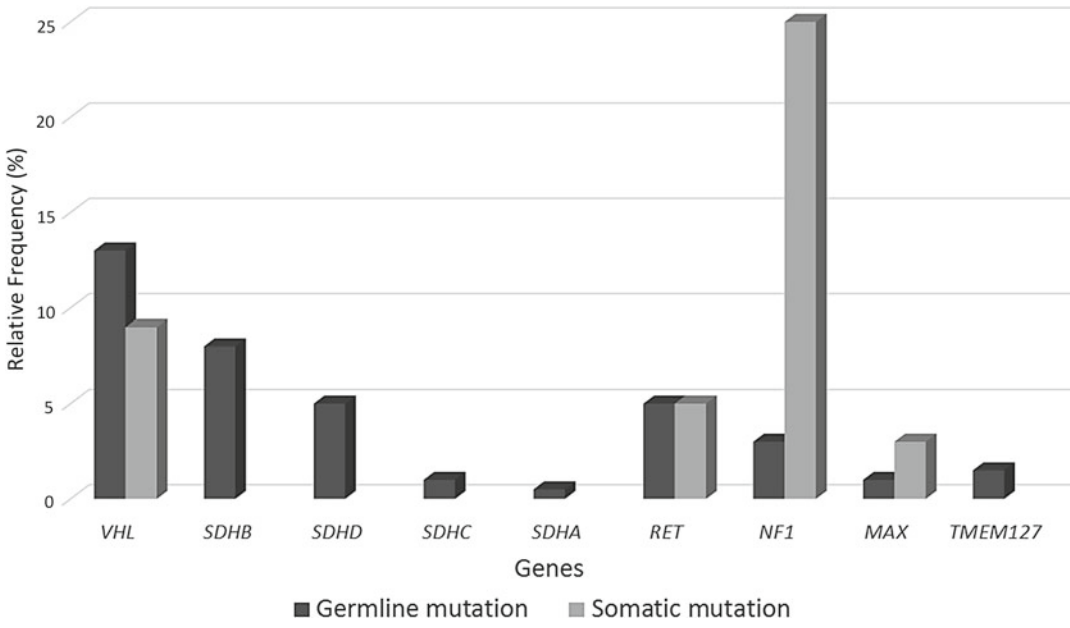


**Fig. 5.1** Relative frequency of germline and somatic mutations of specific genes in pheochromocytomas and paragangliomas. Germline and somatic mutations are shown in *dark gray* and *light gray*, respectively. *VHL* von Hippel–Lindau; *SDH* succinate dehydrogenase; *NF1* neurofibromin 1; *MAX* MYC-associated factor X; *TMEM127* transmembrane protein 127. (Adapted from Dahia [2] with permission)

## Germline Susceptibility Genes in PCC/PGL

The frequency of germline mutations in PCC/PGL is summarized in Fig. 5.1 [2]. The susceptibility genes associated with hereditary PCC/PGL are categorized into two groups based on gene expression profile and the dysregulated pathways associated with driver mutations [2].

Cluster 1 or pseudo-hypoxia cluster: Genes in this cluster are involved in oxygen-independent stabilization of hypoxic-inducible factor (HIF) and activation of the downstream oncogenic signaling pathways. The susceptibility genes in this cluster include posttranslational regulators of HIF stability such as *VHL* and, in rare cases, propyl hydroxylase (*PHD2* or *EGLN1*) [5, 6]. In addition, mutation of genes involved in the Krebs cycle, such as the four subunits of *SDH* (*SDHA-SDHD*), SDH assembly factor (*SDHAF2*), and fumarate hydratase (*FH*), causes accumulation of intracellular metabolites that inhibit the function of *PHD2* [7–12]. These mutations result in a pseudo-hypoxic effect, leading to aberrant accumulation of the HIF transcription factor [2]. HIF is composed of two subunits, HIF1A and HIF2A. HIF1A interacts with HIF2A via dimerization and acts as a transcription factor that allows adaptation to hypoxia (Fig. 5.2). When this mechanism is lost due to mutation, angiogenesis occurs [13].

Cluster 2 or kinase signaling cluster: This cluster includes genes associated with tyrosine kinase receptor and activation of downstream signaling activity. These genes include *RET*, neurofibromin 1 (*NF1*), transmembrane protein 127 (*TMEM127*), MYC-associated factor X *(MAX),* and *KIF1Bβ* [14–18]. Activation of tyrosine kinase receptors and downstream signaling pathways, such as PI3K-AKT-mTOR and MYC, affects glycolysis and synthesis of proteins, nucleic acids, amino acids, and fatty acids and promotes cell growth (see Fig. 5.2) [2].

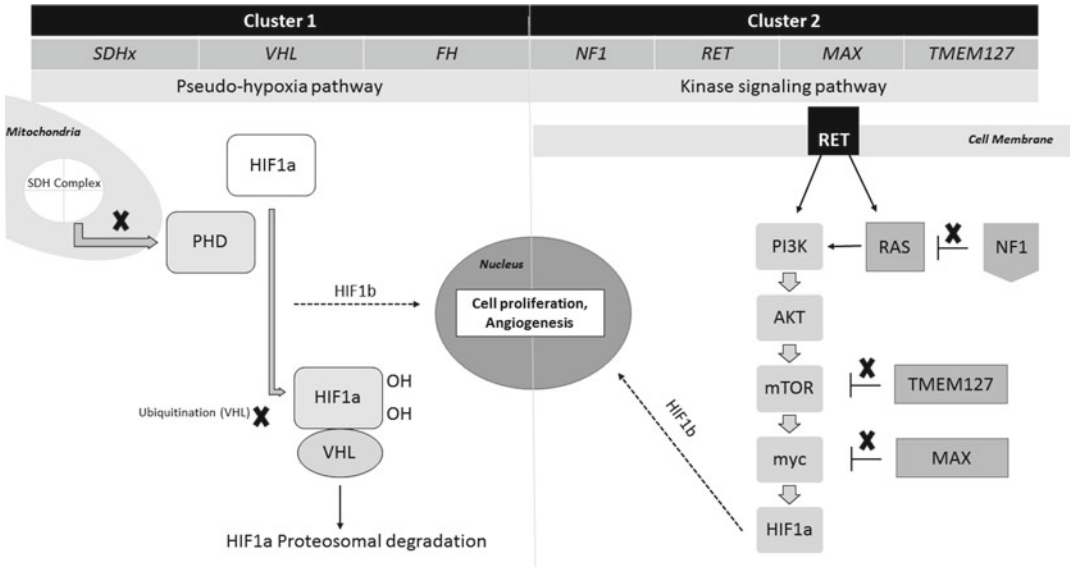


**Fig. 5.2** Two groups of susceptibility genes and signaling pathways associated with pheochromocytomas and paragangliomas. *Solid arrows:* normal pathway. *Dashed arrows*: pathway in hypoxia and/or with gene mutations. *Solid cross*: presence of mutation affecting pathway

## SDH-Related Mutations and Familial Paraganglioma Syndromes

Familial paraganglioma syndromes are caused by mutations in the genes that encode for the subunits of the SDH complex, also known as mitochondrial complex II. Complex II is part of the Krebs cycle and the electron transport system within the mitochondria. The function of the electron transport system is to produce ATP via an electrochemical gradient in the membrane of the mitochondria. Complex II catalyzes the oxidation of succinate to fumarate with the reduction of ubiquinone to ubiquinol. The core of SDH complex is made up of two subunits: a flavoprotein (SDHA) and an iron-sulfur protein (SDHB). The core is anchored to the inner mitochondrial membrane by the other two subunits (SDHC and SDHD). The SDH complex catalyzes oxidation of succinate to fumarate. In turn, electrons are transferred to ubiquinone in the electron transport chain (Fig. 5.3).

*SDH* mutations result in defective SDH proteins with shorter half-lives. Insufficient SDH complex causes pseudo-hypoxia as succinate accumulates in the mitochondria. Excessive succinate is exported to the cytosol where it stabilizes HIF1A and decreases degradation by inhibiting PHD-mediated hydroxylation of HIF1A. Stabilized HIF1A translocates into the nucleus, forming heterodimeric HIF, and inducing a pseudo-hypoxic response [19].

Familial paraganglioma syndromes have distinct clinical phenotypes based on the specific SDH mutation. The first gene to be identified was *SDHD*, followed by *SDHC*, *SDHB*, and *SDHA* [7, 9–12]. Recently, SDH-related genes have been discovered, including *SDHAF1* and *SDHAF2* that encode cofactors [20]. Because of the rarity, clinical information from patients with mutations in *SDHA, SDHC,* and *SDHAF2* is limited. *SDH* mutations are frequently discovered without prior family history, perhaps due to a low rate of penetrance. Furthermore, maternal imprinting has been found in patients with *SDHD* and *SDHAF2,* an epigenetic modification that may obscure familial inheritance [21]. *SDH* mutations cause PCC and extra-adrenal PGL, including head and neck PGL. Additional tumors associated with *SDH* mutations include renal cell carcinoma and gastrointestinal stromal tumors. Renal cell carcinoma has been reported in

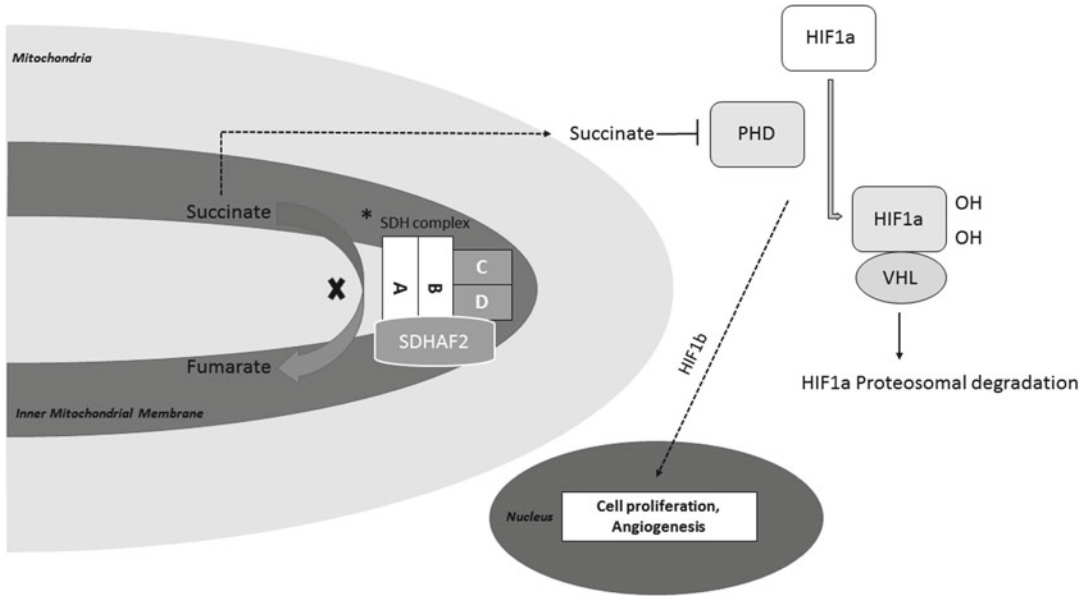


**Fig. 5.3** Pathogenesis of familial paraganglioma syndromes is caused by mutations in the *SDH* gene that form the SDH complex, also known as mitochondrial complex II (*). Excessive succinate results in pseudo-hypoxic response. *Solid arrows:* normal pathway. *Dashed arrows*: pathway in hypoxia and/or with gene mutations. *Solid cross*: presence of mutation affecting pathway

patients with *SDHB, SDHC,* and *SDHD* mutations. It is estimated that 14 % of *SDHB* carriers develop renal cell carcinoma [22–24]. The Carney–Stratakis dyad consists of PCC/PGL and gastrointestinal stromal tumors, while the Carney triad includes additional pulmonary chondromas [25, 26]. Germline *SDHB, SDHC,* and *SDHD* mutations have been reported in patients with Carney–Stratakis dyad; however, the genetic defect in patients with Carney triad remains unknown [27]. Pituitary adenoma and neuroblastoma have also been reported in patients with *SDH* mutations [28, 29]. Clinical characteristics of patients with germline *SDH* mutations are summarized in Table 5.1 [30].

### Succinate Dehydrogenase Subunit D

*SDHD* mutation was discovered in 2000 in patients with hereditary PGL of the head and neck, predominantly at the carotid body. *SDHD* mutation causes familial paraganglioma 1 (PGL1) syndrome. The gene is located on chromosome 11q23 and encodes for a protein that is present in a hydrophobic component of complex II [10]. The SDHD protein is a subunit of cytochrome b that functions along with SDHC to anchor subunits A and B to the inner mitochondrial membrane. Specifically, SDHC interacts with SDHB and attaches mitochondrial complex II to the inner mitochondrial membrane. Inheritance of these mutations is predominantly paternal [31], although recently maternal transmission has been described [32].

The tumors in patients with *SDHD* germline mutations predominantly are nonfunctioning head and neck PGL. However, subsequent studies found that patients infrequently present with PCC and extra-adrenal PGL of the abdomen [33]. The risk of malignancy of PCC/PGL with *SDHD* mutations is low.

### Succinate Dehydrogenase Subunit C

The discovery of *SDHD* led to identification of other *SDH* subunits. *SDHC* was discovered shortly after *SDHD*; mutations are associated with familial paraganglioma syndrome 3 (PGL3). Of all the SDH mutations, *SDHC* mutation is the least common germline mutation. *SDHC* mutations are most commonly associated clinically with head and neck PGLs. Extra-adrenal PGLs

**Table 5.1** Characteristics of hereditary diseases associated with pheochromocytomas and paragangliomas

| Disease | Gene | Protein function | Chromosomal location | Phenotypic features | Focality (single vs. multiple) |
|---|---|---|---|---|---|
| von Hippel–Lindau | *VHL* | E3 ubiquitin ligase | 3p25.3 | Central nervous system and/or retinal hemangioblastoma, renal cell carcinoma, pheochromocytoma, paraganglioma, pancreatic neuroendocrine tumors and cysts, endolymphatic sac tumors, papillary cystadenoma of the epididymis and broad ligament | Multiple |
| Multiple endocrine neoplasia type 2 | *RET* | Receptor tyrosine kinase | 10q11.21 | (2A) Medullary thyroid cancer, hyperparathyroidism, pheochromocytoma<br>(2B) Medullary thyroid cancer, marfanoid habitus, mucocutaneous neuromas, gastrointestinal ganglioneuroma, pheochromocytoma | Multiple |
| Neurofibromatosis type 1 | *NF1* | GTPase-activating protein | 17q11.2 | Café-au-lait spots, axillary and inguinal freckling, Lisch nodules, neurofibroma, osseous lesions, optic gliomas, pheochromocytoma | Single |
| Familial hereditary paraganglioma syndrome | *SDHx* | Succinate dehydrogenase/ mitochondrial complex | | Pheochromocytoma, paraganglioma | |
| – PGL 1 | *SDHD* | Anchoring subunit | 11q23.1 | Head and neck paraganglioma with rare extra-adrenal abdominal paraganglioma or pheochromocytoma | Multiple |
| – PGL 2 | *SDHAF2* | Assembly factor | 11q12.2 | Head and neck paraganglioma | Multiple |
| – PGL 3 | *SDHC* | Anchoring subunit | 1q23.3 | Head and neck paraganglioma with rare extra-adrenal abdominal paraganglioma or pheochromocytoma | Multiple |
| – PGL 4 | *SDHB* | Catalytic subunit | 1p36.13 | Extra-adrenal paraganglioma, rarely pheochromocytoma or head and neck paraganglioma | Multiple |
| – PGL 5 | *SDHA* | Catalytic subunit | 5p15.33 | Pheochromocytoma or extra-adrenal paraganglioma | Single |
| Hereditary pheochromocytoma | *MAX* | Transcription factor | 14q23.3 | Pheochromocytoma | Single |
| Hereditary pheochromocytoma | *TMEM127* | Transmembrane protein involved with mTOR | 2q11.2 | Pheochromocytoma | Single |

Adapted from Dahia [2], and from Favier et al. [30], with permission

*SDHx* succinate dehydrogenase mutation, *mTOR* mechanistic target of rapamycin, *PGL* paraganglioma

have been reported [11, 34]. The gene is located on chromosome 1q21 and encodes a large subunit of cytochrome b (cybL) in the SDH complex [11]. *SDHC* does not have a parental origin of transmission like *SDHD*. The tumors are mostly benign and seldom multifocal [35].

### Succinate Dehydrogenase Subunit B

Following *SDHD*, *SDHB* mutations were discovered. *SDHB* is the most commonly mutated subunit of SDH and is frequently associated with abdominal and extra-adrenal PGL [2]. *SDHB* is located on chromosome 1q36. The SDHB protein is composed of three iron-sulfur clusters, creating a hydrophilic domain. It binds with the SHDA protein to create a core, which then binds to SDHC and SDHD to anchor the core to the inner mitochondrial membrane. The clinical phenotype of patients with *SDHB* mutations is primarily extra-adrenal PGL. Patients with *SDHB* germline mutations commonly present with multiple synchronous or metachronous tumors at a younger age. There is a higher risk of metastatic disease and a poor prognosis associated with *SDHB* mutations [36–38]. However, *SDHB* mutations have lower penetrance compared to other PGL syndromes with PCC/PGL, occurring in 30 % by 80 years of age to 45 % by 40 years of age [23, 36, 39].

### Succinate Dehydrogenase Subunit A

*SDHA* is located on chromosome 5p15.33 and encodes a large hydrophilic protein subunit of the SDH complex. However, for many years after its discovery, no known germline mutations were identified and associated with a clinical hereditary presentation of PCC or PGL. Mutation in *SDHA* is associated with Leigh syndrome, which was first described in 1951 and is also referred to as juvenile subacute necrotizing encephalomyelopathy. Unlike *SDH*-related PGL, Leigh syndrome exhibits recessive inheritance requiring mutation in both *SDHA* alleles. It is an inherited neurometabolic disorder that affects the central nervous system and leads to progressive muscular weakness. It was not until 2011 that Burnichon et al. discovered a germline mutation in a patient with an extra-adrenal PGL [40]. Evaluation of a larger cohort indicated that *SDHA* mutations accounted for 4.5 % of germline mutations of PGL and PCC [40]. PCC, head and neck PGL, extra-adrenal PGL, and pituitary adenoma have been reported in patients with *SDHA* germline mutations [40–42].

### SDHAF2

*SDHAF2* is an SDH-related gene residing on chromosome 11q12.1. The encoded protein functions as an assembly protein for SDHA, a flavoprotein that requires SDHAF2 for flavination and thereby activation. Mutations in *SDHAF2* cause SDHA to be inactive by loss of flavination. Individuals who harbor mutation in *SDHAF2* develop head and neck PGL [12], and mutations have been associated with at least 15 cases of PGL arising from parasympathetic ganglia. The penetrance is 100 % by the age of 45 years [43, 44].

### SDHAF1

SDHAF1 (succinate dehydrogenase complex assembly factor 1) is a protein involved in the assembly of SDH complex. It currently does not have a direct link to the development of PCC/PGL. Mutations in the gene are associated with mitochondrial complex deficiency and were described in 2009 as being involved in the development of infantile leukoencephalopathy [45].

## von Hippel–Lindau disease

*VHL* mutations cause von Hippel–Lindau disease, an autosomal dominant familial cancer syndrome. Primary manifestations of the disease include retinal or central nervous system hemangioblastomas and clear cell renal cell cancer. Other tumors include PCC, pancreatic neuroendocrine tumors, and endolymphatic sac tumors [46] and very rarely PGL of the head and neck [47]. Individuals presenting with one of these tumors and a family history are diagnosed with VHL. In those with no family history, at least two hemangioblastomas or presence of hemangioblastoma and one of the other types of tumors is required for diagnosis; in these individuals, there

**Table 5.2** Clinical characteristics of different von Hippel–Lindau disease subtypes (1, 2A, 2B, 2C)

| VHL disease subtype | Mutation type | Phenotype |
|---|---|---|
| 1 | Deletion, nonsense, frame shift | Clear cell renal cell cancer (ccRCC), hemangioblastoma |
| 2a | Missense | Hemangioblastoma, pheochromocytoma |
| 2b | Missense | ccRCC, hemangioblastoma, pheochromocytoma |
| 2c | Missense | Pheochromocytoma |

Each is characterized by degree of penetrance of each type of tumor. von Hippel–Lindau (VHL) disease subtype, type of mutation, and phenotype expression

*ccRCC* clear cell renal cell cancer

is a high likelihood of a *VHL* de novo germline mutation [48].

VHL disease has different clinical subtypes (1, 2A, 2B, 2C), each characterized by the degree of penetrance of each tumor type. Table 5.2 summarizes the tumors associated with each clinical subtype. Extra-adrenal PGL have been described in VHL [47, 49]; however, the predominant tumors are PCC [48]. Clinical subtype 1 of VHL disease never has PCC, in comparison to clinical subtype 2 in which individuals develop PCC. Clinical subtype 2C exclusively presents with PCC, while 2A and 2B are differentiated based on the risk of developing clear cell renal cancer, with 2A having low risk in comparison to the high-risk 2B subtype [50]. Patients with VHL disease typically develop norepinephrine or normetanephrine-producing PCC, with a high rate of synchronous or metachronous bilateral PC. The age of onset of PCC/PGL in patients with VHL disease is approximately 30 years. Although malignant PCC/PGL in patients with VHL syndrome is rare, recurrent and multifocal PCC can occur.

Germline mutation of *VHL* was first described in 1993, and its location was mapped to chromosome 3p25.3. The gene spans three exons and encodes for two proteins [51], both of which have tumor suppressor activity. The proteins encoded by the *VHL* gene form a complex that is involved in protein degradation. The VHL protein complex causes polyubiquitylation of its substrates that marks them for proteolytic degradation. Two of the polyubiquitylated substrates are HIF1A and HIF2A. A decrease of HIF1α and HIF2α degradation in *VHL*-mutant tumors leads to HIF accumulation and subsequent induction of multiple downstream targets [50]. HIF2A has more oncogenic properties as it promotes the activity of the oncogenic MYC protein. Most *VHL* mutations preferentially impair degradation of the HIF2A subunit compared with HIF1A in vitro [52], and HIF2A is preferentially upregulated in *VHL*-mutant PCC/PGL [53–55]. The role of HIF1A in PCC/PGL remains unclear. Unlike renal cell carcinoma, no mutations or deletions in *HIF1A* have been reported in sporadic PCC/PGL [56]. The function of HIF1A in cancer may be tissue dependent or may be epigenetically regulated. Unlike renal carcinoma-related *VHL* mutations that involve deletions and truncations that severely destabilize VHL function, *VHL* mutations associated with PCC/PGL are predominantly missense [50].

Although the role of HIF in pathogenesis of VHL-associated tumors is clear, mutant *VHL* can be tumorigenic independently of HIF activation [57]. HIF-unrelated *VHL* mutations were initially identified in patients with VHL type 2C who develop only PCC without other VHL-related manifestations [58].

## Multiple Endocrine Neoplasia Type 2

Multiple endocrine neoplasia type 2 (MEN2) is a rare autosomal dominant hereditary syndrome caused by germline activating mutations of the *RET* proto-oncogene. The prevalence of these mutations varies between 1 per 30,000 and 1 per 50,000 individuals [59–62]. Because *RET* encodes a transmembrane tyrosine kinase receptor, the study of *RET* provides insights into the oncogenic effect of aberrant activation of tyrosine kinase signaling and the development of multiple kinase inhibitors currently used clinically. In addition, knowledge of the genotype–phenotype association derived from patients with various *RET* mutations enables tailored disease

**Table 5.3** Genotype–phenotype associations of *RET* protooncogene and risk of developing pheochromocytoma, medullary thyroid cancer, and other phenotypes

| *RET* mutation | Incidence of pheochromocytoma (%) | Medullary thyroid cancer risk | Incidence of hyperparathyroidism (%) | Presence of CLA/HD |
|---|---|---|---|---|
| **533** | 10 | + | – | N/N |
| **609** | 10–30 | + | 10 | N/Y |
| **611** | 10–30 | + | 10 | N/Y |
| **618** | 10–30 | + | 10 | N/Y |
| **620** | 10–30 | + | 10 | N/Y |
| **630** | 10–30 | + | 10 | N/N |
| **631** | ~50 | + | – | N/N |
| **634** | ~50 | ++ | 10–30 | Y/N |
| **666** | 10 | + | – | N/N |
| **768** | – | + | – | N/N |
| **790** | 10 | + | – | N/N |
| **V804L** | 10 | + | 10 | N/N |
| **V804M** | 10 | + | 10 | Y/N |
| **883** | ~50 | ++ | – | N/N |
| **891** | 10 | + | 10 | N/N |
| **912** | – | + | – | N/N |
| **918** | ~50 | +++ | – | N/N |

Adapted from Wells et al. [63]
*CLA* cutaneous lichen amyloidosis, *HD* Hirschsprung disease, + = moderate risk, ++ = high risk, +++ = highest risk, Y = present, N = not present

management based on patients' risk of developing medullary thyroid cancer, PCC, and other phenotypes (Table 5.3) [63].

Mutations in the *RET* gene were first linked to MEN2 in 1993 [64]. *RET* is located on chromosome 10q11.2 and is composed of 21 exons. The encoded tyrosine kinase receptor comprises an extracellular domain containing a ligand-binding site, a cysteine-rich region (exons 10 and 11), a transmembrane domain, an intracellular part with two tyrosine kinase domains (exons 13–15), and an intracellular catalytic core (exon 16) (Fig. 5.4) [60, 65]. RET is expressed in neural and neuroendocrine cell lineages such as thyroid C-cells, adrenomedullary chromaffin cells, and parathyroid cells [66]. RET is essential for neural crest cell migration; the development of enteric nervous system; and for the proliferation, differentiation, and survival of these cell types once they reach their destinations [67]. RET is the receptor for a family of soluble neurotrophic factor ligands, the glial cell line-derived neurotrophic factors (GDNFs). Upon binding, RET dimerization and autophosphorylation of intracellular tyrosine residues recruits adaptor and signaling proteins to stimulate multiple downstream pathways such as PI3K/AKT/mTOR and RAS/MAPK/ERK [67]. Thus, constitutive activation of *RET* causes hyperplasia of thyroid C-cells, adrenal medulla, and parathyroid gland. Alteration in the intracellular catalytic core (mutations in codon 918, classical MEN2B genotype) has the highest transforming capacity; mutations that disrupt ligand-independent dimerization and cross-phosphorylation (mutations in codon 609, 611, 618, 630, and 634) have intermediate activity; and mutations that interfere with ATP binding (mutations in codons 768, 790, 791, 804, and 891) have the lowest transforming activity [65, 68]. This results in variable risks of developing medullary thyroid cancer and distinct clinical manifestations of MEN2. MEN2A-type *RET* mutations occur in the extracellular domain of RET and cause ligand-independent homodimerization and aberrant activation of PI3K–AKT, RAS, p38 MAPK, and JUN N-terminal kinase

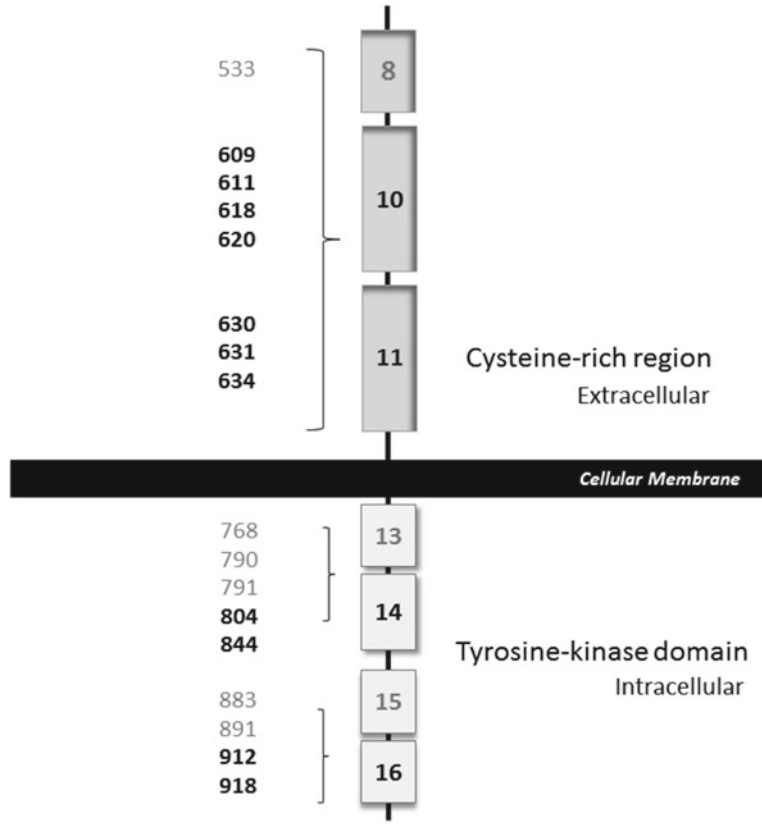


**Fig. 5.4** The RET tyrosine kinase receptor comprises an extracellular domain containing a ligand-binding site, a cysteine-rich region (exons 10 and 11); a transmembrane domain, an intracellular part with two tyrosine kinase domains (exons 13–15), and an intracellular catalytic core (exon 16)

pathways, resulting in the stimulation of cell growth, differentiation, and survival [69, 70]. Patients with MEN2A, in addition to PCC, develop bilateral medullary thyroid cancer and hyperparathyroidism secondary to parathyroid hyperplasia. MEN2B-causing mutations, by contrast, are in the few codons that affect the catalytic site of the kinase and lead to the loss of substrate specificity [70]. MEN2B, in addition to PCC and bilateral medullary thyroid cancer, is characterized by a spectrum of different manifestations, including mucosal neuroma, intestinal ganglioneuromas, and skeletal abnormalities (e.g., marfanoid habitus) (Fig. 5.5) [71]. *RET* mutation at codon 634 is by far the most common, accounting for 85 % of MEN2A cases. The penetrance of PCC in patients with *RET* codon 634 mutations is 20 % of individuals by age 20, and 67 % by the age of 50. In the same cohort, 21 % of individuals presented with PCC prior to diagnosis of medullary thyroid cancer [72]. The significance of this observation is that despite the more commonly assumed presentation of thyroid nodules or diagnosis of medullary thyroid cancer, those that present with PCC alone should be considered for genetic screening for *RET* mutation. While de novo *RET* germline mutations are common (95 %) in patients with MEN2B, de novo *RET* germline mutations occur in 4–10 % of patients with MEN2A [73]. Individuals with MEN2 commonly develop bilateral PCC [74]. However, the risk of malignancy is very low. Thus, partial adrenalectomy to preserve adrenal cortical function is recommended in select cases.

## Neurofibromatosis Type 1

There are two types of neurofibromatosis, neurofibromatosis 1 and neurofibromatosis 2 (NF1 and NF2, respectively). NF1 is a relatively common autosomal dominant inherited syndrome affecting about 1 in 3,000 individuals [75]. NF1 was previously known as von Recklinghausen's

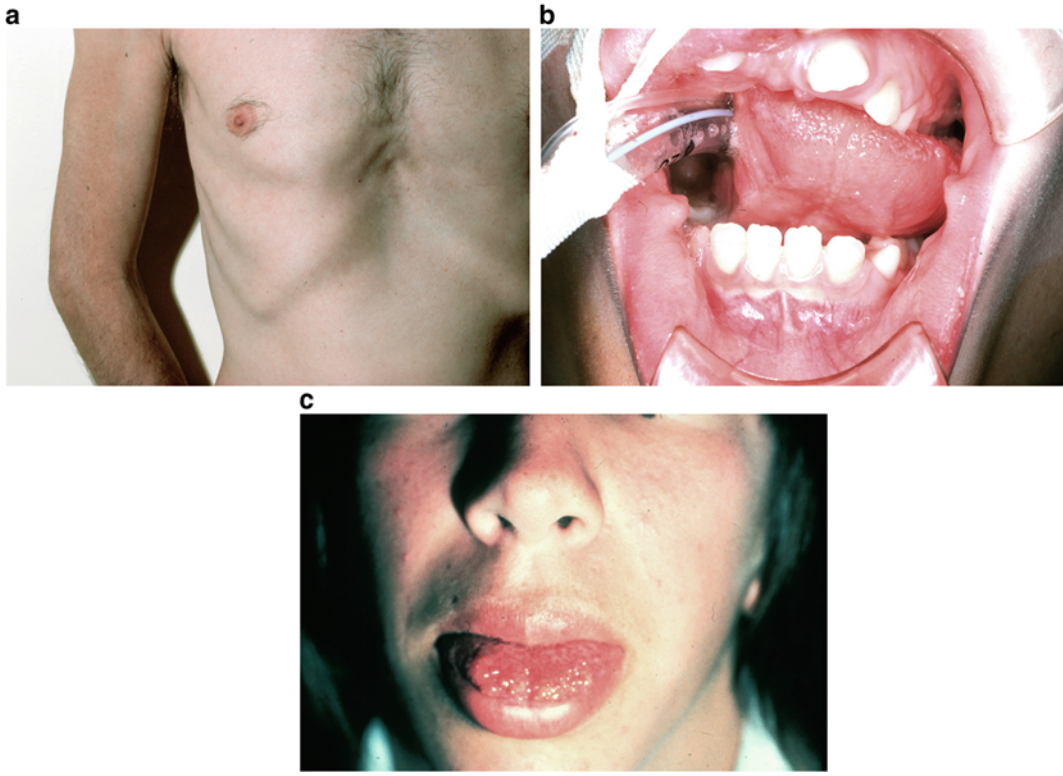


**Fig. 5.5** MEN2B physical exam features including marfanoid body habitus with pectus excavatum (**a**), poor dentition (**b**) and mucosal neuromas (**c**). (From Kebebew et al. [71] with permission)

neurofibromatosis or peripheral neurofibromatosis and represents the majority of neurofibromatosis cases (90%). Patients with NF1 are at risk of developing benign and malignant tumors of the central and peripheral nervous system as well as other malignant tumors. Tumors associated with NF1 include glioma of the optic pathway, glioblastoma, malignant nerve sheath tumor, gastrointestinal stromal tumor, breast cancer, leukemia, PCC, duodenal carcinoid tumor, and rhabdomyosarcoma [76]. NF2 is an autosomal dominant disorder caused by a different genetic mutation (merlin) that affects about 1 in 50,000 individuals. NF2 is characterized by acoustic and central neurofibromas and is not associated with PCC.

NF1 is diagnosed based on clinical presentation, which guides genetic testing. *NF1* gene testing is usually reserved for uncommon presentations or reproductive decision-making [76]. A National Institutes of Health consensus from 1987 outlines the diagnostic criteria for clinical diagnosis of NF1 [77]. NF1 is diagnosed if an individual has two or more of the following presentations: six or more café-au-lait macules, two or more neurofibromas, axillary or inguinal region freckling, optic glioma, two or more iris hamartomas (Lisch nodules), an osseous lesion or thinning of long bones, or a first-degree relative with diagnosis of NF1 [78]. PCC occur in less than 1% of individuals with NF1 and are predominantly unilateral (84%) [79]. The age of onset is typically in the 4$^{th}$ decade of life, similar to those with sporadic disease [80].

NF1 is caused by mutations in the *NF1* gene, a gene located on chromosome 17q11.2 that is composed of 60 exons and encodes a 220-kDa cytoplasmic GTPase activating protein called neurofibromin [81]. Neurofibromin functions as a negative regulator of the *RAS* proto-oncogene. Neurofibromin converts guanosine triphosphate bound Ras to an inactive guanosine

diphosphate bound configuration. When neurofibromin expression is decreased or lost secondary to mutation, the unhindered presence of active Ras leads to increased cell growth thereby promoting tumor formation [82]. mTOR is an important downstream signaling molecule that is aberrantly activated in *NF1*-deficient malignant peripheral nerve sheath tumors and in PCC/PGL [83, 84].

Currently, there are no specific guidelines for surveillance of patients with NF1 for PCC. Previously it was believed that when presenting with hypertension, the individual should be screened for PCC since studies have shown that such individuals have a 20–56 % chance of having a PCC [75]. However, a small cohort of NF1 patients were all diagnosed with PCC incidentally, and only one of six NF1 patients had hypertension. This suggests that patients with NF1 should be screened for PCC prior to development of hypertension [85].

## Pheochromocytomas Associated With *TMEM127* Mutations

*TMEM127* encodes a transmembrane protein involved in regulating the mTOR signaling pathway. Qin et al. [16] initially described TMEM127 in 2010 as a transmembrane-encoding gene on chromosome 2q11.2 which was identified from a cohort of 103 cases without previously defined genetic mutation who predominantly had PCC. Prior to this, the *TMEM127* gene was referred to as the FP gene for familial PCC, stemming from its identification via linkage analysis and characterization as a tumor suppressor gene with loss-of-function mutations. In the study by Qin et al., truncating *TMEM127* germline mutations were found in 30 % of patients with familial PCC, and 3 % of patients with "sporadic" PCC had germline *TMEM127* mutations [16]. PCC associated with *TMEM127* mutations have a transcriptional profile similar to that of PCC associated with *RET* and *NF1* mutations [16, 86]. *TMEM127* is believed to be involved in the mTOR pathway; human *TMEM127*-mutant PCC and cell lines depleted of *TMEM127* have increased mTOR signaling [87, 88]. The loss of *TMEM127* disrupts the early-to-late endosomal transition and enhances lysosomal biogenesis which affects mTOR distribution [88]. The exact function of *TMEM127* and the mechanisms by which it affects mTOR signaling are not fully understood. Thus far, about 30 *TMEM127* germline mutations have been identified [2]. These mutations are insertions or deletions, nonsense or splice site mutations that lead to truncated TMEM127 protein [16, 86].

Although no specific hereditary syndrome is associated with *TMEM127* mutations, initial studies have made several observations about the characteristics of individuals harboring mutations. The penetrance of germline *TMEM127* is low; only 20 % of patients carrying *TMEM127* germline mutations report a family history of PCC [89]. *TMEM127* mutations were predominantly found in individuals with PCC [89, 90], although subsequent studies showed that *TMEM127*-mutation carriers may present with extra-adrenal PGL, including head and neck PGL [91]. Bilateral PCC are common [16, 86], but the majority are unilateral with a similar mean age of presentation as those with sporadic tumors (42.8 years and 43.2 years, respectively). The risk of malignant PCC/PGL in *TMEM127* mutation carriers is low [89].

## Pheochromocytomas Associated With *MAX* Mutations

The association between MYC associated factor X (*MAX*) gene and hereditary PCC was first described in 2011 [17]. *MAX* is located on chromosome 14 and encodes a transcription factor that belongs to the basic helix–loop–helix leucine zipper (bHLHZ) family of proteins. Similar to *TMEM127* mutations, mutations in *MAX* have not been linked to a specific hereditary syndrome. Commonly, mutations in MAX result in an early stop codon, which causes exon skipping. These mutations affect the ability of MAX to appropriately interact with MYC within the MYC/MAX/MXDI complex; MYC is an oncoprotein involved in many human cancers. MAX can form complexes with other bHLHZ proteins

that oppose MYC-mediated activation and inhibit cell growth and promotes cell differentiation [92]. In vivo studies using PCC xenografts show that the reintroduction of MAX in MAX-depleted tumors results in growth arrest, supporting the repressive function of MAX in PCC [92, 93]. In its original description, a paternal mode of transmission was proposed as the mutated allele was paternal in origin in the majority of cases. In this original group, three of eight first-affected family members had metastatic disease, suggesting *MAX* mutations are associated with more aggressive disease [17]. Overall, the rate of germline mutation is low (1.12 %), and it has been predominantly found in individuals with bilateral PCC or multiple PCC in one adrenal gland (68.4 %) [94].

## Other Diseases Associated with Pheochromocytomas and Paragangliomas

Other syndromes have been described that are associated with PGL. Some do have a known mutation while others do not.

Carney triad was described in 1977. It is a triad that manifests with gastric stromal tumors (GIST), pulmonary chondroma, and abdominal PGL. The presence of at least two of the tumor types is needed for diagnosis. Other tumor types, including esophageal leiomyomas and adrenocortical tumors, have also been described [95] some of which may be functioning [96].

Females have a higher prevalence of Carney triad, and the first tumor often identified is a GIST in young patients. It has been recommended that young patients diagnosed with GIST have regular follow up to screen for development of the other tumor types [97]. To date, there is no known causative mutation for Carney triad. Recent studies evaluated the role of SDH complex mutation; although no association was identified, it is believed that Carney triad is caused by alterations in the mitochondrial complex [98].

Carney–Stratakis syndrome manifests with GIST and extra-adrenal PGL [99]. The GIST described as part of this dyad were once classified as wild-type GISTs, lacking the common *KIT* and *PDGFRA* mutations. Germline *SDHB, SDHC,* and *SDHD* have been reported in patients with Carney–Stratakis dyad [27]. The GISTs in this dyad have SDHx deficiency [100].

## Biochemical Profiles in Patients with Hereditary PCC/PGL

Plasma free-metanephrines and/or urine fractionated metanephrines are routinely used to screen for PCC/PGL as they are highly sensitive and specific for PCC/PGL. In addition to clinical presentations, biochemical profile can help select appropriate genetic testing. Because those with VHL syndrome have low expression of phenylethanolamine-N-methyltransferase (PNMT), patients commonly have elevated norepinephrine and normetanephrine but not epinephrine and normetanephrine [101, 102]. On the contrary, MEN2-associated PCC frequently overexpress *PNMT,* and patients present with predominantly elevated epinephrine [102]. Patients with *NF1* germline mutations have elevated norepinephrines and normetanephrines. Patients with *SDHx* mutations have a normetanephrine predominant biochemical profile. In addition, patients with *SDHx* germline mutations can have high levels of dopamine and methoxytyramine [102]. The head and neck PGL usually are biologically inactive. The biochemical profile of PCC/PGL associated with *TMEM127, SDHA,* and *MAX* mutation has not been well documented.

## Somatic Gene Mutations in PCC/ PGL

Sporadic PCC/PGL is defined as a tumor that occurs in individuals without a family history and who do not have a germline mutation in known susceptibility genes. Somatic mutations of *NF1, VHL, RET, HIF2A, HRAS, BRAF, TP53,* and *MAX* can be detected in sporadic PCC/PGL [2, 103]. *NF1* is the most common somatic mutation in sporadic PCC, with inactivating *NF1* mutations recently detected in 20–40 % of

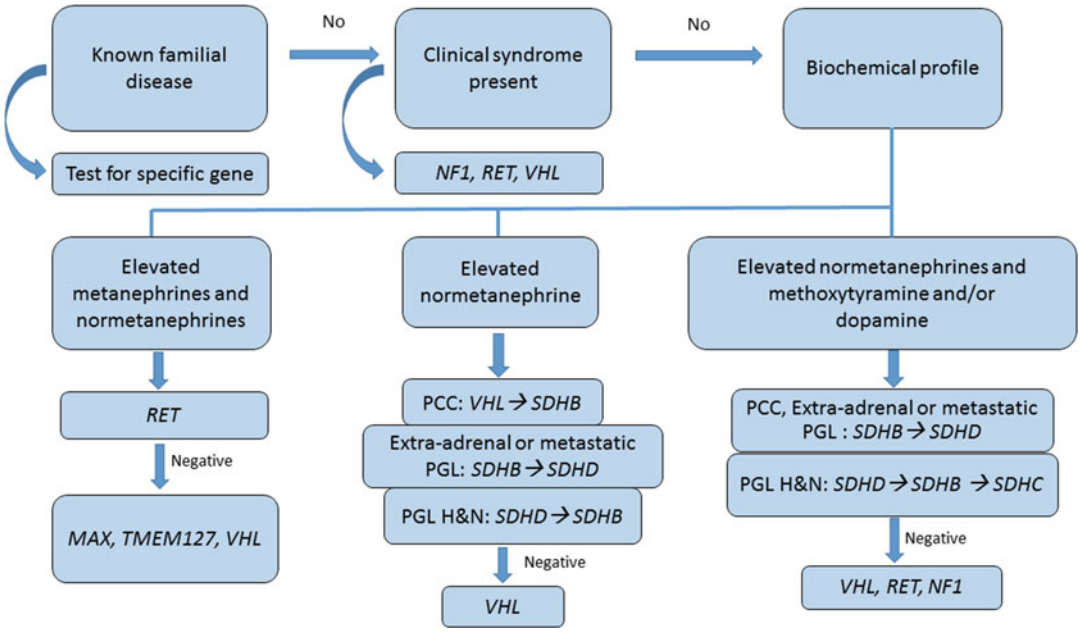


**Fig. 5.6** Genetic testing algorithm for patients with pheochromocytomas (PCC) and paragangliomas (PGL). *H&N* head and neck

sporadic PCC [104, 105]. These somatic variants were predominantly truncating and were often accompanied by the loss of the wild-type allele in the tumor [105]. Somatic *NF1* mutations can be found in tumors with somatic *RET* or somatic *VHL* mutations. Somatic *RET* or somatic *VHL* mutations were identified in 14 % of sporadic PCC/PGL [106]. Somatic *HRAS* mutations occur in 7 % of sporadic PCC/PGL [103, 107]. HRAS mutant tumors were exclusively found in men; all had benign tumors with increased norepinephrine and/or epinephrine secretion [107]. No somatic *HRAS, BRAF* V600E or *TP53* mutations have been found in hereditary PCC/PGL, suggesting that these mutations might be mutually exclusive. The rates of *BRAF* V600E and *TP53* mutations in sporadic PCC/PGL were 1.2 and 2.3 %, respectively [103]. Somatic *HIF2A* mutations were found in 12 % of sporadic PCC/PGL. Tumors with mutated *HIF2A* displayed a pseudo-hypoxic gene expression pattern, similar to those in cluster 1 [108, 109]. Genetic mosaicism of *HIF2A* has been reported in patients with multiple PCC/PGL and congenital erythrocytosis [109].

## Genetic Testing

Genetic testing has evolved in the last several years. First-generation sequencing, also known as Sanger sequencing, was introduced in 1977 by Frederick Sanger et al. [110]). This method requires single-stranded DNA template and a DNA primer, along with normal (dNTPs) and modified (either radiolabeled or fluorescently labeled) deoxynucleotides (ddNTPs). The modified ddNTPs incorporated during PCR terminate DNA elongation and resulting DNA fragments can be resolved to provide a DNA sequence. In the late 1990s, next-generation sequencing was introduced and has been used readily over the past 10 years. Similar to Sanger sequencing, DNA is sequenced by synthesis through addition of nucleotides. However, next-generation sequencing platforms are able to process and sequence DNA more quickly at a lower cost. These improvements have made genetic testing more readily available and accessible.

To reduce cost and remain effective, the algorithm for determining germline genetic testing in patients with PCC/PGL should be based

on family history, clinical features of hereditary PCC/PGL, and biochemical profiles. The algorithm is summarized in Fig. 5.6.

- If both metanephrines and normetanephrines are elevated:
  - Patients that exhibit no clinical features of neurofibromatosis type I should undergo genetic testing for *RET* proto-oncogene first, followed by *MAX, TMEM127,* and *VHL.*
- If methoxytyramine and/or dopamine is elevated:
  - Patients should be tested for *SDHx* mutations (*SDHD* for head and neck PGL first, followed by *SDHB,* and, *SDHB* for PCC, extra-adrenal PGL, or metastatic PCC/PGL first, followed by *SDHD*).
  - Patients with PCC, elevated methoxytyramine and that have no *SDHx* mutations should be tested for *RET, VHL,* and *NF1* mutations.
- If normetanephrines are elevated:
  - Patients with PCC should be tested for *VHL* mutations first, followed by *SDHB.*
  - Patients with PGL should be tested for *SDHx* mutations first (*SDHD* for head and neck PGL first, followed by *SDHB,* and, *SDHB* for extra-adrenal PGL, or metastatic PCC/PGL first, followed by *SDHD*), followed by *VHL.*

Although the earlier algorithm may be cost effective, it is time consuming. A next-generation sequencing strategy has been developed [111] for analysis of germline mutations in individuals with PCC/PGL; this method has a 98.7 % sensitivity and rapid turnaround time as it can simultaneously analyze multiple genes [112].

Although the Endocrine Society Clinical Practice guideline for PCC/PGL recommends that all patients with PCC/PGL should be counseled for germline mutation testing [113], the likelihood of *not* having a germline mutation is increased when an individual presents at an older age, without family history, with a unilateral PCC, and with no evidence of metastatic disease. As such, current guidelines caution against routine testing of germline mutations in these individuals in light of financial costs and limited incremental value of genetic testing in this setting [113]. However, studies are emerging that despite lack of family history, individuals being classified as having sporadic disease may in fact harbor a germline mutation. Neumann et al. showed that in individuals with no family history, a germline mutation could be detected in upward of 24 % [3]. In a group of individuals with apparent sporadic disease, as defined by a lack of family history or syndrome and presenting with a single PCC or PGL, germline mutations were found in 14 % of individuals, and at a higher rate in those with a PGL compared to single PCC (28.7 % vs. 4.5 %, respectively). In the same cohort, somatic mutations were found in 43 % of individuals [114]. As the cost of genetic testing continues to decrease with the advances in next-generation sequencing methods, routine genetic testing for known germline mutations will likely become more accessible and used in clinical practice.

## Management of Patients with PCC/PGL Based on Genetic Information

Management of individuals with PCC/PGL involves a multidisciplinary approach. Once a diagnosis of PCC/PGL is confirmed, germline mutation testing should be obtained prior to a medical or a surgical intervention because the optimal time of intervention, treatment options, and surgical approach may differ based on the risk of malignancy, multifocality, and persistent or recurrent disease. For example, surgical resection of a carotid body tumor in patients with germline *SDHB* mutations should be considered earlier than in patients with *SDHD* mutations because of the higher rate of malignancy and worse disease-free survival [115]. An open approach may be preferable in patients with *SDHB* mutations who present with extra-adrenal PGL to remove surrounding lymph node compartments. A cortical preserving minimally invasive adrenalectomy in patients with VHL or MEN2 syndrome should be considered due to the low risk of malignancy; this reduces the risk of

long-term adrenal steroid replacement [116]. The clinical management of other manifestations associated with hereditary PCC/PGL, such as medullary thyroid cancer in patients with MEN2 syndromes, can benefit from genetic information. It is best to obtain genetic testing especially in patients with no obvious family history or apparent clinical syndrome to appropriately counsel the patients regarding the risks of malignancy and recurrent disease as well as the need for postoperative surveillance for PCC/PGL and other manifestations.

A recent guideline from European Society of Endocrinology recommends annual biochemical, anatomical, and functional imaging with plasma or urine metanephrines to surveil for recurrent disease for at least 10 years. Anatomical imaging may be started 3 months postoperatively and should be continued for a minimum of 10 years. The recommendations are based on data from the European Network for the Study of Adrenal Tumors that demonstrated a 10 % risk of recurrence over the first 5 years of follow-up. For patients with a high risk for persistent or recurrent PCC/PGL, such as young patients, those with germline mutations, or patients with malignant (defined as metastasis in lymph nodes or other distant sites) or large PCC/PGL, lifelong surveillance is recommended [117].

For sporadic disease, one may argue that apparently sporadic appearing presentations in individuals in the fourth or fifth decade may need minimal follow-up. However, these individuals may still have 7 % risk of recurrent disease over 5 years, compared to 17 % in patients with germline mutations [117]. The assessment of younger individuals, from the pediatric population to those in their 30s, remains unclear as they may harbor germline or somatic mutations not yet identified.

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