Chapter 5
Pheochromocytoma and Paraganglioma

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Abstract Pheochromocytoma (PCC) and paraganglioma (PGL) are neuroendocrine tumors that originate in the neural crest. While PCCs develop from chromaffin cells in the adrenal medulla, PGLs develop either from paraganglia in the sympathetic nervous system (and are distributed symmetrically along the entire paravertebral axis from the neck to the pelvis, giving rise to thoracic and abdominal/retroperitoneal PGL) or more rarely from parasympathetic paraganglia (giving rise to head and neck PGL and rarely thoracic PGL). PCCs/PGLs have the highest heritability of all human neoplasms being a good example of diseases with underlying genetic heterogeneity. In this regard, at least 40% of PCC/PGL patients carry a germline mutation in 1 of the 19 genes described so far as related to the disease. In addition to the complexity of the genetics of PCC/PGL, we need to consider the role of somatic mutations, which to date have been identified up to 30–35% of tumors. The latter have been observed to occur not only in the same genes involved in heritable susceptibility but also in the new ones, which have thus recently emerged as key players in the sporadic presentation of these diseases. Despite the increasing proportion of patients already explained by germline or somatic genetic defects, there are still patients with clinical indicators of hereditary disease (i.e., family history, multiple tumors, and/or young age of onset) without a molecular diagnosis, which are being actively investigated.

Keywords Pheochromocytoma · Paraganglioma · Pediatric · Genetics · Neuroendocrine tumor · Diagnostic and prognostic markers

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5.1 Epidemology

The annual incidence of PCC/PGL in the Spanish population is 2.06 per million (2–8 per million in the USA), which classifies the disease as rare, although results from autopsies suggest a higher incidence [1]. Between 10% and 20% of patients with PCC/PGL are diagnosed during childhood or adolescence [2]. PCC is the most frequently diagnosed endocrine tumor in children [3].

5.2 Clinical Presentation

Both PCC and sympathetic PGL usually manifest as symptoms related to the excessive production of catecholamines [4] and less frequently as symptoms caused by the tumor mass. Parasympathetic PGLs are fundamentally non-functional and therefore almost always present symptoms caused by the tumor mass (Table 5.1).

The average age at presentation of PCC/PGL in pediatrics is 11–13 years, with a male preponderance of 2:1. The classic triad of symptoms (palpitations, sweating, and headache), usually accompanied by hypertension, only occurs in a minority of cases and is particularly unusual in infants. Adrenergic crises can last from seconds to hours, with variable time between crises, from hours to months. They can present spontaneously or result from physical activity (more common in children), changes in posture or an increase in intra-abdominal pressure due to defecation, pregnancy, trauma, or certain diagnostic tests. The clinical presentation is variable, with sustained hypertension seen in 60–90% of pediatric cases, but PCCs/PGLs are the cause of hypertension in only 0.5–2% of pediatric cases [5, 6].

5.3 Tumor Behavior

PCC/PGL diagnosed in infants is more often extra-adrenal (8–43%), multifocal or bilateral (7–53%), malignant (10–47%), and familial (9–50%) [7].

Catecholamine excess, local growth, and metastatic disease all contribute to increased morbidity and mortality in patients with PCC/PGL. Those with sympathetic PCC/PGL have an almost ten times higher incidence of cardiovascular events before their diagnosis. However, mortality is caused mainly by metastatic disease.

The risk of metastasis is greater for sympathetic PGL than for PCC or parasympathetic PGL; however, parasympathetic PGL gives rise to substantial morbidity due to local tumor growth [8]. The following factors are associated with a greater risk of metastasis: carrying a mutation in *SDHB*, young age, persistent postoperative hypertension, large tumor size (>4–5 cm), extra-adrenal or dopamine-producing tumors, and tumors not detected by ^{123}*I-MIBG scintigraphy* [2, 3, 9]. Prognostic data on PCC/PGLs are heterogeneous. Goffredo et al. using data from 18 US

Table 5.1 Clinical presentation [4, 5, 7–10]

PCC and sympathetic PGL	Symptoms in adults: Headache (70–90%), sweating (60–70%), palpitations (50–70%), thoracic and abdominal pain (20–50%), nausea (26–43%), nervousness (35–40%), asthenia (15–40%), blurred vision (3–21%), anxiety and panic attacks (20%), dyspnea (11–19%), heat intolerance (13–15%), dizziness (3–11%), constipation (10%), diarrhea (6%), tremors, weight loss without anorexia, polyuria, and polydipsia Children usually present with headache (81%), sweating (36–68%), weight loss (44%), nausea and vomiting (27–56%), polyuria and polydipsia (25%), constipation (8%), visual disturbances, seizures, and panic attacks
	Signs: – Due to hypersecretion of catecholamines: hypertension (90%), tachycardia (50–70%), fever (66%), pallor (30–60%), vomiting (26–43%), hyperglycemia (42%), flushing (18%), seizures (3–5%), leukocytosis, elevated hematocrit, and hyperglycemia. The onset of an episode of hypertension, tachycardia, and/or arrhythmia related to an invasive diagnostic or therapeutic procedure, anesthesia, the intake of foods rich in tyramine, or the intake of certain drugs should raise the suspicion of PCC/PGL (1). Less frequent in children than in adults are tachycardia, fever, flushing, and hyperglycemia. Dysrhythmia, mood swings, and character changes are rare in children – Due to local compression: Hydroureteronephrosis, renovascular hypertension, etc. Hypertension is the most common sign, yet only 0.05–0.6% of adults and 1–2% of children with hypertension have PCC/PGL – Presentation of hypertension: In adults: Persistent (50%) and paroxysmal (50%); 5–15% of cases have normal blood pressure In children: Persistent in most cases (60–90%) and paroxysmal in only 10%, 20% have normal blood pressure, 80% of pediatric cases present with orthostatic hypotension with or without hypertension Other presentations: "Manic-depressive behavior" of blood pressure: Extreme oscillations over short periods of time, non-*dipper* nocturnal pattern, and hypotension, especially orthostatic hypotension, in adrenaline- or dopamine-secreting tumors
	Medical emergency (occasionally): Hypertensive crisis (often with headache, visual disturbances, and/or seizures), stroke, arrhythmias, ischemic heart disease, cardiomyopathy, congestive heart failure, or pulmonary edema (especially if these become worse on initiating treatment with β-adrenergic blockers), multiorgan failure and even death
Parasympathetic PGL	Unilateral hearing loss, pulsatile tinnitus, dysphonia, cough, pain, Horner syndrome, headache, blurred vision, and dysphagia

registries (time frame 1988–2009) reported a 5-year overall survival rate of 58% for PCCs and 80% for PGLs [10]. More recently, in a retrospective study of 18 European centers, with a follow-up from 1998 to 2010, Hescot et al. reported a global 5-year survival rate of 62% and a median OS of 6.7 years [11].

In the 2017 WHO classification of adrenal tumors, the term benign PCC/PGL was abolished to highlight the fact that all PCC/PGLs should be considered to have metastatic potential. Malignancy occurs in approximately 12% of pediatric/

adolescent patients, which is associated with a 5-year survival rate of 40–95% in adults and 98% in children [12].

5.4 Diagnosis

The diagnosis of PCC and sympathetic PGL is based on biochemical evidence of the levels of catecholamines, while parasympathetic PGL is diagnosed using imaging techniques [13].

The secretion of catecholamines (adrenaline, noradrenaline, dopamine) can be variable and intermittent. In contrast, their conversion to the corresponding metabolites (metanephrine, normetanephrine, methoxytyramine) is continuous and independent of their secretion, which means that the measurement of plasma-free or urine-fractionated metanephrines is more effective in the initial diagnostic screening [14–16].

While determination of metanephrines in plasma has greater specificity and sensitivity (98% and 100%, respectively) than that in urine (both 96%), it requires that the patient meet the following conditions in order to minimize the number of false positives: free of stress, 8–12 h fasting, supine position, and extraction after 20–30 min following insertion of the venous cannula. It is also often much more appropriate in children for which the collection of urine over 24 h can be very challenging. If measured in urine, the level of excreted creatinine should be determined to verify that the sample was collected appropriately [3]. Dietary restrictions are not normally applied, except in the measurement of deconjugated normetanephrine or methoxytyramine in plasma or urine [17].

The range of levels of catecholamines and metanephrines in plasma and urine tends to be higher for hypertense and hospitalized patients compared to normotensive volunteers, for adults compared to children, and for men compared to women. Given that the normal range is usually determined as the 95% central range in a normotensive reference population, defining as positive any result above the upper limit of "normal" may result in an excess of false positives. While age and sex have minimal influence on the normal range for adults, this is not the case for pediatric patients, and so ranges specific for age and sex should be established for biochemical studies carried out in children [16]. In general, when levels more than four times greater than the upper limit of the normal range are observed, the probability of PCC/PGL is high, and further analysis to determine the tumor location is indicated. When increases less than four times the upper limit are observed or when the result is unclear, checks should be carried out for technical errors, inadequate sample extraction, and other clinical conditions that could elevate catecholamines. The result could be confirmed in plasma if originally determined in urine or vice versa [3]. When paroxysmal symptoms are observed in the presence of normal levels, another sample should be collected during or immediately after a paroxysm. It may be informative to carry out a clonidine suppression test, where suppression of $\geq$40% of plasma metanephrines signifies the absence of a tumor [16]. Stimulation tests

have fallen into disuse because of the risks implied and the technical improvements in biochemical tests. Suppression and stimulation tests have not been validated in children and so are generally not recommended in such cases [18].

Chromogranin A is a protein that is co-stored and co-secreted with the amines contained in the secretory granules of neuroendocrine tumors and is therefore a non-specific marker that can be used in clinical follow-up because of its correlation with tumor burden, even in non-secreting tumors [2, 19].

5.5 Determination of Tumor Location

Studies to determine tumor location should only be carried out following biochemical confirmation of diagnosis, except in the case of possible parasympathetic PGL and the follow-up of patients and mutation carriers for which the probability of developing the disease is high [20].

The technique of first choice is computerized tomography (CT) for adults and magnetic resonance imaging (MRI) for children and pregnant women. CT scans allow the visualization of adrenal tumors larger than 1 cm and extra-adrenal tumors larger than 2 cm. The most common image without contrast is that of highly dense ($\geq$10 Hounsfield units) and heterogeneous masses, with an increase in enhancement and a delayed washing following intravenous infusion of contrast. The main drawbacks of this test include exposure to radiation and that tumor identification can be complicated by scaring from prior surgery. MRI is a more expensive technique but has three key advantages: (1) intravenous contrast is not required, (2) it is better than CT in detecting extra-adrenal tumors, and (3) it does not emit ionizing radiation [3, 16].

Head and neck PGLs are highly vascularized tumors that are typically found to be associated with blood vessels and nerve structures. Via CT, the involvement of bone structures can be more clearly defined, and PGL appears as a homogeneous mass with intense contrast enhancement. Using MRI, tumors may appear on T1 sequence surrounded by a matrix of intermediate density, with disperse areas without signal but with intense contrast enhancement, which correspond to the surrounding blood vessels, and on T2 sequence as “salt and pepper” images. In order to establish the involvement of the surrounding vascular structures, it is usually necessary to carry out a selective arteriography, which at the same time allows the embolization of the main artery to reduce hemorrhaging and facilitates surgical resection [21].

Functional tests may be carried out to determine tumor location when other methods fail or require confirmation or in staging in order to identify or rule out metastatic disease or multiple tumors where the location has been established. However, there are no established criteria for their use and no consensus regarding their application prior to surgery. ^{123}I-MIBG scintigraphy is considered the functional test of choice. Somatostatin analogue scintigraphy (Octreoscan®) could be useful for parasympathetic PGL and for those tumors not detected by ^{123}I-MIBG

scintigraphy. In addition, somatostatin analog and MIBG uptake are also predictive factors for targeted internal radiation therapy that can be relevant in the case of progressive metastatic or unresectable disease.

Positron emission tomography (PET) with a radiotracer, combined with CT, is less commonly available but has higher resolution and is more sensitive than *^{123}I-MIBG* scintigraphy to detect extra-adrenal PGL. ^{68}Ga-DOTATATE PET/CT could be used in *SDH*- and *FH*-mutated cases and ^{18}F-DOPA in *VHL*-, *EPAS1*-, *RET*-, *NF1*-, *MAX*-, *TMEM127*-, and *HRAS*-related tumors, both being superior to ^{18}F-FDG PET/CT. At the NIH, the use of ^{68}Ga-DOTATATE PET/CT has extended to pediatric patients with PCC/PGL. Their preliminary results demonstrate the superiority of ^{68}Ga-DOTATATE PET/CT in localization of SDHx-related PCC/PGLs in pediatric population compared to 18F-FDG PET/CT and CT/MR imaging with the exception of abdominal (excluding adrenal and liver) lesions [12, 19, 22].

5.6 Susceptibility to Develop PCC and PGL

These tumors can develop in an apparently sporadic manner or as part of one of several inherited tumor syndromes associated with alterations in distinct genes. Particularly in the latter case, PCC/PGL often presents with other pathologies within a family and even in the same individual. This variable clinical phenotype is testament to the genetic complexity that underlies the development of this disease.

For years PCC/PGL was known as "the 10% tumor," given that 10% were metastatic, 10% hereditary, 10% bilateral, and 10% extra-adrenal. However, the evidence emerging over the last two decades has shown this alias to be erroneous; we now know that more than 30% of patients develop extra-adrenal tumors [23, 24], that the percentage of metastatic cases depends on location of the primary tumor and/or the gene mutated (from approximately 3% for tumors associated with *RET* or *VHL*, up to 70% for those due to mutations in *SDHB*) [6, 25], and that approximately 40% of tumors are due to a germline mutation in one of the known susceptibility genes. In fact, PCC/PGL is the most heritable human tumors, and there are still patients with multiple PGLs and/or bilateral PCC and/or a family history of the disease for which the genetic cause has not been identified. This as-yet unexplained heritability presents a substantial challenge in the quest to understand the tumor biology and correctly genetically classify each patient in order to be able to offer them the most appropriate clinical follow-up.

Since the discovery in 1990 of the first susceptibility gene for PCC, 18 additional genes have been described (Fig. 5.1), highlighting the importance of studies that systematically scan for germline mutations in apparently sporadic cases of PCC/PGL [25].

The 40% of PCC/PGL that is known to be hereditary develops primarily in the context of three familial tumor syndromes: von Hippel-Lindau disease (VHL), multiple endocrine neoplasia type 2 (MEN2), and familial PCC/PGL. Other syndromes

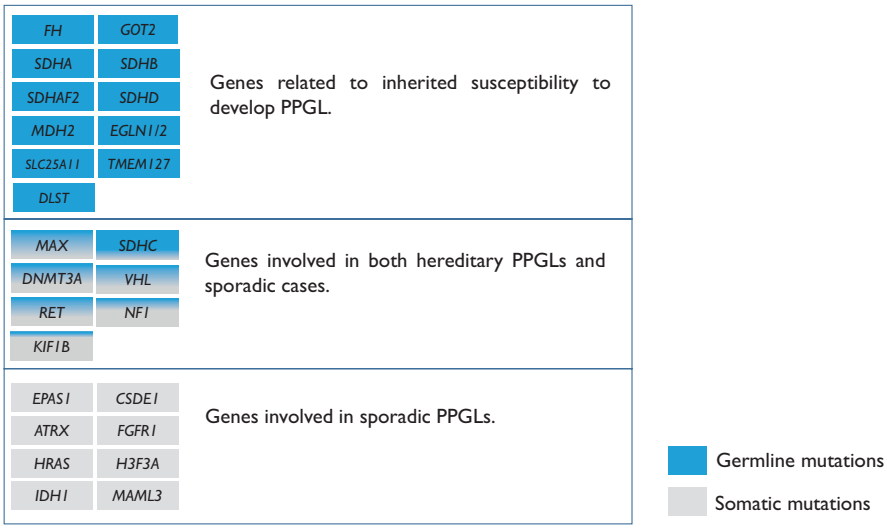

Fig. 5.1 PCC/PGL is a paradigm of genetic heterogeneity. Prior to the discovery by Baysal et al. in 2000 of the first case of PCC caused by one of the genes implicated in mitochondrial respiratory chain complex II, the proportion of cases that were hereditary was 10%, mainly associated with three syndromes, MEN2, VHL, and NF1. Since then, several additional genes have been identified, thereby increasing the proportion of hereditary cases to 40%. There remains a proportion of patients with a personal or family history of PCC/PGL in which no germline mutation has been found in one of the known susceptibility genes. Furthermore, somatic mutations mainly of *RET*, *VHL*, *NF1*, *MAX, EPAS1*, *HRAS,* and *FGFR1* can be detected in an additional 30–35% of the tumors. Overall, the proportion of cases harboring either a germline or a somatic mutation reaches 75%. The proportion of gray indicates the described percentage of somatic mutations for each gene.

are presented in Table 5.2. Patients diagnosed with *neurofibromatosis type I* (NF1) can also develop PCC but do so less frequently. PGLs present almost exclusively as part of familial PCC/PGL.

The proportion of pediatric patients with germline mutations in one of the known susceptibility genes is higher than that found in adults. It has been reported that up to 70–80% of children with PCC are mutation carriers, regardless of their family history [7, 26]. An as-yet unknown proportion of patients with clinical characteristics indicative of hereditary disease (bilateral PCC, multiple PGLs, family history, and/or early-onset disease) does not carry mutations in any of the known genes, suggesting that other loci remain to be discovered (Fig. 5.1); their identification will likely add additional complexity to the genetics underlying the pathogenesis of this disease. This is exemplified by the discovery of post-zygotic somatic alterations in the *EPAS1* (*HIF2A*) gene in patients with multiple PCCs/PGLs, particularly those diagnosed during adolescence [27, 28]. Another example is the case of post-zygotic somatic mutations in the *H3F3A* gene [29], although the prevalence of mutations in this gene has not been addressed so far.

Table 5.2 Genetic and clinical characteristics of genes associated with syndromes related to PCC/PGL development

Gene	Inheritance	Locus	Related syndrome Associated tumors/features
RET	Autosomal dominant	10q11.2	MTC, PHPT, PCC, infrequently PGL
VHL	Autosomal dominant	3p25–26	HB (SNC and retina), ccRCC, neuroendocrine pancreatic tumors, pancreatic cystadenoma, renal cysts, endolymphatic sac tumors, PCC, PGL, etc.
SDHD	Autosomal dominant with maternal imprinting	11q23	Carney-Stratakis syndrome, PGL1, renal cell carcinoma, GIST, pituitary adenoma
SDHC	Autosomal dominant	1q21	Carney-Stratakis syndrome, PGL3, ccRCC, GIST, pituitary adenoma
SDHB	Autosomal dominant	1p35–36.1	Carney-Stratakis syndrome, PGL4, ccRCC, GIST, pituitary adenoma
NF1	Autosomal dominant	17q11.2	von Recklinghausen's disease (0.1–5.7% present PPGL, 3.3–13% based on autopsy studies); Café au lait spots, neurofibromas, axillary and inguinal freckling, Lisch nodules (iris hamartomas), bony abnormalities, optic/CNS gliomas, malignant peripheral nerve sheath tumors, macrocephaly, and cognitive defects
MEN1	Autosomal dominant	11q13	MEN1 syndrome: <1% present PCC Primary hyperparathyroidism, pituitary adenoma, gastroenteropancreatic NET, adrenal cortical tumors, carcinoid tumors, facial angiofibromas, collagenomas, and lipomas
SDHAF2	Autosomal dominant with maternal imprinting	11q13.1	PGL2; H&N PGL> > PCC
TMEM127	Autosomal dominant	2q11.2	PCC, infrequently PGL (head and neck), ccRCC
SDHA	Autosomal dominant	5p15	PGL5, Leigh syndrome (homozygous patients, but no PPGL described), ccRCC, GIST, and pituitary adenoma
MAX	Autosomal dominant by paternal transmission	14q23	PCC (single, bilateral, multiple), up to 20% of patients also develop PGL (thoracic and abdominal), pituitary adenomas
FH	Autosomal dominant	1q43	Reed syndrome or hereditary leiomyomatosis and renal cell cancer (HLRCC), multiple cutaneous and uterine leiomyomatosis (MCUL), cutaneous and uterine leiomyomas, and type 2 papillary renal carcinoma
MDH2	Autosomal dominant	7q11.23	Early-onset severe encephalopathy (homozygous patients, but no PPGL described)

(continued)

Table 5.2 (continued)

Gene	Inheritance	Locus	Related syndrome Associated tumors/features
EGLN1/ PHD2	ND	1q42.1	Hereditary polycythemia, polycythemia
EGLN2/ PHD1	ND	19q13.2	Hereditary polycythemia, polycythemia
EPAS1/ HIF2A	Somatic/ somatic mosaic	2p21	Familial erythrocytosis type 4, Pacak-Zhuang, polycythemia, and somatostatinoma
KIF1B	Autosomal dominant	1p36.22	PCC, neuroblastoma (?), ganglioneuroma (?), leiomyosarcoma (?), lung adenocarcinoma (?), colorectal carcinoma (?)
SLC25A11	Autosomal dominant	17p13.2	PGL6; PGL, PCC
GOT2	Autosomal dominant	16q21	PGL, PCC
DNMT3A	Autosomal dominant	2p23.3	Gain of function mutations: H&N PGL
DLST	Autosomal dominant	14q24.3	PGL7; PGL (multiple) > > PCC

(?) – the association is not clearly demonstated
PCC pheochromocytoma, *PGL* paraganglioma, *H&N* head and neck, *MTC* medullary thyroid carcinoma, *PHPT* primary hyperparathyroidism, *GIST* gastrointestinal stromal tumor, *ccRCC* clear cell renal cell carcinoma, *HB* hemangioblastoma, *ND* no data, although but presumably autosomal dominant

5.7 Syndromic PCC

Some patients develop PCC or PGL as part of a hereditary tumor syndrome; they present with other clinical signs that can point to the gene in which defects are most likely to be involved and therefore help prioritize genetic testing. Such patients have often developed other neoplasms or have a family history indicative of a strong genetic etiology, as is the case for PCC associated with MEN2, VHL or, NF1 and, to a lesser extent, other syndromes such as Carney triad, Carney-Stratakis syndrome, and MEN1. Patients with germline mutations in *RET* more often have been previously diagnosed with medullary thyroid carcinoma (MTC), while those from NF1 families show *cafe au lait spots*. As described in detail below, one exception to this tendency to have particular comorbidities are patients with particular germline mutations in *VHL*, who tend to develop PCC as the sole manifestation of their disease.

5.7.1 MEN2-Associated PCC

MEN2 (OMIM 171400) has an estimated annual incidence of 0.5×10^{-6} and a prevalence of 1 in 30,000. MEN2 follows an autosomal dominant mode of inheritance; causal mutations have variable clinical expression and a penetrance that depends on their transformative capacity. MEN2 patients can develop MTC, PCC, and/or PHPT, the latter resulting from hyperplasia or from parathyroid adenomas. This syndrome is classified into three subtypes: MEN2a, MEN2b, and MTC familiar (MTCf), each defined according to the combination of pathologies developed by the individuals affected. MEN2a patients may develop all three pathologies. In addition, they are more likely to develop a disorder known as "cutaneous lichen amyloidosis," a pruritic skin lesion in the upper area of the back caused by the uncontrolled deposition of amyloid protein between the dermis and epidermis. Rarely they may also develop Hirschsprung disease (HSCR). Patients are classified as MEN2b if they develop, in the absence of parathyroid disease; MTC; PCC; multiple mucocutaneous neuromas involving the lips, tongue, and eyelids; corneal nerve myelination; intestinal ganglioneuromas (*hyperganglionic megacolon*); and *Marfanoid habitus*, including skeletal deformities and hypermobility of joints. Finally, families in which an affected member has developed exclusively MTC or C-cell hyperplasia (CCH) are considered to have the third subtype, MTCf, but only if more than ten members have MTC. An exhaustive clinical follow-up of these families is required to rule out the presence of other tumors characteristic of MEN2, especially in older family members.

Susceptibility to develop MEN2 is caused by germline mutations in the proto-oncogene *RET*. *RET* spans 55 kilobases, includes 21 exons, and encodes a tyrosine kinase receptor that is mainly expressed in cells derived from the neural crest (C cells, parafollicular thyroid cells, and adrenal medulla cells, among others) and in urogenital system precursor cells [30]. Despite its medium size, the genetic testing of *RET* is relatively simple, since the mutations associated with the development of MEN2 affect only a small number of amino acids located on specific exons. Mutations on exons 5, 8, 10, 11, 13, and 14 are related to MEN2a and MTCf, while those on exons 15 and 16 are found in MEN2b patients. The established genotype-phenotype relationships for MEN2 syndrome are based on the classification of individual mutations according to their transforming ability and therefore the expected associated aggressiveness [31]. The impact of *RET* mutation testing on the management of MEN2 patients is without doubt one of the most robust examples of the utility of genetic diagnosis in personalizing clinical follow-up.

Approximately 50% of MEN2 patients develop PCC in their lifetime, and the mean age at diagnosis is 35 years. *RET* mutations are very rarely found in cases diagnosed before age 20 [7, 14, 26], and so *RET* is not a priority in the genetic testing of pediatric patients, although it should still be included in genetic diagnosis algorithms [26]. Between 50% and 80% of tumors are bilateral; they tend to show an adrenergic biochemical phenotype, and a low proportion of tumors are metastatic. A PCC is the first manifestation of MEN2 in only 12–15% of cases, and so

RET explains relatively few cases of non-syndromic disease (around 5%), compared to other syndromes [32] (see Reference [8] for a review of *RET* and MEN2). A recent study by the COMETE consortium reported the presence of somatic mutations in *RET* in a substantial proportion (14%) of sporadic PCC [33]; this finding highlights the importance of working with germline and tumor DNA from the same patients in order to provide a comprehensive genetic diagnosis.

The identification by whole-exome sequencing (WES) of two or more deleterious *RET* mutations in the same patient [34] raises questions regarding their capacity to jointly influence phenotype in MEN2 families. It is likely that the availability of data from large-scale sequencing studies will also shed light on the role of single-nucleotide polymorphisms (SNPs) in modifying phenotype. There are conflicting results from studies focused on the role of SNPs in the development or progression of MTC or PCC in MEN2A patients. Recent studies suggest that p.G691E, or a combination of SNPs, may affect the development of PCC in MEN2A patients [35]. These findings should be confirmed in sufficiently informative families where the co-segregation of these SNPs with the development of PCC is analyzed.

The American Thyroid Association guidelines for PCC surveillance in patients with MEN2 syndromes recommend screening high-risk and moderate-risk patients starting at the age of 11 and 16 years, respectively [36].

5.7.2 VHL

VHL (OMIM 193300) is a hereditary tumor syndrome with a prevalence of 1 in 36,000 and variable clinical manifestation. The penetrance of causal mutations is age-dependent. Affected patients are at higher risk of developing hemangioblastomas (HBs) of the retina and central nervous system (CNS), PCC and/or PGL, clear cell renal cell carcinoma (ccRCC), renal and pancreatic cysts (serous cystadenoma), neuroendocrine pancreas tumors, endolymphatic sac tumors, pancreatic serous cystadenomas, and papillary cystadenomas of the epididymis in men and of the broad ligament in women (Table 5.2) [2, 37, 38].

The diagnosis of VHL is based primarily on the following clinical criteria: patients with a family history and at least one HB of the retina or CNS, PCC, or ccRCC; patients with no family history and at least two HBs or one HB of the CNS; and a visceral injury (other than renal or epididymal cysts, which are both common in the general population). A classification of the disease, including practical information for screening and genetic counseling, has been established and is widely accepted. VHL type 1 families have a low risk of developing PCC but may present with any of the other tumors associated with the disease. Type 2 families develop PCC and HBs and are sub-classified according to the associated low (type 2A) or high (type2B) risk of ccRCC. Finally, type 2C families have PCC as the only clinical sign of the disease.

5.7.2.1 The *VHL* Gene, its Protein (pVHL), and Tumorigenesis

VHL is caused by mutations in *VHL*, a tumor-suppressor gene that has three exons and encodes three gene products: a protein comprising 213 amino acids and two shorter isoforms, one produced by alternative splicing (excluding exon 2) and the other by alternative initiation. While the protein (pVHL) is involved in multiple processes, its best characterized role is in the regulation of the proteasomal degradation of hypoxia-inducible factors (HIFs) [39]. Under normal circumstances, the HIFs mediate the response to hypoxia, augmenting glucose uptake and increasing the expression of angiogenic, metabolic, and growth factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGFB), transforming growth factor (TGF), and erythropoietin (EPO) [39]. Inactivation of pVHL leads to the stabilization of HIF-1α and HIF-2α and therefore to the activation of genes whose transcription depends on these HIFs; this explains the highly vascularized nature of the tumors associated with VHL syndrome [37].

5.7.2.2 PCCs/PGLs Associated with VHL

Approximately 20% of patients with VHL develop PCC or PGL (sympathetic and parasympathetic), although the latter is much less frequent. Tumors show noradrenergic biochemical phenotype, are multifocal or bilateral in 43–45% of cases, and are metastatic in less than 5% [40, 41]. The median age at diagnosis of PCC/PGL is 29 years, which is lower than for other syndromes and particularly relevant for genetic testing since between 12% and 32% of patients with PCC diagnosed during childhood are found to carry a germline mutation in *VHL*. Of note is that PCC (principally) and PGL (occasionally) are the first manifestation for 30–50% of patients with VHL [40]. For these reasons, *VHL* mutation screening is essential in patients diagnosed before age 18. Furthermore, *VHL* has a high mutation rate (20–21%) [42, 43], and so mutation testing of this gene is recommended specifically for apparently sporadic and non-syndromic cases.

The development of VHL-related tumors has been linked to the alteration of specific interactions between pVHL and other proteins with which it forms complexes. The most accepted hypothesis in this regard is that the development of PCC in the context of VHL is associated with a partial retention in the function of pVHL [44]. A hot-spot in *VHL* that is associated with the development of PCC affects residue 167, located in the alpha domain. This domain has the role of interacting with other proteins, so that mutations giving rise to amino acid changes in this region do not result in the loss of function of pVHL. The finding that 23% (7/30) of patients with PCC who carry deleterious germline variants in *VHL*, but have no signs of either VHL or MEN2, have a mutation that affects this residue is consistent with this hypothesis [45].

On the basis of the above findings, it has been proposed that the measurement of the change in pVHL stability could be used as an additional tool to understand the clinical features developed by a VHL patient [44]. Indeed, the use of this tool led to

the identification of an association between ccRCC and missense mutations that significantly alter pVHL stability. A subsequent study classified these mutations as "surface" or "deep," depending on the location of the affected residue in the protein structure, and found a clear difference between them in the associated risk of PCC [38].

Based on the earliest described age at diagnosis, it is recommended that screening be initiated at age 5 years [2]. For more information regarding the genetics of VHL we recommend consultation with the international consensus [46].

5.7.3 Neurofibromatosis Type 1

Neurofibromatosis type 1 (NF1), formerly known as von Recklinghausen disease, is a common hereditary disease with an incidence of 1 per 2500–3300 newborns. It is normally diagnosed in children and is characterized by the appearance of multiple neurofibromas; cafe au lait spots; freckling in the armpits and groin; iris hamartomas (Lisch nodules); bone lesions such as scoliosis, sphenoid dysplasia, or pseudoarthrosis; macrocephaly; learning disorders; cognitive deficits; predisposition to optic and CNS glioma; and leukemia [2, 47]. Although it has been established that NF1 has an autosomal dominant inheritance, close to 50% of patients have de novo mutations, which if they occur post-zygotically can give rise to mosaic phenotypes [48].

5.7.3.1 The *NF1* Gene and its Protein

The gene responsible for NF1, *NF1* (17q11.2), which acts as a tumor suppressor, comprises 60 exons and has one of the highest rates of spontaneous mutation of any gene in the human genome. It encodes the protein neurofibromin, which is expressed primarily in the nervous system and has the role of suppressing cell proliferation by inactivating RAS proteins. Loss-of-function mutations in *NF1* lead to the activation of RAS and the PI3K/AKT/mTOR pathway, which depends on RAS [47].

5.7.3.2 PCCs Associated with NF1

An estimated 0.1–5.7% of NF1 patients develop PCC, although this figure is 3.3–13% based on autopsy studies. NF1-associated PCCs tend to develop at a later age (mean 41 years), can be unilateral or bilateral, are rarely extra-adrenal, and slightly more often metastatic (up to 10%) than those in VHL and MEN2 cases. Recent findings have demonstrated that *NF1* is responsible for a substantial portion of sporadic PCC, with 14–20% of apparently sporadic tumors presenting with somatic mutations in the gene [49–51]. This finding once more highlights the need to study in parallel both normal and tumor tissues from the same patient in order to

carry out a comprehensive genetic diagnosis that is informative for genetic counseling. The prevalence of *NF1* alterations in pediatric PCC is low, and the syndrome is relatively easily identified based on its associated clinical features.

The earliest recorded age at diagnosis of PCC is 7 years, but given the low penetrance of *NF1* mutations, screening is only recommended in cases of hypertension or symptoms suggestive of disease [2]. Other groups have proposed biochemical screening every 3 years starting at age 10 years [52].

5.8 Non-syndromic PCC/PGL

Here we review the genes related to susceptibility to develop PCC or PGL as the only manifestation of the disease. Associations with other tumors have been reported but only in a limited number of patients. We will outline the functions of the SHD and *FH* genes, as well as *TMEM127* and *MAX*, and detail the clinical manifestations associated with mutations in each of these.

5.8.1 Non-syndromic PCC/PGL Associated with Mutations in the SDH *and* FH *Genes*

5.8.1.1 SDH Gene Function

The connection between the SDH genes and the development of neuroendocrine tumors was established in 2000 when germline mutations in *SDHD* were first described in patients with familial PGL [53]. The SDH genes encode Complex II subunits of the mitochondrial respiratory chain, or succinate dehydrogenase (SDH), which plays a key role in both the electron transport chain and the *tricarboxylic* acid cycle. This complex is made up of four subunits: two catalytic (SDHA and SDHB) and two structural (SDHC and SDHD). Heterozygous mutations in the *SDHA*, *SDHB*, *SDHC*, and *SDHD* genes adversely affect the ability of the complex to detect oxygen and cause pseudo-hypoxia, which activates the angiogenic pathway mediated by HIF-1α and VEGF (reviewed in [25]). An additional gene, *SDHAF2*, is also involved in mitochondrial Complex II and in the development of PCC/PGL, confirming the importance of this complex for the disease [54]. SDHAF2 controls the flavination of SDHA, which is critical for the correct functioning of Complex II. The accumulation of succinate caused by mutations in the SDH genes likely causes oncogenesis via the inhibition of prolyl hydroxylases, which are required for the regulation of HIF-1α, mediated by pVHL [55]. This link between mutations in the SDH genes and the HIF-1α pathway is also corroborated by results from tumor expression profiling studies [56]. Mutations in any of the SDH genes, both catalytic and structural, cause defects in the enzymatic activity of the complex, along with the

absence of the protein SDHB [57]; this represents a great advantage in the selection of patients for genetic testing, since if paraffin-embedded tumor material is available, SDHB expression can be determined via immunohistochemistry, and its absence used to indicate the likely involvement of these genes in disease etiology.

5.8.1.2 Mutations in the SHD Genes: Genotype-Phenotype Relationship

Clinical Presentation Associated with Mutations in *SDHD*

The estimated penetrance of germline mutations in *SDHD* (11q23.1) is 86% to age 50 years. Mutations predispose carriers primarily to the development of PGL (84% of cases), although up to 22% also develop thoracic and abdominal PGL and 12–24% PCC, the latter rarely being bilateral [24, 58, 59] (Table 5.2). *SDHD* mutation carriers normally present with multiple PGLs at a mean age of 35 years. While it has been established that *SDHD* defects have an autosomal dominant mode of inheritance, the gene is also subject to maternal imprinting. That is, mutation carriers will only develop the disease if their mutation came from their father; if it came from their mother, they will not be affected, although they will still be able to pass on the mutation to their children (with a probability of 50% for each gestation). This means that the hereditary nature of disease is complete masked in families in which by chance the mutation has been transmitted from generation to generation only from mother to child. In these cases, the disease skips generations, and these can only be identified in genetic counseling centers that collect information from second- and third-degree relatives. Nevertheless, there are reports of families in which the disease has developed in individuals with a germline-mutated maternal chromosome [60, 61]. It has been suggested that the molecular mechanism explaining this involves a loss of the imprinting of the maternal allele, although the probability of this occurring is very low. A key issue in clinical follow-up is the fact that 43.2% of non-proband carriers of a germline mutation in *SDHD* develop malignancies [62]. In the case of pediatric patients, despite the possible lack of family history due to imprinting, it has been suggested that a diagnosis of at least one head or neck PGL is sufficient to justify genetic testing; in fact, 8–16% of patients under age 20 years carries a germline alteration in *SDHD* [7, 26].

In relation to the development of other tumors, it should be noted that there has been some controversy around two variants in *SDHD*, p.H50R and p.G12S. Both were initially reported to be associated with the development of Merkel cell carcinoma and familial CCH and even Cowden-like syndrome. However, they were subsequently classified as SNPs, present in several healthy populations (http://www.lovd.nl/3.0/home), and their associations with the proposed diseases have therefore been ruled out [63]. Screening was recommended starting at 10 years of age for patients carrying a mutation in *SDHD* [64].

Clinical Presentation Associated with Mutations in *SDHB*

An estimated 67% of patients carrying mutations in *SDHB* (1p36.13) develop primarily thoracic and abdominal PGL, 27% develop head and neck PGL, and 17–29% adrenal PCC, which is rarely bilateral [24, 57, 58]. It has been shown that clinical disease penetrance in non-proband *SDHB* mutation carriers is 16%, 22%, and 44% at 50, 60, and 80 years, respectively [62].

Of all the known susceptibility genes for hereditary PCC/PGL, *SDHB* constitutes a paradigm of heterogeneity in and of itself. Mutations in this gene are usually associated with the presence at diagnosis of a single retroperitoneal tumor [65]; this differentiates it from other susceptibility genes that often give rise to bilateral or multiple tumors. However, 23–70% of these single *SDHB* mutation-linked tumors metastasize [6, 24, 66], meaning that it is widely accepted that the identification of a mutation in *SDHB* is a marker of poor prognosis and the need to clinically monitor the patient more closely. An additional issue contributing to the complexity in managing these patients is that while *SDHB* is one of the main genes responsible for pediatric cases of the disease [25, 26, 58], as mentioned above the average penetrance to age 80 years of mutations is only 44% [62]. That is, most mutation carriers never develop PCC/PGL; furthermore, most of those that do have no family history of disease at the time of their diagnosis. This fact, along with the frequent appearance of a single tumor in affected individuals, makes it very difficult to identify potentially hereditary cases. For all these reasons, and principally because of the high associated malignant potential of resulting tumors, all the current algorithms used to guide genetic diagnoses include testing of *SDHB* in patients with PCC/PGL.

In a study of 64 pediatric PCC/PGL patients with *SDHB* germline mutations, most of the patients (78.13%) presented with extra-adrenal sympathetic tumors, and median size of the primary tumor was 5.7 cm. Metastases developed in 70% of patients at a median age of 16 years and were t diagnosed either in the first 2 years or in years 12–18 post-diagnosis. Around 19% of pediatric patients with *SDHB* mutation-related PCC/PGL presented with metastatic disease at the initial diagnosis, which warrants whole body studies to be performed at initial imaging evaluation. Thorough follow-up is crucial in the first 2 years post-diagnosis, and more frequent follow-ups are needed in years 10–20 post-diagnosis due to the increased risk of metastases. Most common site of metastases were bones, followed by the retroperitoneum and lungs. The estimated 5-, 10-, and 20-year survival rates were 100%, 97.14%, and 77.71%, respectively. Although this age group developed metastasis as early as 5 years from diagnosis, the overall 20-year prognosis and survival are good [6].

Carrying a mutation in *SDHB* has been associated with an increased risk of developing ccRCC, 4.7% at 60 years [62, 67], and so it is generally recommended not only that mutation carriers be screened for this disease but that mutation carriers with ccRCC be clinically worked up to rule out the existence of PGL.

Although the earliest reported age at diagnosis is 6 years, screening is recommended from age 5 years with initial work-up focusing on abdominal region [2]. If abdominal imaging is negative, evaluation of pelvic, chest, and head and neck

regions needs to follow. Abdominal MRI is recommended every 18 months with MRI of the neck, thorax, and abdomen and pelvis every 3 years. Currently, ^{68}Ga-DOTATATE PET/CT shows the highest per-lesion detection rate (93.5%) of primary and metastatic lesions compared to ^{18}F-FDG PET/CT and CT/ MRI scans. However, the use of ^{68}Ga-DOTATATE PET/CT seems to be limited to non-abdominal, especially bone, lesions probably due to reduced expression of SSTR2 in abdominal PCC/PGL [6].

Clinical Presentation Associated with Mutations in *SDHC*

Since relatively few mutations in *SDHC* (1q23.3) have been described worldwide, the associated clinical manifestations have not been clearly defined; nevertheless, it is known that mutation carriers tend to develop PGL (93% parasympathetic and 7% sympathetic) and infrequently adrenal PCC or GIST. Tumors are generally benign, although it has also identified metastatic extra-adrenal PGLs [68]. Seventeen percent of affected individuals have multiple PGLs, and 25% have a family history, suggesting that mutations have incomplete penetrance [23–25]. In fact, it has been shown that the estimated risk for *SDHC* non-probands carriers is 25% at 60 years of age [62]. While very little is known about the involvement of this gene in pediatric disease, the mean age at diagnosis of 43 years suggests that its genetic testing might not be a priority in cases with no family history of PGL [69].

Screening was recommended starting at 10 years of age for patients carrying a mutation in *SDHC* [64].

Clinical Presentation Associated with Mutations in *SDHA*

Based on the currently available information, *SDHA* (5p15.33) appears to account for approximately 3% of PCC/PGL [57]. These carriers had developed PCC, head and neck PGL, or thoracic and abdominal PGL (Table 5.2). One of the peculiarities of *SDHA* is that the mutations described to date have been reported to have low frequencies in unaffected population controls; this finding indicates that these mutations have incomplete penetrance and adds additional complexity to the genetic counseling offered to carriers. Nevertheless, *SDHA* should be considered in genetic testing for patients presenting with clinical evidence of familial PCC/PGL who test negative for the other known susceptibility genes. As previously mentioned, mutations in any of the SDH genes have the effect of suppressing the enzymatic activity of Complex II, and a key indicator that this has occurred is to detect negative immunostaining for SDHB. Furthermore, mutations in *SDHA* also give rise to negative immunostaining for SDHA [57]. This relatively easily implemented clinical screening tool should be incorporated into molecular diagnostic protocols to ensure that appropriate mutation testing is carried out in the most efficient and cost-effective manner. Screening was recommended starting at 10 years of age for patients carrying a mutation in *SDHA* [64].

Clinical Presentation Associated with Mutations in *SDHAF2*

The gene *SDHAF2* (11q12.2) is similar to *SDHD* in that it has an autosomal dominant mode of inheritance but is subject to maternal imprinting. To date, only head and neck PGLs have been reported in *SDHAF2* mutation carriers, most diagnosed at an early age and all with a family history of the disease ([54] and references contained therein). Available data suggest that mutations in *SDHAF2* do not explain a substantial portion of cases. Nevertheless, genetic testing of *SDHAF2* should be offered to patients with head and neck PGLs with negative tumor staining for SDHB and who test negative for mutations in *SDHD*, *SDHC*, and *SDHB*. Only two distinct *SDHAF2* mutations have been described in five independent families [54, 70–72]. While currently too few data are available to draw clear conclusions, none of the affected mutation carriers developed PGL before age 20 years, suggesting a priori that mutations are not relevant to the development of pediatric tumors.

FH: Clinical Presentation Associated with Mutations in *FH*

FH is the Krebs cycle enzyme involved in the reversible hydration/dehydration of fumarate to malate. It is known that germline mutations in *FH* (1q43) predispose to leiomyomas and ccRCC in an autosomal-dominant hereditary syndrome named hereditary leiomyomatosis and renal cell cancer (HLRCC) [73]. Loss-of-function mutations of *FH* lead to the accumulation of fumarate in the tumors which, like succinate, promotes the inhibition of the αKG-dependent dioxygenases [74]. In 2013, Letouze et al. [75] identified a germline mutation in *FH* by WES applied to blood and tumor DNA obtained from a 63-year-old female presenting with one PCC. The patient was selected to be sequenced because the tumor showed a methylome- and transcriptome-based profile very similar to that found in tumors carrying mutations in the SDH genes. The subsequent screening of almost 600 patients with PCC/PGLin whom no mutations in the major susceptibility genes had been found, revealed that five carried pathogenic germline *FH* mutations, providing further evidence of the involvement of this gene in the development of PCC/PGL [76]. Clinically, metastatic phenotype and multiple tumors were significantly more frequent in patients with *FH* mutations than those without such mutations. *FH* should thus be added to the list of PCC/PGL susceptibility genes and should be considered in mutation screening, to assess the risk of malignant disease.

5.8.2 *Non-syndromic PCC/PGL Associated with Mutations in* TMEM127

5.8.2.1 The TMEM127 Gene and its Protein

TMEM127 (2q11) was identified as a new PCC susceptibility gene in 2010, via an integrated analysis of results from studies using several genomic platforms, including linkage analysis, gene expression profiling, and mapping of chromosomal gains and losses [77]. Loss of heterozygosity (LOH) of the wild-type allele was observed in all available tumors from carriers of *TMEM127* mutations, suggesting that the gene acts as a classic tumor suppressor.

TMEM127 encodes a transmembrane protein with no known functional domains. Functional studies suggest that the protein (TMEM127) localizes to the plasma membrane and cytoplasm and is associated with a subpopulation of vesicular organelles, including the Golgi and lysosomes. TMEM127 is dynamically distributed at the subcellular level in response to nutrient signals [77]. It has also been demonstrated that TMEM127 modulates mTOR Complex 1 (mTORC1), which promotes cell growth and the translation of proteins and the phosphorylation of 4EBP1 and S6K. A detailed analysis of the global expression profile of *TMEM127*-mutated tumors grouped them with those associated with *RET* and *NF1* mutations.

5.8.2.2 Clinical Presentation Associated with Mutations in *TMEM127*

Few studies have been published to date based on patient series genetically tested for mutations in *TMEM127*. The most relevant of these reported the genetic findings in 990 patients with PCC or PGL who tested negative for mutations in *RET*, *VHL*, and *SDHD/B/C* [78]; 2% carried germline *TMEM127* mutations and presented with disease at a mean age of 43 years. Subsequent reports have described two mutation-carrying patients with PGL, one thoracic and abdominal and the other with multiple head and neck tumors. In addition, patients with renal carcinoma have been described, which would have an impact on surveillance and management of *TMEM127* mutation carriers [79, 80]. As for other susceptibility genes, the findings published to date suggest that mutations have incomplete penetrance, which would tend to mask the underlying hereditary disease and, in many cases, mean that patients may not meet the selection criteria for genetic testing. Given the mean age at disease onset for mutation carriers studied to date and the reported absence of bilateral disease and family history, genetic testing of *TMEM127* is not recommended in pediatric patients with PCC/PGL.

5.8.3 *Non-syndromic PCC/PGL Associated with Mutations in* MAX

5.8.3.1 The *MAX* Gene and its Protein

MAX (14q23.3) encodes a transcription factor that plays an important role in the regulation of cell proliferation, cell differentiation, and apoptosis, as part of the MYC/MAX/MXD1 axis. These proteins form dimers that bind to DNA; in fact, MYC forms a heterodimer with MAX to bind to specific DNA sequences called "E-boxes," which are located in MYC target genes, and this entire complex acts as a transcription activator. Both the lethal character demonstrated in *MAX*-knockout mice and the fact *MAX* is constitutively expressed in many cell types make it difficult to understand how MYC can carry out its function without the presence of MAX. However, the PC12 cell line, derived from PCC in rat, carries a homozygous *Max* mutation, which points to the existence of an additional unknown factor that is able to regulate the function of MYC (reviewed in [81]).

The identification of *MAX* as a PCC susceptibility gene was the result of a study of WES of three unrelated patients with PCC and a family history of the disease [81]. These patients had been selected because their tumors had a common transcription profile that differentiated them from tumors related to other known susceptibility genes [56]. LOH in the tumors of germline *MAX* mutation carriers, along with the absence of MAX protein shown by an immunohistochemical analysis, suggested that *MAX* acts as a tumor suppressor gene.

5.8.3.2 Clinical Presentation Associated with Mutations in MAX

Following the identification of pathogenic mutations in *MAX* in the three initial families, the genetic study was extended to 59 patients that had tested negative for the key known susceptibility genes. These 59 patients were chosen because they were diagnosed with PCC before age 30 years, had bilateral disease, or had a family history of the disease. *MAX* mutations were found in 8.5% of them; 67% of mutation carriers had bilateral disease, and 25% had developed metastases. One of the most striking findings was that the mutated allele had to have been inherited paternally in order for the carrier to develop the disease, as is the case for *SDHD* and *SDHAF2*, although the mechanism behind this remains unknown.

A subsequent study screened for mutations in *MAX* in a series of 1694 patients and 245 tumors in order to establish their prevalence and the associated clinical presentation. This study was made possible through the collaboration of 17 reference centers from around the world [82]. Pathogenic germline mutations were identified in 1.3% of patients; 21% of them had developed thoracic and abdominal PGL in addition to PCC, 37% had a family history of the disease, and 10% had metastases. The mean age of diagnosis for mutation carriers was 32 years, and 21% were diagnosed at or before age 18. These findings suggest that *MAX* should be included

along with *VHL* and *SDHB* in genetic testing protocols for pediatric cases [26]. This study also established that the frequency of somatic mutations is 1.65% and that the associated biochemical-secretor profile is characterized by elevated levels of normetanephrine and associated with normal or slightly increased levels of metanephrine [82]. Later, somatic *MAX* mutations have been found in many other cancers such as small cell lung cancer, GIST, multiple myeloma, and Wilms' tumors [83–86].

5.8.4 Non-syndromic PCC/PGL Associated with Mutations in Other Recently Identified Genes

During the last 5 years, several genes have been found to be involved in the hereditary predisposition to PCC/PGL, and so far, no mutations affecting these new genes have been found in pediatric cases. However, it is too soon to know which is their role in the pediatric presentation of PCC/PGL.

Although the first germline mutation found in one of the members of the Egl-9 family of hypoxia-inducible factors (EGLN) was reported in 2008 [87], recently two new variants have been reported in patients with PCC/PGL. Thus, a total of three germline mutations, two in *EGLN2* and one in *EGLN1* (also known as *PHD2* and *PHD1*, respectively), have been described in patients with PCC/PGL-polycythemia disorder [88]. Mutations in these genes cause substantial loss of protein stability of both PHD1 and PHD2, resulting in the upregulation of HIF-α target genes and therefore in the activation of hypoxic pathway.

MDH2 encodes malate dehydrogenase 2, which is essential for the reversible oxidation of malate to oxaloacetate in the TCA cycle. This tumor suppressor gene was first reported mutated, with an incomplete penetrance, in a single family with multiple malignant PGLs [89]. Later, the same mutation was found in another patient with malignant PCC, and additional pathological variants have been also reported accounting for <1% of the patients [90].

In 2018, germline mutations in the tumor suppressor gene *SLC25A11* were identified in seven unrelated patients, many of them with metastatic thoracic and abdominal PGLs. *SLC25A11*-mutated tumors showed a reduction of αKG levels with the pertinent accumulation of aspartate as a consequence of the malate-aspartate shuttle disruption. *SLC25A11* has been classified into the transcriptional Cluster 1A due to the SDHx-like molecular phenotype exhibited by the mutated tumors (i.e., pseudohypoxia and a CpG island methylator phenotype [CIMP] profile). *SLC25A11* gene mutations could account for 1% of all PCC/PGL [91]. Interestingly, a gain-of-function mutation in the *GOT2* gene, encoding the mitochondrial aspartate aminotransferase, was also reported in a PGL patient [92], further linking dysfunction of the malate-aspartate shuttle to PCC/PGL development.

Trio-based WES applied to the germline DNA of a selected patient strongly suspected of having hereditary PCC/PGL identified a single, de novo mutation in the

DNA methyltransferase 3A gene (DNMT3A) [93]. Genome-wide methylome analysis of *DNMT3A*-mutated tissues identified a characteristic CIMP profile as well as a significant hypermethylation of homeobox-containing genes, suggesting an activating role of the mutation. The extension of the study to a series of PCC/PGL patients and tumors revealed the presence of somatic sub-clonal mutations affecting the same residue in six additional tumors, all of them PGLs, and a second germline *DNMT3A* mutation (c.952C>T; p.Arg318Trp) in a patient with family history of PCC.

Finally, targeted sequencing of a panel of TCA cycle-related genes allowed the identification of germline variants affecting the dihydrolipoamide S-succinyltransferase (*DLST*) gene in seven unrelated patients [94]. A recurrent mutation (p.Gly374Glu) found in four unrelated patients with multiple PCCs/PGLs disrupted the TCA cycle triggering the accumulation of 2-hydroxyglutarate. In addition, p.Gly374Glu-*DLST* tumors exhibited LOH (by means of uniparental disomy), highly positive DLST immunostaining, as well as homogeneous expression and methylation profiles.

5.9 Sporadic PCC/PGL

5.9.1 PCC/PGL with Mutations in EPAS1

The HIF family of transcription factors (HIF-1α, HIF-2α [EPAS1], and HIF-3α) plays a key role in the regulation of hypoxia response to counteract the lack of oxygen in normal homeostasis. HIF-1α has been suggested to preferentially drive genes implicated in apoptosis and glycolysis, while HIF-2α is involved in cell proliferation and angiogenesis [95, 96]. A few years ago, a new and direct link between HIF proteins and PCC/PGL development has been found [27]; post-zygotic somatic mutations in *EPAS1* (2p21) were found in two unrelated patients with multiple PGLs, somatostatinomas, and polycythemia. The mutations were found in the residues located close to the prolyl hydroxylation site of the protein (proline 531) which was shown to disrupt the recognition of EPAS1 by the PHD family members, its hydroxylation, and the consequent degradation by VHL [97, 98]. Thus, mutations affecting the *EPAS1* gene stabilize the protein, causing the aforementioned pseudohypoxia, indicating that *EPAS1* behaves as an oncogene. A germline alteration affecting *EPAS1* was found in a patient with multiple PGLs and polycythemia. Although it has been demonstrated that the variant stabilizes the protein, its location outside the prolyl hydroxylation sites and the absence of segregation with the disease in the carrier's family make this result somewhat controversial [99].

5.9.2 *PCC/PGL with Mutations in* HRAS

The members of the RAS family of oncoproteins (e.g., *HRAS*, *NRAS*, and *KRAS*) are small GTP-binding proteins that affect multiple downstream pathways related to cell growth and homeostasis. They were first linked to cancer in 1982 [100], and nowadays it is known that together they represent around 30% of the total oncogenic activating mutations distributed across many different cancers [100, 101]. Mutations in *KRAS* appear in 21.6% of human cancers, *NRAS* is mutated in 8.0% of tumors, and *HRAS* mutations are found in 3.3% of cancers (www.sanger.ac.uk/genetics/CGP/cosmic/) [102]. A mutation affecting *HRAS* (11p15.5) was first described in one PCC by Yoshimoto et al. [103]. Crona et al. [104] applied WES to 58 PCCs and found that four harbored somatic mutations in the gene. The subsequent study of a large series of tumors determined that 10% of sporadic PCCs have mutations in *HRAS* and ruled out the involvement of *NRAS* and *KRAS* in the disease [105]. The presence of mutations in one of the isoforms of RAS is not a new issue in the development of endocrine tumors since they are present in around 10–20% of follicular cell-derived thyroid cancers and in 18% of *RET*-negative sporadic MTCs [106–108]. The pivotal role of RAS genes in the PIK3CA-AKT1-mTOR pathway explains why they group within the so-called transcriptional Cluster 2 [109].

5.9.3 *PCC/PGL with Mutations in* ATRX

The presence of somatic loss-of-function mutations in *ATRX* (alpha thalassemia/mental retardation syndrome X-linked) in PCCs/PGLs was first described in 2015 mostly coexisting with SDHx mutations (and therefore associated with Cluster 1A) [110]. However, there are also cases without any further driver mutation which are related to the transcriptional Cluster 3, which includes Wnt signaling-related tumors [109, 111]. Mutations in *ATRX* have been associated with alternative lengthening of telomeres and clinically aggressive behavior, and a recent study suggests that they are independent risk factors for metastatic PCC/PGL [112].

5.9.4 *PCC/PGL with Rearrangements Affecting* MAML3

The Cancer Genome Atlas (TCGA) project revealed PCCs/PGLs carrying somatic gene fusions affecting the *MAML3* (mastermind-like transcriptional coactivator 3) transcription factor gene, with increased transcription levels of a chimeric MAML3 [109]. One of the fusions observed in PCCs/PGLs, UBTF-MAML3, leads to the activation of the Wnt target expression and Hedgehog signaling pathway, something already detected in neuroblastoma, a tumor with a similar developmental origin to

PCCs/PGLs. Another important finding of this study is that the presence of MAML mutations in PCCs correlates with poor clinical outcome.

5.9.5 Other Somatic Mutations Observed in PCC/PGL

Somatic mutations in the *IDH1* gene, frequently found in central nervous system tumors [113], have been also identified in PCCs/PGLs leading to a neomorphic production of D-2HG that finally causes the characteristic CIMP profile. However, they are low-frequent events in PCC/PGL (<1%) [92, 109, 114]. Very recently, a single HN-PGL carrying a somatic mutation in IDH2 has also been reported [115].

In the TCGA study, somatic loss-of-function mutations in *CSDE1* (cold shock domain containing E1) were also reported. *CSDE1* encodes an RNA-binding protein not hitherto associated with cancers [109].

Postzygotic mosaic mutations in *H3F3A* (H3 histone family member 3A) cause PCC/PGL together with giant cell tumor of the bone and lead to the upregulation of MYCN [116]. *H3F3A*-mutated PCCs/PGLs have been proposed to be part of Cluster 2, although due to their function they may fit better into Cluster 1. Other chromatin-remodeling genes found mutated in PCCs/PGLs are *EZH2*, *HIST1H1T*, *HIST4H4*, *JMJD1C*, *KDM2B*, *KMT2B*, or *SETD282*.

5.9.5.1 Treatment

Surgery is the treatment of choice for both PCC and sympathetic PGL. For PCC, laparoscopic intervention has a lower associated morbidity and mortality than open procedures. Laparoscopic option is not contraindicated for large, multiple, bilateral, malignant, or recurrent tumors, and the final decision regarding the surgical approach usually depends on the experience of the surgeon. An alternative for bilateral complete adrenalectomy in hereditary PCC is bilateral partial adrenalectomy (also known as subtotal, function-preserving adrenalectomy or adrenal sparing surgery). This alternative should be raised with the patient, considering their advantages (avoid the adrenal insufficiency and other morbidities associated with long-term corticotherapy) and disadvantages (greater risk of recurrence and remaining possibility of adrenal insufficiency). Their use in cases of sporadic unilateral PCC in patients with previous damage of the contralateral adrenal gland remains controversial [3, 13].

Patients with head and neck PGL are generally differentiated into (1) those with asymptomatic small-medium-sized tumors for which ongoing observation is usually indicated and (2) those with large, symptomatic, or fast-growing tumors, for which both surgery and radiotherapy/radiosurgery are viable options. The therapeutic decision depends on the center, the patient, and the possible side effects predicted [117, 118].

The therapeutic options for metastatic cases are limited and rarely curative. Surgery has not shown to improve survival, but it can reduce the size of the mass that produces catecholamines or that produces local compression and can be used as an adjuvant treatment to radiotherapy or chemotherapy. In cases where surgical resection is not viable, who are positive on 123I-MIBG scintigraphy and have slowly growing metastatic lesions, radiotherapy with *^{131}I is recommended but with the goal of maintaining disease stability and less for regression or disease cure.* A new preparation of ^{131}I-MIBG, produced on the Ultratrace® platform, may increase tumor uptake and treatment efficacy, but the spectrum of side effects on the Ultratrace® platform has yet to be presented.

If the ^{123}I-MIBG scintigraphy is negative and/or the tumor is fast-growing, chemotherapy (cyclophosphamide, vincristine, dacarbazine CVD) alone or, in combination with radiotherapy, is an option in malignant disease in both pediatric and adult cases [119].

Our improved understanding of the molecular biology of these tumors has helped to broaden the therapeutic options. Anecdotal reports have suggested several other treatment approaches, a few of which deserve further evaluation:

(a) Pro-apoptotic: somatostatin analogues, histone deacetylase (HDAC) inhibitors, eicosapentaenoic acid, triptolide/capsaicin, gamitrinib, and camptothecin.
(b) Anti-proliferative: everolimus (mTOR1 inhibitor), AEZS-131 (ERK inhibitor), AZD-8055 (mTOR1 and mTOR2 inhibitor), sunitinib and other tyrosine kinase inhibitors (pazopanib, axitinib, and cabozantinib), LB1 (inhibitor of serine/threonine protein phosphatase 2A) combined with temozolomide, and inhibitors of carboxypeptidase E [119].
(c) DNA methyl transferase inhibitors: guadecitabine.
(d) Checkpoint inhibitors.
(e) Topoisomerase or PARP inhibitors.
(f) Glutaminase inhibitors [12].

Other treatment options include radiotherapy to alleviate pain or symptoms due to local compression (especially for bone metastases) and local treatment with cryoablation, radiofrequency ablation, radionuclides, and/or embolization [9, 13]. Because of the lack of curative treatments, most pediatric patients are treated only if they present with reduced quality of life [16].

5.9.5.2 Perioperative Clinical Management

For all patients with elevated norepinephrine or metanephrine, the present-day recommendation is to offer preoperative pharmaceutical "blockade" regardless of symptoms for at least 7–10 days before proceeding with the surgery.

Appropriate clinical management of patients, including those with non-functional disease, is essential to prevent intraoperative hypertensive crises and minimize the adverse effects of the anesthesia and tumor manipulation (Table 5.3) [13, 120]. Randomized controlled trials have not been conducted, and therefore no consensus

Table 5.3 Preoperative management

Condition	Treatment	Characteristics
Hypertension	α-Adrenergic blockers	
	– Phenoxybenzamine 0.2 mg/kg/day (max. 10 mg/dose) Increase by 0.2 mg/kg/day every 4 days to goal 0.4–1.2 mg/kg/day divided every 6–8 h (max. 2–4 mg/kg/day)	– α1- and α2-adrenergic blocker that is irreversible, non-selective, non-competitive, and long-acting – Side effects: Nasal stuffiness, fatigue, dizziness, reflex tachycardia, retrograde ejaculation, orthostasis and hypotension (up to 36 hours following surgery) – Contraindicated in cardiopulmonary disease
	– Prazosin, terazosin, doxazosin. 1–2 mg/day Increased to 4–16 mg, daily or divided two times daily	– α1-adrenergic blocker that is reversible, selective, competitive, and short-acting – Treatment of choice for cases requiring long-term treatments – Higher risk of intraoperative complications: The binding to the receptor may be lost if an abundant amount of catecholamines is released – Lower risk of postoperative hypotension, tachycardia, and side effects
	Calcium channel blockers	
	– Dihydropyridines: Slow-releasing nifedipine and nicardipine – Non-dihydropyridines: Diltiazem	– Used to supplement α-adrenergic blockers if blood pressure remains uncontrolled or as a second-line treatment if side effects are not tolerated
	Competitive inhibitor of *tyrosine hydroxylase*	
	Metyrosine 20 mg/kg/day, divided every 6 h or 125 mg daily Increase up to 60 mg/kg/day divided every 6 h or increase by 125 mg every 4–5 days to max. 2.5 g/day	– Normally used only when other treatments have been ineffective or poorly tolerated or in cases of metastatic or inoperable disease, cardiopulmonary disease or where substantial tumor manipulation is foreseen – Side effects: Sedation, diarrhea, extrapyramidal signs, nightmares, depression, urolithiasis, and galactorrhea
Hypertensive crisis	– Short-acting α-adrenergic blockers such as intravenous phentolamine. Not recommended where there is risk of *cardiogenic shock* – Vasodilators such as nitroprusside, nicardipine, fenoldopam, or nitroglycerin – Magnesium sulfate, typically as a second-line treatment	

(continued)

Table 5.3 (continued)

Condition	Treatment	Characteristics
Angina, arrhythmia, or reflex tachycardia	Cardioselective β-adrenergic blockers: Propranolol 1–2 mg/kg/day, divided 2–4 times daily 4 mg/kg/day, up to 640 mg/day, divided 2–4 times daily Atenolol 0.5–1 mg/kg/day, daily or divided two times daily 2 mg/kg/day, up to 100 mg/day, daily or divided two times daily Labetalol 1–3 mg/kg/day, divided 2–3 times daily 10–12 mg/kg/day, up to 1200 mg/day, divided 2–3 times daily Metoprolol or bisoprolol	– If an adequate α-adrenergic blocker has not been previously initiated, β-adrenergic blockers can cause hypertensive crisis and, in the case of underlying cardiomyopathy, acute pulmonary edema Can be started at least 3 days prior to surgery. Common sides effects: Dizziness, fatigue, and asthma exacerbation
	Esmolol or lidocaine	– In the event of intraoperative tachycardia
Intravascular volumen expansion	Abundant oral intake of fluids. Intravenous infusion of 2–3 liters of saline the day before surgery. Diet high in salt content (6–10 g) and in some cases prescription of sodium chloride tablets. Caution should be taken with children, patients with heart or kidney disease and patients with increased risk of pulmonary edema.	

protocol exists for the medical management of these tumors in adults or children; and the procedures followed in most cases depend on the experience of the institution involved. The goal is blood pressure reduction of <50 percentile for age and height. Dilated cardiomyopathy can develop from chronic catecholamine-induced hypertension, making an echocardiography valuable preoperatively.

The possibility of particular postoperative complications, apart from the common ones such as hemorrhage, hematoma, and infection, should also be taken into account (Table 5.4). In head and neck PGLs, due to their proximity to vascular and nerve structures, the resection can give rise to specific complications.

5.9.5.3 Clinical Follow-Up

In order to ensure that the resection has been complete, biochemical testing is recommended 2–6 weeks after surgery, depending on patient recovery. If persistent elevation is observed, it is important to determine whether this is due to residual tumor, occult metastases, or the presence of additional primary tumors.

Smaller pediatric and adult case series recommend follow-up at 6 weeks and between 6 months and 1 year following initial surgery and then annually. All patients with genetic mutations should be followed throughout their lifetime given the risk of recurrence and malignancy. A multidisciplinary management including

Table 5.4 Postoperative complications [5, 7, 10, 11]

Complication	Causes
Hypotension	The cause may be multifactorial: – Loss of peripheral vasoconstriction – Persisting effect of the drugs used in the preoperative work-up, particularly if phenoxybenzamine or metyrosine was taken – Blood volume depletion – Adrenocortical insufficiency
Hypertension or *blood pressure liability*	It is important to differentiate whether the occurrence or persistence of hypertension is due to: – Postoperative pain – Volume overload – Autonomic instability – Incomplete tumor resection – Existence of an undetected tumor – *Coexisting essential hypertension* – Accidental ligation of a renal artery or renal failure
Hypoglycemia	– Loss of the suppressive effect of catecholamines on the secretion of insulin – Adrenocortical insufficiency

endocrinologists, genetic counselors, radiologists, oncologists, and surgeons for the optimal follow-up is important.

All patients with PCC/PGL are at risk for tumor recurrence even after complete resection without residual disease, and the current WHO classification stresses that all PCC/PGLs have a metastatic potential [19].

Nevertheless, no universal consensus exists with regard to the biochemical or imaging tests to be used or the frequency with which they should be applied [2, 9]. Biochemical tests should be carried out at least annually, with a focus on the biochemical phenotype particular to the known mutation. Imaging tests are generally reserved for those cases with positive results in biochemical analyses and to monitor the development of non- or low-secreting tumors, in those with a higher risk especially TCA cycle-related PCC/PGLs. Periodic image testing is recommended for carriers of mutations in the SDH genes because of the possibility of developing non-functional PGLs; MRI is preferable because it is more sensitive in detecting extra-adrenal tumors and minimize radiation exposure.

5.10 Conclusions

Faced with the complex genetic scenario described in this chapter, in order to offer an appropriate and efficient genetic test to a patient, it is essential to collect information related to age at diagnosis, tumor location, bilaterality, multiplicity, family history of disease, and the development of metastases, as well as the biochemical and immunohistochemical characteristics of the tumor. Also necessary for a comprehensive molecular diagnosis is tumor DNA, since 30–40% of patients will have

somatic mutations mainly in *NF1*, *HRAS*, *VHL*, *or RET*. The detection of a germline or somatic mutation in one of the genes related to the development of these tumors has clear implications for genetic counseling and the clinical follow-up of the patient.

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