Chapter 3

# Pheochromocytomas and Paragangliomas: A Focus on Genetics

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**Abstract**

Pheochromocytomas and paragangliomas are rare tumors, characterized by catecholamine synthesis, release, and metabolism, with the same embryological origin. Pheochromocytomas develop from chromaffin cells of the adrenal medulla, whereas paragangliomas arise extra-adrenal from sympathetic and parasympathetic nervous chains. During the past 10 years, there have been significant advances in the understanding of these tumors as it is now known that 30–40% of pheochromocytomas and paragangliomas have an underlying genetic cause. Pheochromocytomas and paragangliomas have classically been associated with three syndromes: von Hippel-Lindau (VHL), multiple endocrine neoplasia type 2 (MEN 2), and neurofibromatosis type 1 (NF1). To date, more than 21 gene mutations have been identified that are involved in the development of these tumors. Identification of such gene mutations associated with pheochromocytomas and paragangliomas will ensure early diagnosis, prompt treatment, and better prognosis for patient and family members. The recent developments in molecular pathogenesis of pheochromocytomas and paragangliomas will provide future treatment options toward personalized therapy for patients. This chapter summarizes the most important aspects of genetics and clinical characteristics, together with a focus on the new susceptibility genes.

**Keywords:** genetics, pheochromocytomas, paragangliomas, susceptibility genes

## 1. Introduction

The tumor classification system by the World Health Organization (WHO) in 2004 defines pheochromocytomas as catecholamine-secreting tumors originating from chromaffin cells

found in the medulla of adrenal glands, and paragangliomas as extra-adrenal tumors developing from ganglia along the sympathetic and parasympathetic autonomic nervous chains [1]. Head and neck paragangliomas (formerly, called glomus tumors) are derived from parasympathetic paraganglia, arising in the vagal and carotid bodies, jugulotympanic region (middle ear), orbit, or nasal cavity. They are nonchromaffin tumors (negative staining to chromium salts) and usually nonfunctional with the exception of a minority of these tumors (20–30%) that produce mostly methoxytyramine [2, 3]. Head and neck paragangliomas demonstrate usually a slow growth rate benign in nature with symptoms dependent on their location. As a result, they can cause clinical signs of compression and infiltration of adjacent neurovascular structures, such as cranial nerve palsy, hearing loss, and tinnitus [4].

Sympathetic paragangliomas are found in the mediastinum (from the thoracic sympathetic nervous chain), pre- and paravertebral autonomic nervous chains and para-aortic sympathetic chains of the thorax, abdomen, and pelvis, including the organ of Zuckerkandl. These tumors often secrete catecholamines, especially norepinephrine, and are chromaffin-positive tumors. Due to their catecholamine secretory profile, pheochromocytomas and sympathetic paragangliomas can share the same clinical symptoms with the "the classic triad" of palpitations, diaphoresis, and headache. Clinical signs can appear suddenly or be precipitated by physical activity, drugs (e.g. metoclopramide and glucocorticoids), foods containing tyramine, diagnostic procedures, or micturition [5].

Pheochromocytomas and paragangliomas affect about 1 in 2500–6500 persons, but this incidence may be more significant because of this disease diagnosis made after the person's death [6]. Pheochromocytomas are found in approximately 4–5% of adrenal incidentalomas, and together with sympathetic paragangliomas, can be a secondary cause of hypertension in about 0.2–0.4% of the cases [7]. Paragangliomas can appear in all ages, but their highest incidence is between 40 and 50 years with no sex differences.

Although mostly benign, 10–15% of such tumors are malignant, consisting of metastatic spread of chromaffin cells especially in the liver, lungs, bone, and lymph nodes [8]. Despite much investigation, no definite biochemical, genetic, or histological markers to determine the malignant character of these particular neuroendocrine tumors has been identified. In this regard, the Pheochromocytoma of the Adrenal Gland Scaled Score (PASS) was created to predict the malignant character of pheochromocytomas with a PASS score of <4 suggesting benignity, whereas a PASS score >4 indicating a potentially malignant tumor [9]. Nevertheless, a large retrospective study indicated no correlation of predictive risk and PASS score for malignancy [10]. Moreover, Ki-67 index of proliferation used commonly as a marker of malignancy in other tumors demonstrated no utility for distinguishing between benign or malignant pheochromocytomas and paragangliomas [11]. In addition, studies have revealed certain factors that predict malignancy for pheochromocytomas and paragangliomas: the extra-adrenal tumors in the mediastinum or the organ of Zuckerkandl, tumor size >5 cm, increased expression of angiogenesis-related genes by immunohistochemistry, increased plasmatic levels of 3-methoxytyramine or chromogranin A, younger

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age at diagnosis, and identification of a SDHB mutation [12–15]. This chapter summarizes recent data regarding pheochromocytomas and paragangliomas with a focus on genetics. Furthermore, a genetic testing algorithm is proposed for the clinical management of such patients with these tumors.

## 2. Genetics of pheochromocytomas and paragangliomas

Before 2000, pheochromocytomas and paragangliomas were thought to follow the 10% rule: 10% familial, 10% bilateral, 10% extra-adrenal, 10% malignant, 10% children, 10% not hypertensive, and 10% calcified. It was previously believed that familial pheochromocytomas and paragangliomas were caused by three gene mutations that determined three distinct clinical syndromes: multiple endocrine neoplasia type 2 (MEN2) syndrome involving RET proto-oncogene mutations, neurofibromatosis type I caused by NF1 gene mutations and von Hippel-Lindau disease produced by VHL gene mutations. Recent studies, however, reveal that 30–40% from pheochromocytomas and paragangliomas have an underlying genetic pathogenesis, and some sporadic tumors are caused by somatic mutations in 10–24% of the cases [16, 17].

Currently, more than 21 genes (RET, NF1, VHL, SDHA, SDHB, SDHC, SDHD, SDHAF2, MAX, TMEM127, HIF2A, KIF1Bβ, H-RAS, K-RAS, PHD2/EGLN1, IDH, ATRX, MDH2, PHD1, FH, and BAP1) are known to cause hereditary pheochromocytomas and paragangliomas. By the main signaling pathways, these genes have been grouped into two clusters:

1. Cluster 1, a pseudohypoxic group, referring to mutations in VHL, HIF2A, SDHx, EGLN1/PHD2, IDH, MDH2, and FH genes

2. Cluster 2, a kinase receptor-signaling group of genes, involving mutations in NF1, RET, MAX, TMEM127, and KIF1B genes.

Recent studies have demonstrated that the two clusters are connected by particular signaling pathways, all leading to tumorigenesis of pheochromocytomas and paragangliomas [18]. This elevated percentage of tumors with molecular pathogenesis indicates genetic testing as a mandatory step in the clinical management of patients with pheochromocytomas and paragangliomas.

### 2.1. Syndromic tumors

#### *2.1.1. RET proto-oncogene*

The REarranged during Transfection (RET) proto-oncogene is located on chromosome 10, and its translated protein is a transmembrane receptor from the tyrosine kinase family that

controls cellular growth, differentiation, and apoptosis. Germline mutation of RET activates this RET receptor with "gain of function." This activation consequently leads to cell proliferation, causing an autosomal-dominant syndrome called multiple endocrine neoplasia type 2 (MEN2). This syndrome is classified into two subcategories: MEN2A and MEN2B. MEN2A syndrome is further divided in four types: classical MEN2A, MEN2A associated with cutaneous lichen amyloidosis, MEN2A associated with Hirschprung's disease, and familial medullary thyroid cancer (FMTC).

The classical form of MEN2A syndrome is most frequent, occurring in approximately 90% of cases. It is characterized by medullary thyroid carcinoma (MTC) in 95% of cases, pheochromocytoma in 50% of cases and hyperparathyroidism in 15–30% of cases. FMTC is represented by this thyroid cancer as the only clinical feature [5]. MEN2B syndrome is composed of MTC in all the cases, and pheochromocytoma in half of patients together with intestinal ganglioneuromas, multiple mucosal neuromas, and marfanoid habitus.

The MEN2 subcategories are linked with the RET gene defects. Most mutations of the RET gene in MEN2A and FMTC patients involve codons: 609, 611, 618, 620 (exon 10) or 630, and 634 (exon 11). The mutation at codon 634, consisting in a majority of cases in the substitution of the amino acid cysteine to arginine, is the most frequent genetic defect in patients with MEN2A syndrome who have a high risk of pheochromocytoma. Regarding MEN2B, the majority of cases are determined by the codon 918 mutation (exon 16), leading to the substitution of methionine to threonine. Additionally, rare mutations in codon exon 14 and 15 have been associated with this type of MEN2 [19].

Pheochromocytomas associated with MEN2A and MEN2B syndromes are frequently benign and bilateral in a majority of cases at a young age diagnosed between 30 and 40 years. The malignancy rate is less than 1–5%. The secretory profile is characterized by hypersecretion of epinephrine, caused by the overexpression of phenylethanolamine-N-methyltransferase [16].

#### *2.1.2. Neurofibromatosis type 1 tumor suppressor gene*

Neurofibromatosis type 1 (NF1) syndrome, also called von Recklinghausen's disease, is an autosomal dominant condition caused by mutations of the large neurofibromatosis type 1 tumor suppressor gene (NF1). This gene is located on chromosome 17q11.2 and encodes for the protein neurofibromin, which enters nervous cells (e.g., neurons, Schwann cells, and oligodendrocytes), leukocytes, keratinocytes, and adrenal medulla [5].

NF1 gene defects can cause the inhibition of RAS signaling cascade, acceleration of phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), and mammalian target of rapamycin (mTOR) pathways. As a result, NF1 defects play a role in the alteration of cell growth, differentiation, migration, and apoptosis. Nevertheless, more than a half of NF1 patients present *de novo* defects of the NF1 gene. Diagnosis of NF1 is based on clinical criteria

(at least two): six or more café- au- lait spots, two or more iris hamartomas (Lisch nodules), two or more cutaneous neurofibromas, optic-nerve glioma, freckling in the axilla, neck, or inguinal, pseudoarthrosis or dysplasia of the sphenoid bone, and a first-degree family member with a diagnosis of NF1.

Patients with NF1 also have an increased risk for brainstem gliomas, astrocytomas, gastrointestinal stromal tumors, rhabdomyosarcomas, chronic myeloid leukemias, and pheochromocytomas. The incidence of pheochromocytomas in NF1 patients is approximately 0.1–5.7%, typically by 42 years of age. Cases of sympathetic paragangliomas are very rarely reported. Most pheochromocytomas are unilateral, benign, and have an epinephrine secretory profile. The rate of malignancy for pheochromocytomas in NF1 patients is approximately 12%, a little higher than in MEN2, and VHL patients [20, 21].

*2.1.3. Von Hippel-Lindau disease tumor suppressor gene*

Von Hippel-Lindau (VHL) tumor suppressor gene is located on the short arm of chromosome 3, and this gene encodes for VHL protein that controls the actions of hypoxia-inducible factor alpha (HIF$\alpha$). Germline mutations of this tumor suppressor gene cause VHL disease, an autosomal dominant disorder that comprises a large variety of tumors: retinal angiomas, cerebellar and spinal hemangioblastomas, clear cell renal carcinoma, neuroendocrine tumors of the pancreas, epididymal cystadenomas, cysts of the broad ligament, endolymphatic sac tumors, pheochromocytomas, and paragangliomas. Depending on the presence of pheochromocytomas and paragangliomas, VHL disease is divided into type 1 and type 2. Type 1 VHL disease is the most common form, characterized by the absence of pheochromocytomas and paragangliomas. Conversely, type 2 VHL disease is distinguished by the development of pheochromocytomas and paragangliomas. Type 2 VHL disease is further classified into three subcategories: 2A without clear renal cell carcinoma, 2B with clear renal cell carcinoma, and 2C with only pheochromocytomas and paragangliomas [16].

VHL gene mutations are variable, and >500 germline transformations have been described (i.e., insertions, deletions, missense, nonsense, and splice site mutations) with a rate of occurrence of *de novo* mutations of up to 20%. In type 1 VHL disease, the most frequently encountered are deletions and nonsense mutations, whereas in type 2 VHL diseases, the most common are missense mutations. It is important to note that missense mutations in codon 167 increase patient's risk to develop pheochromocytomas.

Sympathetic head and neck paragangliomas cases are rarely reported. Pheochromocytomas develop in 10–20% of VHL patients, more than a half of them being bilateral, with a young age of onset around 30 years. Pheochromocytomas and paragangliomas within this disorder secrete mainly norepinephrine due to the absence of the phenylethanolamine N-methyltransferase. Furthermore, malignant pheochromocytomas have been reported in few cases, lower than 5% of VHL patients [22].

*2.1.4. Genes encoding succinate dehydrogenase mitochondrial complex*

Gene mutations encoding for the succinate dehydrogenase (SDH) complex (SDHA, SDHB, SDHC, SDHD, and the cofactor SDHAF2) lead to clinical manifestations of the familial paraganglioma (PGL) syndromes (PGL1, PGL2, PGL3, and PGL4). The SDH complex plays a role in the Krebs cycle and electron transport chain, and its genetic defects effect the increase of succinate and reactive oxygen species and stabilization of hypoxia-inducible factor 1 (HIF-1). Mutations of the SDH complex resemble VHL disease where the hypoxia signaling pathways are activated [23].

*2.1.4.1. PGL 1 syndrome*

Paraganglioma 1 (PGL1) syndrome is an autosomal-dominant condition, with maternal imprinting, caused by SDHD gene defects. This particular gene is localized on the long arm of chromosome 11. The clinical characteristics of this syndrome are multifocal nonsecretory parasympathetic head and neck paragangliomas and, less commonly, sympathetic thoracic or abdominal paragangliomas. Furthermore, pheochromocytomas have rarely been described. Tumor malignant transformation for SDHD mutation carriers is <5% [24].

*2.1.4.2. PGL 2 syndrome*

Familial paraganglioma 2 (PGL2) syndrome is a rare autosomal-dominant transmitted condition, with maternal imprinting, caused by genetic defects in SDHAF2/SDH5 gene that is localized on the long arm of chromosome 11. The gene product is succinate dehydrogenase assembly factor 2 (SDHAF2), which ensures the flavination of SDHA subunit. Studies have reported that mutations carriers have exclusively multifocal head and neck paragangliomas with an early onset from 30 to 40 years [25].

*2.1.4.3. PGL 3 syndrome*

Familial paraganglioma 3 (PGL3) syndrome, also with an autosomal dominant mode of transmission, is caused by SDHC gene mutations that are localized on the long arm of chromosome 1. This disorder is characterized by the presence of benign parasympathetic head and neck in paragangliomas up to 4% of patients. However, few cases of pheochromocytomas and sympathetic paragangliomas have been described. The biochemical phenotype is frequently associated with noradrenergic or dopaminergic secretion, but they can also be nonsecreting tumors. The malignancy rate of these tumors is very low [26].

*2.1.4.4. PGL 4 syndrome*

The most frequently encountered modification, germline mutations of the SDHB gene found in locus 1 p36.13 cause familial paraganglioma 4 (PGL4) syndrome. The clinical manifestations of this disorder consist in thoracic, abdominal, and pelvic catecholamine-secreting paragangliomas, parasympathetic head and neck paragangliomas. Tumors associated with

SDHB gene mutations are frequently multiple, large, presenting a noradrenergic or dopaminergic secretory profile. Diagnosis is usually made at a young age (approximately 30 years), and they are linked to a high risk of developing metastases (ranging from 30 to 72% of cases). As a result, all patients with malignant pheochromocytomas and paragangliomas should undergo genetic screening, searching initially for SDHB gene modifications. Patients with PGL4 syndrome present a high risk for developing clear cell renal carcinoma, neuroblastoma, papillary thyroid carcinoma, breast neoplasms and gastrointestinal stromal tumors (GIST) [18, 21, 23, 27, 28].

SDHA gene mutations localized on the short arm of chromosome 5 which were initially found only in homozygous carriers, were linked to the rare juvenile encephalopathy recognized also as Leigh syndrome. Additionally, paragangliomas were described in the heterozygous carriers of the SDHA gene mutations, having a malignancy rate of 0–14%. [29]. Germline mutations of the SDHB, SDHC, and SDHD genes were also identified in the Carney-Stratakis dyad that consists of gastrointestinal stromal tumors (GIST) and paragangliomas, and the Carney triad, composed of paragangliomas, GIST, and pulmonary chondromas. The secretory profile of SDHx-related paragangliomas is characterized by an overproduction of norepinephrine together with dopamine, dopamine hypersecretion (or its metabolite 3-methoxytyramine) only, or a silent biochemical phenotype [5, 23, 30].

### 2.2. Nonsyndromic tumors

#### *2.2.1. Other susceptibility genes*

##### *2.2.1.1. TMEM127 gene*

The TMEM127 gene is localized on the long arm of chromosome 2, which encodes for transmembrane protein 127. Expression of this gene is associated with endosomes, lysosomes, and Golgi complex, acting as a negative factor of mTOR kinase pathways that modulates cell growth and apoptosis. Mutations of this gene induce the development of pheochromocytoma (unilateral or bilateral), abdominal and head and neck paragangliomas with adrenergic or noradrenergic biochemical phenotypes. Further studies report a 2% prevalence of TMEM127 germline mutations in patients with pheochromocytomas and paragangliomas at a mean age of 43 years. Risk for malignant transformation is approximately 1%. Other tumors such as breast and papillary thyroid carcinoma have been described in patients carrying this gene mutation [5, 18, 31, 32].

##### *2.2.1.2. MAX gene*

The MYC-associated factor X (MAX) gene localized on the short arm of chromosome 14 encodes for MAX protein, and its main role is in the modulation of cell growth and apoptosis as a component of the MYC-MAX-MXD1 complex. MAX gene germline defects have been described in patients with pheochromocytomas and paragangliomas. The tumors are frequently bilateral with a mean age of onset at 32 years. They also present with an increased

malignant potential (25% of patients). Additionally, mutations of MAX gene have a paternal mode of transmission with maternal imprinting. They lead to the development of tumors with a norepinephrine secretory profile [5, 18, 33].

#### *2.2.1.3. HIF2A gene*

Hypoxia-inducible factor 2-alpha (HIF2A) gene mutations found in the locus 2p21 have recently been correlated with the development of pheochromocytomas and paragangliomas. HIF2A gene mutations determine a decreased rate of HIF2*α* protein degradation. They lead to an enhanced action of vascular endothelial growth factor A (VEGF-A) and erythropoietin that regulate cell proliferation, angiogenesis, and erythropoiesis. Somatic HIF2A gene mutations have been described in patients who presented with multiple pheochromocytomas and paragangliomas with a noradrenergic secretory profile, polycythemia, and somatostatinomas, which comprise the clinical features of the recently described Pacak-Zhuang syndrome. Of note, all patients with this syndrome were women, but further studies are required in order to establish the genetic basis [18, 34].

#### *2.2.1.4. EGLN1/PHD2 gene*

The egg-laying-defective nine 1 gene (EGLN1) also named prolyl hydroxylase domain 2 (PHD2) gene is located on chromosome 1q42.1, and encodes for the EGLN1/PHD2 protein that hydroxylates HIF*α* protein. Germline mutations of EGLN1/PHD2 gene have been associated with the development of multiple sympathetic abdominal paragangliomas and erythrocytosis [18, 35].

#### *2.2.1.5. KIF1Bβ gene*

The kinesin family 1Bβ gene (KIF1Bβ) is located on chromosome 1p36.22 and its product plays an important role in regulating cell apoptosis. Very rare cases of patients with germline KIF1B gene mutations have been reported to have unilateral or bilateral pheochromocytomas, neuroblastoma, ganglioneuroma, medulloblastoma, leiomyosarcoma, and lung adenocarcinoma [5, 18, 36].

#### *2.2.1.6. IDH gene*

The isocitrate dehydrogenase (IDH) gene is located on chromosome 2q33.3, and its mutations are frequently associated with glioblastoma multiforme. Studies conducted on patients with pheochromocytomas and paragangliomas indicate only one somatic mutation of the IDH gene associated in the development of carotid paragangliomas [18, 37].

#### *2.2.1.7. FH gene*

The fumarate hydratase (FH) gene is found on chromosome 1q43 and its germline mutation has been reported in one patient with a pheochromocytoma and noradrenergic secretory profile.

Furthermore, FH gene mutations have also been reported in patients with clear cell renal carcinoma and leiomyomatosis [11, 18, 38].

*2.2.1.8. BAP1 gene*

Germline mutations of BRCA-1 associated protein-1 (BAP1) gene located on chromosome 3p21.1 have been reported in a limited number of patients with paragangliomas. Moreover, BAP1 mutations were identified in some patients with meningioma, melanoma, and mesothelioma, but correlations with familial paragangliomas require further studies [18, 39].

*2.2.1.9. HRAS and KRAS genes*

The RAS genes (Harvey rat sarcoma—H-RAS virus gene located on chromosome 11p15, and Kirsten rat sarcoma virus gene—K RAS found on chromosome 12p12.1) are the most common oncogenes associated with malignancies. H-RAS and K-RAS gene mutations cause the constitutive activation of the GTPase domain of RAS, inducing cell growth through activated RAS-ERK (extracellular signal-regulated kinase) pathway. Somatic mutations in H-RAS and K-RAS have been described in limited cases of pheochromocytomas and paragangliomas with adrenergic or noradrenergic biochemical phenotype. Recently, one study regarding pheochromocytomas and paragangliomas stated that 6.9% of patients had somatic H-RAS mutations [40, 41].

*2.2.2. Miscellaneous*

One family with a germline mutation of Malate Dehydrogenase type 2 (MDH2) gene with multiple metastatic paragangliomas producing norepinephrine has been described [41, 42]. Furthermore, somatic mutations of Alpha Thalassemia/Mental Retardation Syndrome X-Linked (**ATRX)** gene have been described in aggressive cases of pheochromocytomas and paragangliomas. This gene, localized on the X chromosome, plays an important role in telomere function [41, 43]. Moreover, a novel germline mutation involving prolyl hydroxylase domain 1 (**PHD1)** also known as egg-laying-defective nine 2 gene (EGLN2) mutation was reported in one case of pheochromocytoma/paraganglioma, and polycythemia [41, 44].

Recent studies have also stated that the following genes play roles in the development of pheochromocytomas and paragangliomas: glial cell-derived neurotrophic factor (**GDNF**) localized on 5p13.2, guanine nucleotide binding protein (g protein) alpha stimulating (**GNAS**) found on 20q13.32, cyclin-dependent kinase inhibitor 2a (**CDKN2A**) localized on 9p21.3, breast cancers **BRCA 1 and 2** found on 17q21.31 and 13q13.1, respectively, lysine methyltransferase 2d (**KMT2D**) found on 12q13.12, and **tumor protein p53** localized on 17p13.1 [18, 46–48, 50].

All genes currently known to be involved in the pathogenesis of pheochromocytomas and paragangliomas, specifying the chromosomal location of the gene, hormonal secretory profile, malignancy risk, and the main clinical characteristics of the patients and associated diseases are summarized in **Table 1** [5, 16–18, 21, 22, 27, 30, 41, 50, 55–59].

| Gene | Locus | Syndrome | Hormonal secretory profile | Malignancy risk | Clinical features and associated tumors |
|---|---|---|---|---|---|
| **RET** | 10q11.2 | MEN 2 | Adrenergic | Low 1–5% | Frequently benign, bilateral pheochromocytomas associated with<br>• MEN2A: Medullary thyroid carcinoma (95%) and hyperparathyroidism (15–30%)<br>• MEN2B: marfanoid habitus; mucosal<br>• Ganglioneuromas |
| **NF1** | 17q11.2 | NF1 | Adrenergic | 12% | Mostly unilateral, benign pheochromocytomas and very rare sympathetic paragangliomas, associated with:<br>• Café- au- lait spots<br>• Cutaneous neurofibromas<br>• Iris hamartomas (Lisch nodules)<br>• Optic nerve gliomas<br>• Inguinal or axillary freckles<br>• Sphenoid bone dysplasia or pseudoarthrosis |
| **VHL** | 3p25.5 | VHL | Noradrenergic | Less than 5% | Usually bilateral, benign pheochromocytomas and rare sympathetic or parasympathetic paragangliomas together with<br>• Cerebellar and spinal hemangioblastomas<br>• Retinal angiomas<br>• Epididymal cystadenomas<br>• Cysts of the broad ligament<br>• Endolymphatic sac tumors<br>• Clear cell renal carcinoma<br>• Islet cell tumors of pancreas |

| Gene | Locus | Syndrome | Hormonal secretory profile | Malignancy risk | Clinical features and associated tumors |
|---|---|---|---|---|---|
| **SDHA** | 5p15 | | Unknown | 0–14% | Pheochromocytomas and paragangliomas<br>• Leigh syndrome in homozygous patients |
| **SDHAF2/SDH5** | 11q13.1 Paternal transmission | PGL2 | Unknown | Unknown | Head and neck paragangliomas |
| **SDHB** | 1p36.13 | PGL4 | Noradrenergic or dopaminergic | 30–72% | Frequently malignant pheochromocytomas and sympathetic or parasympathetic head and neck paragangliomas associated with<br>• Gastrointestinal stromal tumors<br>• Renal cell carcinoma<br>• Breast carcinoma<br>• Papillary thyroid carcinoma<br>• Neuroblastoma |
| **SDHC** | 1q21 | PGL3 | Noradrenergic, dopaminergic or silent | Low | Mostly benign parasympathetic head and neck paragangliomas and rarely cases of pheochromocytomas and sympathetic paragangliomas |
| **SDHD** | 11q23 Paternal transmission | PGL1 | Noradrenergic, dopaminergic or silent | Less than 5% | Frequently multiple head and neck paragangliomas, less commonly sympathetic paragangliomas and rarely pheochromocytomas |
| **MAX** | 14q23.3 Paternal transmission | – | Adrenergic or noradrenergic | 25% | Frequently bilateral pheochromocytomas and paragangliomas |
| **TMEM127** | 2q11.2 | – | Adrenergic or noradrenergic | Low 1% | Pheochromocytomas (unilateral or bilateral), sympathetic and head and neck paragangliomas<br>• Papillary thyroid carcinoma<br>• Breast carcinoma |


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| Gene | Locus | Syndrome | Hormonal secretory profile | Malignancy risk | Clinical features and associated tumors |
|---|---|---|---|---|---|
| **HIF2A** | 2p21 | Pacak-Zhuang | Noradrenergic or adrenergic | Unknown | Multiple pheochromocytomas or paragangliomas associated with:<br>• Multiple somatostatinomas<br>• Polycythemia |
| **IDH** | 2q33.3 | – | Unknown | Unknown | Carotid paragangliomas with<br>• Glioblastoma multiforme |
| **FH** | 1q43 | – | Noradrenergic | Unknown/ potentially high | Pheochromocytomas associated with<br>• Leiomyomatosis<br>• Clear renal cell carcinoma |
| **KIF1Bβ** | 1p36.2 | – | Unknown | Unknown | Unilateral or bilateral Pheos, associated with<br>• Neuroblastoma<br>• Ganglioneuroma<br>• Medulloblastoma<br>• Leiomyosarcoma<br>• Lung adenocarcinoma |
| **PHD2/EGLN1** | 1q42.1 | – | Unknown | Unknown | Multiple paragangliomas associated with<br>• Erythrocytosis |
| **H-RAS**<br>**K-RAS** | 11p15<br>12p12 | – | Adrenergic or noradrenergic | Unknown | Pheochromocytomas and paragangliomas |

| Gene | Locus | Syndrome | Hormonal secretory profile | Malignancy risk | Clinical features and associated tumors |
|---|---|---|---|---|---|
| **BAP1** | 3p21.1 | – | Unknown | Unknown | Paragangliomas associated with<br>• Meningioma<br>• Melanoma<br>• Mesothelioma |
| **ATRX** | Xq21.1 | – | Unknown | Unknown | Pheochromocytomas and paragangliomas |
| **PHD1/EGLN2** | 19q13.2 | – | Unknown | Unknown | Pheochromocytomas and paragangliomas associated with<br>• Polycythemia |
| **MDH2** | 7q11.23 | – | Noradrenergic | Unknown | Multiple metastatic paragangliomas |

**Table 1.** Genotype-phenotype correlations for pheochromocytomas and paragangliomas.


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## 3. Guidelines for the genetic diagnosis of pheochromocytomas and paragangliomas

It is currently recognized that 30–40% of pheochromocytomas and paragangliomas are caused by the aforementioned genetic mutations, and several studies have concluded that all patients with such tumors should be genetically tested [45, 46]. Hereditary pheochromocytomas and paragangliomas are frequently multifocal, recurrent and, in particular cases, have a high risk for malignant transformation. Therefore, early diagnosis may assure more effective treatment and improved prognosis. Genetically, inherited syndromes are also commonly associated with other neoplasms, justifying the need for early diagnosis and treatment. Furthermore, the identification of a germline defect within a family can ensure early diagnosis, treatment, and better clinical outcomes for other family members. Finally, hereditary pheochromocytomas and paragangliomas should be suspected in all patients, but these cases presenting the following clinical characteristics should be investigated first: patients with clinical features of a specific syndrome, age <45 years, patients with multiple, recurrent, or malignant tumors, and also individuals with a family history or personal medical history of head and neck paragangliomas [45–47].

Management of such patients should start with a careful clinical examination including cutaneous and retinal examination followed by a genetic work-up (family history and pedigree). Advanced medical techniques such as next-generation and whole genome sequencing represent the optimal genetic strategies for discovering gene mutations [46–48]. Several studies have proposed different algorithms for genetic diagnosis in order to prioritize gene testing due to its high price and lack of availability. Patient's age and family history, together with tumor secretory profile and position are considered valuable in the determination for genetic testing. The authors propose an algorithm (**Figure 1**) for genetic testing that comprises information and clinical data from the latest medical reports [16–18, 27, 30, 41, 45–50].

Patients with a known familial disorder, syndromic lesions, or both should be tested for the appropriate gene. Specific clinical characteristics of a particular syndrome should guide the testing protocol. For example, the occurrence of MTC together with pheochromocytoma is suggestive for MEN2 (RET gene); the presence of café- au- lait spots, Lisch nodules, would indicate NF1 gene testing and the presence of hemangioblastomas significant for VHL gene testing [19–22]. In addition, the association of diseases such as GIST, renal clear cell carcinoma, breast, and thyroid carcinoma should guide the clinician to test SDH gene mutations, especially SDHB [23, 27, 28, 30].

Recent discoveries have shown that the order of tested genes in nonsyndromic, nonfamilial cases can be based on the histological evaluation, location, and biochemical phenotype of pheochromocytomas and paragangliomas. Biochemical testing is the least expensive diagnostic method, but it is often overlooked by physicians. There are several types of biochemical phenotypes:

**a.** The adrenergic phenotype consisting of epinephrine or metanephrine secretion orient the diagnosis first to MEN2 syndrome or NF1, then second to TMEM127, HIF2A, MAX, H-RAS, and K-RAS genes.

**b.** The noradrenergic phenotype, resulting in normetanephrines and norepinephrine production, is typical for pheochromocytomas within VHL disease or for SDHB-related

paragangliomas. However, mutations of SDHC, SDHD, MAX, HIF2A, TMEM127, FH, MDH2, K-RAS, and H-RAS genes have been reported.

**c.** A mixed phenotype characterized by the production of both epinephrines/metanephrines and norepinephrines/normetanephrines indicate the involvement of HIF2A, TMEM127, H-RAS, K-RAS, and MAX genes.

**d.** A dopaminergic biochemical phenotype should indicate first, initial SDHB and SDHD gene testing, and second, screening for SDHC gene [2, 3, 51].

**e.** A silent phenotype with no catecholamines/metanephrines production is associated with SDHx mutations.

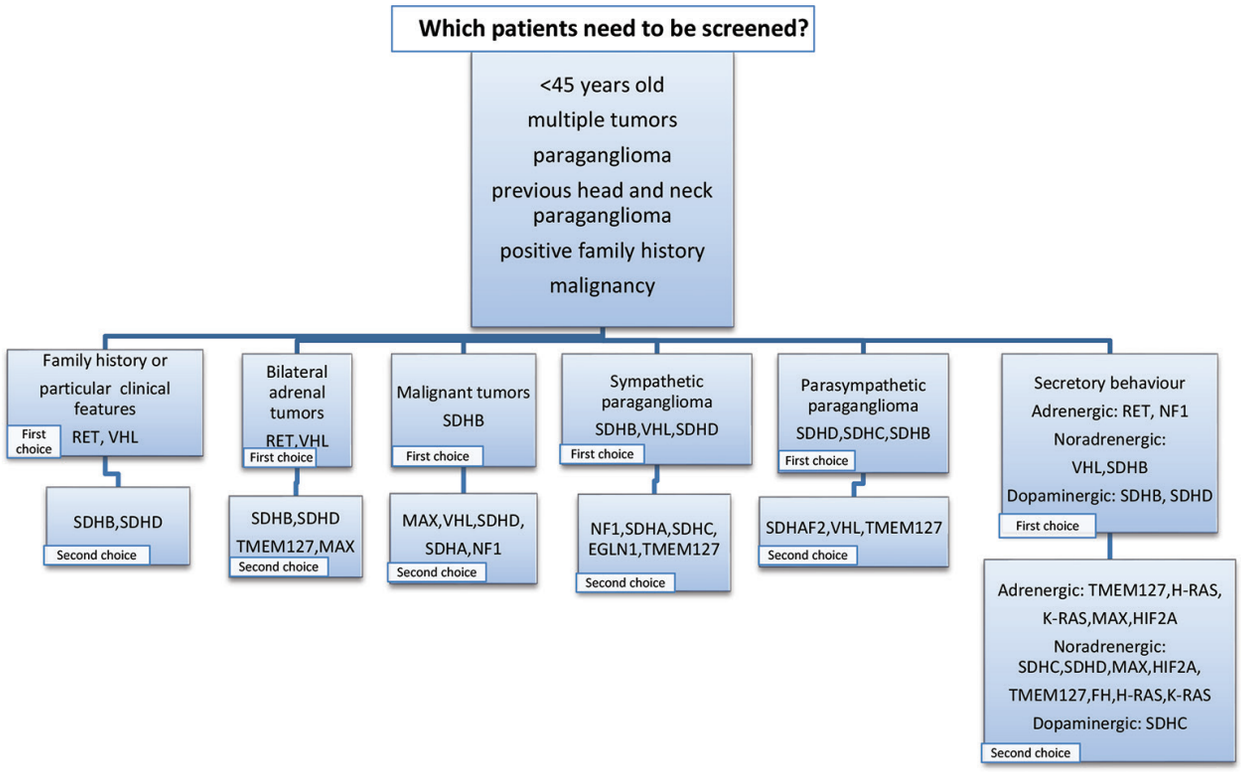


**Figure 1.** Genetic testing algorithm.

Bilateral pheochromocytomas are frequently described in RET and VHL gene mutations, and therefore, testing of these sites should be considered as a first step [19, 21, 41]. Afterward, if negative, mutations in SDHB, SDHD, MAX, and TMEM127 genes should be evaluated [30–33, 52, 53].

Sympathetic paragangliomas are more commonly associated with SDHB, SDHD, and VHL genes mutations; and less frequently with SDHA, SDHC, TMEM127, NF1, and EGLN1/PHD2 gene mutations [24–28, 53, 54]. Parasympathetic head and neck paragangliomas are commonly described in SDHB, SDHC, and SDHD gene mutation carriers, and rarely associated with SDHAF2, VHL, and TMEM127 gene mutations [54, 55]. Malignant pheochromocytomas and paragangliomas have been associated with germline mutations of the SDHB gene, but if

negative, testing for SDHD, SDHA, VHL, MAX, and NF1 gene mutations should be considered [4, 5, 11–14, 56–59]. Moreover, paternal transmission mode of pheochromocytomas and paragangliomas corresponding to maternal imprinting leads to genetic testing toward the SDHD, SDHAF2, and MAX genes [23–25, 30, 33].

## 4. Conclusions

Recent progress in the better understanding of pheochromocytomas and paragangliomas have revealed 30–40% of these tumors have underlying genetic mutations. More than 21 genetic mutations involving the following genes: RET, SDHA, SDHB, SDHC, SDHD, SDHAF2, NF1, VHL, MAX, H-RAS, K-RAS, KIF1B, TMEM127, HIF2A, IDH, FH, PHD2/EGLN1, PHD1, MDH2, BAP1, and ATRX have been linked to the development of pheochromocytomas and paragangliomas. Due to cost and lack of availability of the next-generation sequencing tests used for genetic screening, it is important to classify the risks by other clinical means as well.

Recent studies recommend considering the following criteria: tumor type, position, presence of metastases, secretory profile, or any particular clinical feature. As a result, establishing a genetic testing protocol based on which physicians can guide their diagnosis and treatment is essential. The advantages of the detection of inherited mutations in cases of pheochromocytomas and paragangliomas can provide a proper diagnosis and treatment with an improved clinical outcome for the patient, as well as an early diagnosis for family members. In cases of familial pheochromocytomas and paragangliomas, distant metastases and additional cancers can develop, highlighting the importance of early diagnosis and treatment. The strong genetic determinism of pheochromocytomas and paragangliomas emphasize the need for further studies to better understand the pathogenesis and malignant transformation of these particular tumors, which may have an important role in the development of future treatment options.

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