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Genetic Testing for Pheochromocytoma

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Abstract Pheochromocytomas (PHEOs) and paragangliomas (PGLs) are rare, catecholamine-producing tumors that are usually sporadic. However, about 30% of these tumors have been identified as being of inherited origin. To date, nine genes have been confirmed as participating in PHEO or PGL tumorigenesis. Germline mutations were found in 100% of syndromic cases and in about 90% of patients with positive familial history. In nonsyndromic patients with apparently sporadic tumors, genetic mutations have been found in up to 27%, and genetic testing is now recommended for all patients with PHEOs and PGLs. Patients with syndromic lesions, a positive family history, or both should be tested for the appertaining gene. Recent discoveries have shown that the order of tested genes in nonsyndromic, nonfamilial cases can be based on histologic evaluation, location, and the biochemical phenotype of PHEOs and PGLs—the "rule of three." Identification of a gene mutation may lead to early diagnosis and treatment, regular surveillance, and a better prognosis for patients and their relatives.

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Introduction

Although most pheochromocytomas (PHEOs) are sporadic, genetics in the development of these tumors is becoming more and more central. According to the latest discoveries, nine genes play an important role in the pathogenesis of PHEOs, including the REarranged during Transfection *(RET)* proto-oncogene, the von Hippel-Lindau disease tumor suppressor gene *(VHL)*, the neurofibromatosis type 1 tumor suppressor gene *(NF1)*, genes encoding four succinate dehydrogenase complex subunits (*SDHx*—that is, *SDHA, SDHB, SDHC,* and *SDHD* genes), the gene encoding the enzyme responsible for flavination of the SDHA subunit (*SDHAF2* or *SDH5* gene, for its yeast ortholog), and the newly described tumor suppressor *TMEM127* gene [1–3, 4••, 5••, 6••, 7••]. Furthermore, although previously about 24% of sporadic PHEOs presented genetic mutations [8], now this number is about 30% or more [3, 9••, 10, 11••]. Finally, there are new data linking the specific genotype of these tumors to its specific location, typical biochemical phenotype, or future clinical behavior. For example, *SDHB* gene mutations are associated with extra-adrenal location, overproduction of norepinephrine and dopamine, and a high risk of malignancy [12••, 13••; Eisenhofer G, Pacak K, et al., unpublished observations].

PHEOs are associated with the following familial syndromes: multiple endocrine neoplasia type 2 (MEN 2), von Hippel-Lindau disease (VHL), von Recklinghausen's

neurofibromatosis type 1 (NF1), and familial PGLs. Hereditary forms of PHEOs and PGLs can differ in age at diagnosis, location, malignant potential, and catecholamine phenotype (Table 1).

Familial Syndromes Associated with PHEOs and PGLs

Multiple Endocrine Neoplasia Type 2

MEN 2 is an autosomal-dominant syndrome caused by activating germline mutations in the *RET* proto-oncogene located on chromosome 10q11.2, which encodes a transmembrane receptor tyrosine kinase involved in the regulation of cell proliferation and apoptosis [1, 2, 14]. This syndrome is usually divided into three subgroups:

- MEN 2A is characterized by medullary thyroid carcinoma (MTC) in 95% of patients, PHEO in 50%, and hyperparathyroidism (caused by parathyroid hyperplasia/adenoma) in 15% to 30% of cases.
- MEN 2B is characterized by MTC in 100% of cases, PHEO in 50%, marfanoid habitus, and multiple mucosal ganglioneuromas.
- Familial MTC occurs alone in the third group [1, 14].

In most cases, MTC is the first presentation of MEN 2. Approximately 90% of MEN 2 cases are of the MEN 2A subtype [1, 2, 14–16]. More than 85% of patients with MEN 2A have mutations in codon 634, exon 11, and about 95% of MEN 2B cases are caused by a single missense mutation in codon 918, exon 16 of the *RET* proto-oncogene [1, 2, 15, 16].

In MEN 2, PHEOs are usually adrenal, benign, and are bilateral in more than 50% of patients [1, 2, 9••, 10, 17]. The frequency of malignant transformation is less than 5%, but children with MEN 2B–associated PHEOs have a higher risk of malignancy than those with MEN 2A or sporadic disease [15]. PHEOs are most commonly diagnosed between the ages of 30 and 40 years [2, 9••, 10, 15, 17]. MEN 2–related tumors overexpress phenylethanolamine N-methyltransferase (the enzyme that converts norepinephrine to epinephrine), so the biochemical phenotype is consistent with hypersecretion of epinephrine in large amounts, resulting in an early clinical phenotype characterized by attacks of palpitations, nervousness, anxiety, and headaches rather than the more common patterns of hypertension to be seen in other hereditary tumors [17, 18]. The increased plasma and urinary levels of metanephrine (the catecholamine O–methylated metabolite of epinephrine) in MEN 2 patients distinguish them from those with *VHL* and *SDHx* mutations (Eisenhofer G, Pacak K, et al., unpublished observations).

von Hippel-Lindau Disease

VHL is an autosomal-dominant inherited syndrome with PHEOs (VHL type 2) or without PHEOs (VHL type 1) [1, 2, 19]. VHL type 1 is the most common form, with a predisposition to develop retinal angiomas, central nervous system hemangioblastomas, and clear-cell renal carcinomas. There also could be other tumors: islet tumors of the pancreas, endolymphatic sac tumors, or cysts and cystadenomas of the kidney, pancreas, epididymis, and broad ligament. VHL type 2 is characterized by a predisposition to develop PHEOs. Type 2A is without renal carcinoma, and other VHL type 1 tumors are infrequent; type 2B includes all VHL type 1 tumors; type 2C develops PHEO alone, as an apparently sporadic tumor [2, 3, 17, 19–21]. VHL is caused by heterozygous germline mutations in the *VHL* tumor suppressor gene on chromosome 3p25.5. The gene encodes a VHL protein, which is involved in blood

Table 1 Main familial syndromes associated with pheochromocytomas and paragangliomas

	MEN 2	VHL	NF1	PGL 1	PGL 4
Gene	*RET*	*VHL*	*NF1*	*SDHD*	*SDHB*
Mean age of diagnosis, *y*	30-40	20-40	40-50	30-40	20-40
Adrenal PHEOs	+++	++	+++	-/+	++
Bilateral PHEOs	+++	+++	+	-/+	-/+
Extra-adrenal sympathetic PGLs	-/+	+	-/+	+	+++
Head and neck parasympathetic PGLs	-	-/+	-	+++	+
Biochemical profile	E/MN; NE/NMN	NE/NMN	E/MN; NE/NMN	DA/MT	DA/MT; E/NMN
Malignancy	-/+	-/+	-/+	-/+	+++

DA dopamine, *E* epinephrine, *MEN 2* multiple endocrine neoplasia type 2, *MN* metanephrine; *MT* methoxytyramine, *NE* norepinephrine, *NF1* neurofibromatosis type 1, *NF1* neurofibromatosis type 1 tumor suppressor gene, *NMN* normetanephrine, *PGL 1* paraganglioma syndrome type 1, *PGL 4* paraganglioma syndrome type 4, *PGLs* paragangliomas, *PHEOs* pheochromocytomas, *RET* rearranged during transfection proto-oncogene, *SDHB* succinate dehydrogenase subunit B gene, *SDHD* succinate dehydrogenase subunit D gene, *VHL* von Hippel-Lindau disease, *VHL* von Hippel-Lindau disease tumor suppressor gene

vessel formation by regulating activity of hypoxia inducible factor-1 alpha (HIF-1α). Loss of VHL protein function predisposes *VHL* carriers to both benign and malignant tumors in multiple organs [2, 3, 20, 22]. More than 300 mutations of the *VHL* gene have been identified [1, 21]. Approximately 20% of families with VHL carry *de novo* mutations, highlighting the need for mutation analysis in patients with apparently sporadic PHEOs [21].

VHL catecholamine-producing tumors are most commonly intra-adrenal PHEOs, although rare extra-adrenal sympathetic PGLs and parasympathetic head and neck PGLs have also been found [9••, 10, 23, 24••, 25•]. PHEOs associated with VHL are more likely to be bilateral (up to 50%) and more than half of the VHL patients with PHEOs have multiple tumors [2, 9••, 10, 23, 24••]. VHL-associated PHEOs appear to undergo malignant transformation less frequently than sporadic PHEOs (<5 % of patients) [2, 17, 22, 24••]. PHEOs develop in 10% to 20% of VHL patients, with a mean age of presentation of 30 years [3, 9••, 10, 15]. The biochemical profile of VHL patients differs from that of patients with MEN 2 or NF1. PHEOs in VHL mostly produce only norepinephrine because of a low expression of phenylethanolamine-N-methyltransferase [18]. As a result, VHL patients usually show solitary increases in plasma and urinary normetanephrine levels (Eisenhofer G, Pacak K, et al., unpublished observations).

Neurofibromatosis Type 1

NF1 or von Recklinghausen's disease is an autosomal-dominant genetic disorder caused by inactivating mutations of a tumor suppressor *NF1* gene being localized on chromosome 17q11.2. This large gene encodes a neurofibromin, which is a GTPase-activating protein involved in the inhibition of Ras activity, which controls cellular growth and differentiation. Up to 50% of NF1 patients with indentified germline mutations carry a *de novo* mutation [1, 2, 20]. PHEOs occur in 0.1% to 5.7% of patients with NF1 and in 20% to 50% of NF1 patients with hypertension [26, 27]. The clinical diagnosis of NF1 requires two of the following seven criteria: six or more café-au-lait spots; two or more cutaneous neurofibromas or a plexiform neurofibroma; inguinal or axillary freckles; two or more benign iris hamartomas (Lisch nodules); at least one optic nerve glioma; dysplasia of the sphenoid bone, or pseudoarthrosis; and a first-degree relative with NF1, according to the preceding criteria [28]. In addition, a variety of tumors, including MTC, carcinoid tumors of the duodenal wall, parathyroid tumors, peripheral nerve sheath tumors, and leukemia (particularly chronic myeloid leukemia) have been described with higher frequency in NF1 patients than in the general population [2, 20].

In NF1, the mean age of PHEO diagnosis is in the fifth decade (mean, 42 years), the same as in the general population [9••, 10, 27, 29]. On occasion, NF1 can be diagnosed concurrently with PHEO, but usually the skin lesions typical of NF1 lead to the diagnosis of NF1 in childhood, whereas PHEOs are usually diagnosed in adulthood [26]. In most cases, PHEOs are benign and unilateral, followed occasionally by bilateral PHEOs and rarely by extra-adrenal sympathetic PGLs. Malignant PHEOs have been identified in up to 12% of patients, similar to the frequency of malignancy in the general population [2, 9••, 10, 27, 29]. NF1-related PHEOs produce both epinephrine and norepinephrine. The increased plasma and urinary levels of metanephrine (indicating epinephrine overproduction) help to discriminate NF1 patients from those with *VHL* and *SDHx* mutations [Eisenhofer G, Pacak K, et al., unpublished observations].

Familial Paraganglioma Syndromes

Hereditary PGL syndromes are caused by mutations in the genes encoding the SDH complex subunits [3, 4••, 30–32]. Inactivation of SDH is associated with the accumulation of succinate and increased oxygen free radical production, resulting in the stabilization of HIF-1α [3, 33]. It has been suggested that loss of SDH function mimics chronic hypoxia leading to cellular proliferation. In contrast to normal differentiated cells, which rely primarily on mitochondrial oxidative phosphorylation to generate the energy needed for cellular processes, most malignant tumor cells instead rely on aerobic glycolysis, a phenomenon termed "the Warburg effect" [34]. The SDH enzyme complex consists of four subunits encoded by four *SDHx* genes: the *SDHA, SDHB, SDHC,* and *SDHD* genes. Mutation of the *SDHD* gene is responsible for the PGL 1 syndrome, *SDHC* gene mutation is causative for the PGL 3 syndrome, and *SDHB* gene mutation causes the PGL 4 syndrome. Succinate dehydrogenase complex assembly factor 2 (SDHAF2) encoded by the *SDHAF2* gene (or *SDH5*, for its yeast ortholog) plays the main role in flavination of SDHA, which is necessary for correct function of this subunit. Loss of SDHAF2 results in loss of SDHA subunit function and reduces the stability of the whole SDH enzyme complex. Mutation of the *SDHAF2* gene has been discovered to be responsible for PGL 2 syndrome [5••, 6••].

PGL 1 is an autosomal-dominant syndrome characterized by familial parasympathetic head and neck PGLs, less frequently by sympathetic extra-adrenal PGLs, and rarely by unilateral or bilateral PHEOs [3, 9••, 10, 11••, 29]; it is caused by inactivating mutations in the *SDHD* gene located on chromosome 11q23 [2, 3, 32]. The mean age of diagnosis is about 35 years. Carriers of *SDHD* mutations should be observed especially for head and neck PGLs,

which are often multifocal, sometimes recurrent, and rarely malignant [3, 9••, 10, 11••, 29]. Family history in patients with *SDHD* mutations could be inconclusive because of maternal genomic imprinting [3, 20, 35].

PGL 2 is a very rare autosomal-dominant syndrome defined by familial head and neck PGLs. No cases of PGL 2 syndrome presenting as PHEOs have been described. Hereditary transmission occurs exclusively in children of fathers carrying the gene, pointing to the importance of maternal imprinting [36]. The *SDHAF2* gene has been identified as causative [5••, 6••]. This gene was mapped to chromosome 11q13.1 by genomic sequence analysis [5••].

PGL 3 is a rare autosomal-dominant syndrome linked to mutations in the *SDHC* gene, which is located on chromosome 1q21 [2, 3, 31]. It is characterized by benign, seldom multifocal head and neck PGLs [9••, 10, 36]. *SDHC* mutations were originally believed to be associated only with parasympathetic head and neck PGLs [2, 36], but extra-adrenal sympathetic PGLs and PHEOs have been reported [9••, 11••, 37•]. No genomic imprinting has been described in PGL 3 and PGL 4 [21, 36].

PGL 4 is an autosomal-dominant syndrome characterized by sympathetic extra-adrenal PGLs, followed by intra-adrenal PHEOs and parasympathetic head and neck PGLs [3, 9••, 10, 11••, 29]. The syndrome is caused by inactivating mutations of the tumor suppressor *SDHB* gene located on chromosome 1p35–p36 [2, 3, 30]. An increased risk for renal cell carcinoma, gastrointestinal stromal tumor (GIST), and breast and papillary thyroid carcinoma may be expected in carriers of an *SDHB* mutation [38•, 39•, 40, 41]. The mean age at the moment of diagnosis of PGL 4 is approximately 30 years. Typically, *SDHB*-related PGLs originate in extra-adrenal locations (the organs of Zuckerkandl in the abdomen, the mediastinum, or the pelvis), and are often large, mostly solitary, and have a strong tendency for metastatic spread [9••, 10, 11••, 29, 41, 42]. Diagnosis is frequently delayed by an atypical clinical presentation. Symptoms are caused by tumor mass effect rather by catecholamine excess [12••, 41, 43]. *SDHB* gene mutations have been implicated as the most common cause in the pathogenesis of malignant PHEOs and PGLs in both children and adults [9••, 10, 11••, 12••, 13••, 33, 41, 42]. Recently, about 38% of *SDHB*-related PGLs have been observed to be malignant [11••]. Even if there is no previous history of a familial syndrome related to PHEOs or PGLs, all patients with metastatic tumors should be considered for *SDHB* gene mutation testing. No clear genotype-phenotype correlations were detected for *SDHB* mutations. Genotype-phenotype correlations failed to distinguish differences between different mutations in tumor size, location, malignant potency, aggressive behavior, hypersecretion of dopamine, or metastatic disease at presentation of *SDHB*-related PGLs. Identical *SDHB* mutations of family members may result in tumors that vary in location and severity [33, 42, 43, 44••].

The predominant biochemical phenotype of PHEOs and PGLs related to *SDHB* and *SDHD* consists of dopamine hypersecretion alone, or hypersecretion of both dopamine and norepinephrine (*SDHB*-related tumors) [12••]. Thus increased plasma levels of methoxytyramine (indicating dopamine hypersecretion) could distinguish patients with *SDHB* and *SDHD* mutations from those with *VHL*, *RET*, or *NF1* mutations (Eisenhofer G, Pacak K, et al., unpublished observations).

Recently, immunohistochemistry staining for SHDB of removed tumors has been observed as a cost-effective approach for distinguishing *SDHx*-related PHEOs or PGLs (negative staining due to an absence of SDHB) from other forms (positive staining for MEN 2, VHL, and NF1 due to the presence of SDHB) [45••, 46••]. Completely absent staining is more commonly found with *SDHB* mutation, whereas weak, diffuse staining often occurs with *SDHD* mutation [45••]. In a prospective series, SDHB immunohistochemistry was 100% sensitive and 84% specific in detecting the presence of *SDHx* mutations [46••].

New Genes Related to PHEOs and PGLs

In addition to the *SDHAF2* gene, two other genes recently have been found to be causative for familial PHEOs or PGLs. The *SDHA* gene previously was thought to be associated only with a neurodegenerative disorder known as Leigh syndrome, not with PHEOs/PGLs [3, 20, 21], but a specific mutation of *SDHA* linked with catecholamine-secreting abdominal PGL has been described. This mutation was present in 4.5% of a large series of PHEOs/PGLs [4••]. Recently, the tumor suppressor gene *TMEM127* has been identified as a new PHEO susceptibility gene. This gene, located on chromosome 2q11, encodes transmembrane protein 127 (TMEM 127), which dynamically associates with a subpopulation of vesicular organelles, Golgi complex, and lysosomes, suggesting a subcompartment-specific effect. Germline *TMEM127* mutation was detected in about 3% of sporadic-appearing PHEOs [7••].

Genetic Testing Approaches in Clinical Practice

Genetic Testing in Patients With a Known or Suspected Familial Syndrome

There are two main reasons for genetic testing in patients with a known or suspected familial syndrome. First, the familial syndromes are associated with other malignant tumors, so an early diagnosis of the syndrome (confirmed

by the genetic testing) may lead to regular surveillance and early treatment. Second, hereditary forms of PHEOs are often multiple, extra-adrenal, recurrent, and sometimes malignant, so a strict follow-up is recommended for better prognosis [17]. This approach could extend to other family members, with similar benefits. The personal history, family history, and clinical examination are starting points before the assessment of an appropriate germline mutation. In case of a positive family history or evidence for specific features of the familial syndromes shown on Table 1 and Table 2, targeted genetic testing should be performed (Fig. 1). Germline mutations have been found in 100% of syndromic patients [9••, 47••] and in 41% to 64% of nonsyndromic patients with positive familial history [9••, 48••]. Overall, there is about a 90% chance of finding a specific gene mutation in patients with a positive family history [9••]. Whenever a specific germline mutation has been identified, screening for associated disorders should be performed. Moreover, genetic testing should be offered to the patient's first-degree relatives, to ascertain the presence or absence of this gene mutation. Predictive testing helps to identify asymptomatic individuals at risk of developing the familial syndrome, and early identification of such individuals allows targeted biochemical and radiologic screening, which reduces morbidity and mortality [3, 17, 49].

Genetic Testing in Patients with Nonfamilial, Apparently Sporadic PHEOs/PGLs

PHEOs and PGLs are usually sporadic tumors without known family history or other symptoms of the above-mentioned familial syndromes, but the familial nature of the disease may not be recognized because of genomic imprinting, incomplete penetrance, *de novo* mutation, or incomplete familial history [2, 36]. Previous studies have shown that a significant number (7.5% to 27.0%) of patients with apparently sporadic PHEOs or PGLs were carriers for germline mutations of genes associated with familial syndromes. The frequency of genetic mutations in nonfamilial PHEOs and PGLs without an obvious syndrome varied significantly (*VHL*, 3.5%–11.1%; *RET*, 0.4%–5.0%; *SDHD*, 0.8%–10.0%; *SDHB*, 1.5%–10.0%) and showed geographical differences [49]. Recently, two large studies have found about an 18% to 19% frequency of germline mutations in nonsyndromic patients with negative family history [47••, 48••], but in the case of multiple or recurrent PHEOs and PGLs, the frequency has been estimated to be about 39% [9••]. These findings led to the recommendation that all patients with apparently sporadic PHEOs or PGLs should be screened for hereditary causes. Routine testing of all genes is expensive and time-consuming, but the proper order of gene tests can reduce the expense. In general, the presence of a germline mutation is likely in patients with any of the following features: early onset (<45 years); bilateral, multifocal, or extra-adrenal tumors (especially head and neck PGLs); and recurrent or malignant disease [9••, 36, 47••, 48••, 49].

PHEOs associated with NF1 can be identified by a careful physical examination or positive family history, so genetic testing for the *NF1* gene is not necessary in principle. Other genes (*RET, VHL, SDHB, SDHD, SDHC,* rarely *SDHA, SDHAF2*, and potentially *TMEM127*) remain to be involved in mutation analysis. Patients with a positive personal history, specific syndromic lesions (Table 2), or both should be tested for the corresponding genes. Decision-making for genetic testing of nonsyndromic patients with sporadic PHEOs or PGLs could be based on histologic evaluation, location, and catecholamine production of the tumor (Fig. 1).

Histologic Evaluation of PHEOs/PGLs

Malignant PHEOs and PGLs (especially extra-adrenal PGLs) have been associated mostly with *SDHB* germline mutations [10, 11••, 12••, 13••, 29, 33, 41, 43, 44••, 48••].

Table 2 Clinical features of familial pheochromocytomas and paragangliomas

Syndrome	Clinical features
MEN 2A	MTC, hyperparathyroidism.
MEN 2B	MTC, marfanoid habitus, mucosal ganglioneuromas.
von Hippel-Lindau disease (VHL)	Retinal angiomas; central nervous system hemangioblastomas; renal cell carcinomas; islet tumors of the pancreas; cysts and cystadenomas of the kidney, pancreas, epididymis.
Neurofibromatosis type 1 (NF1)	Café-au-lait spots, mucosal and cutaneous neurofibromas, inguinal or axillary freckles, benign iris hamartomas (Lisch nodules), optic nerve glioma, dysplasia of sphenoid bone.
Paraganglioma syndrome type 1, 2, or 3 (PGL1, 2, 3)	Usually benign, multiple head and neck tumors with symptoms mainly related to their location; maternal imprinting (only in PGL1 and PGL2).
Paraganglioma syndrome type 4 (PGL4)	Mostly extra-adrenal tumors (abdominal organs of Zuckerkandl; thoracic, pelvic) with symptoms caused by large tumor mass effect rather by catecholamine excess; frequently malignant.

MEN multiple endocrine neoplasia, *MTC* medullary thyroid carcinoma

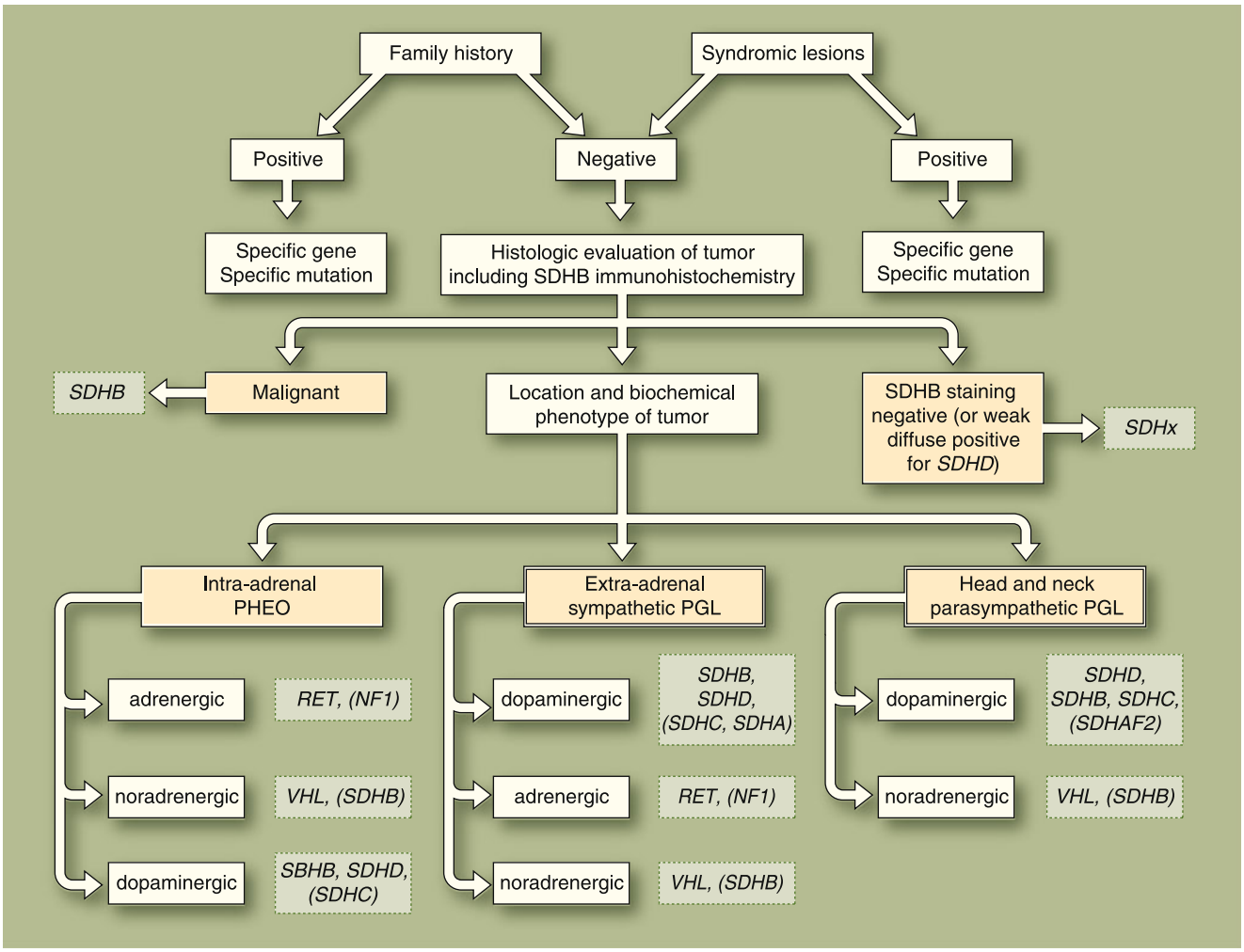

Fig. 1 Genetic testing of a patient with a pheochromocytoma (PHEO) or paraganglioma (PGL). If the patient has a positive family history, syndromic lesions, or both, the appropriate specific gene should be tested. In nonsyndromic patients with apparently sporadic tumors, immunohistochemical staining for SDHB presentation could discriminate *SDHx*-related tumors (SDHB staining is negative, or weak diffuse positive for *SDHD* mutations) from others with positive SDHB staining. In case of tumor malignancy, the patient is probably a carrier of an *SDHB* mutation. In other patients, it may be necessary to consider the location and biochemical phenotype of the tumor. A patient with an intra-adrenal PHEO who has an adrenergic phenotype (>6% of plasma metanephrine, indicating hypersecretion of epinephrine) is probably a *RET* proto-oncogene carrier; NF1 is also possible but should already have been diagnosed by a clinical evaluation. A *VHL*-related PHEO produces only norepinephrine (detected by solitary increased normetanephrine levels). A dopaminergic phenotype (>10% plasma methoxytyramine levels, indicating dopamine hypersecretion) could distinguish *SDHx*-related PHEOs from other inherited forms. *SDHx*-related PHEOs are associated mostly with *SDHB* mutations, less frequently with *SDHD*, and very rarely with *SDHC*. Extra-adrenal sympathetic PGLs of *SDHx* origin usually overproduce dopamine (though *SDHB*-related tumors often produce norepinephrine or both norepinephrine and dopamine). The genetic testing should start with the *SDHB* gene (especially for large, solitary extra-adrenal tumors or simultaneous extra-adrenal PGL and PHEO), followed by *SDHD*; extra-adrenal PGLs involving *SDHC* are rare and *SDHA*-related ones are very rare. Extra-adrenal sympathetic PGLs with an adrenergic phenotype (epinephrine/metanephrine hypersecretion) would probably be associated with *RET* mutations (patients with NF1 having been previously diagnosed clinically). Patients with solitary increased normetanephrine levels (indicating hypersecretion of norepinephrine) would have sympathetic extra-adrenal PGLs due to *VHL* mutations or *SDHB* mutations. Parasympathetic head and neck PGLs are predominantly associated with *SDHx* mutations and may overproduce dopamine. The genetic testing should start with *SDHD* gene testing (especially in multiple tumors). Less frequently, *SDHB* or *SDHC* mutations are related to these tumors. If none of these mutations are found, the *SDHAF2* gene mutation should be tested, especially if the patient is young. *VHL*-related head and neck PGLs are relatively rare and do not produce dopamine. Head and neck PGLs related to *RET* or *NF1* are extremely rare. *NF1* neurofibromatosis type 1, *NF1* neurofibromatosis type 1 tumor suppressor gene, *RET* rearranged during transfection proto-oncogene, *SDHA* succinate dehydrogenase subunit A gene, *SDHAF2* succinate dehydrogenase complex assembly factor 2 gene, *SDHB* succinate dehydrogenase subunit B, *SDHB* succinate dehydrogenase subunit B gene, *SDHC* succinate dehydrogenase subunit C gene, *SDHD* succinate dehydrogenase subunit C gene, *SDHx* succinate dehydrogenase subunits genes, *VHL* von Hippel-Lindau disease tumor suppressor gene

Less than 5% of malignant tumors have been described in carriers of *SDHD* or *SDHC* mutations [3, 10, 11••, 29, 33, 41, 43, 48••]. Malignant NF1-related PHEOs were identified with a frequency similar to that malignancy-like sporadic PHEOs in the general population [2, 9••, 10, 27, 29]. Like VHL-associated PHEOs, MEN 2–associated PHEOs appear to undergo malignant transformation less often than sporadic PHEOs; only children with MEN 2B–associated PHEOs have a higher risk of malignancy than those with MEN 2A [2, 15, 17, 22, 24••].

Immunohistochemistry staining for SDHB positivity could have high sensitivity and specificity in distinguishing between *SDHx*-related PHEOs/PGLs and other familial syndromes (MEN 2, VHL, NF1) or true sporadic tumors [45••, 46••].

Tumor Location and Biochemical Phenotype

Identification of intra-adrenal PHEOs suggests mutation of either the *RET* or *VHL* gene, followed by *NF1, SDHB*, and rarely by *SDHD* or, very rarely, by *SDHC* genes [1, 2, 9••, 10, 11••, 17, 23, 25•, 27, 29, 37•]. If the biochemical profile shows elevated metanephrine values (indicating epinephrine overproduction), then *RET* should be tested first. (NF1 patients are usually diagnosed by clinical investigation.) Tumors due to mutations of *VHL* and *SDHx* are not characterized by increase of epinephrine or metanephrine hypersecretion. Additional measurement of plasma methoxytyramine (indicating dopamine production) could discriminate patients with *SDHx* mutations from those with *VHL* mutations (Eisenhofer G, Pacak K, et al., unpublished observations).

When extra-adrenal PGLs are diagnosed, germline mutations are found most commonly in *SDHx* genes [3, 9••, 10, 11••, 29, 47••, 48••], particularly in cases of dopamine hypersecretion (detected by increased levels of methoxytyramine) (Eisenhofer G, Pacak K, et al., unpublished observations). *SDHx*-related parasympathetic head and neck PGLs are associated mostly with *SDHD* (especially multiple tumors) and less frequently with *SDHB* or *SDHC* mutations [3, 4••, 9••, 10, 11••, 29, 47••, 48••]. If testing for *SDHD, SDHB,* and *SDHC* is negative, testing for an *SDHAF2* gene mutation should be performed, especially in young patients [6••]. If the tumors do not overproduce dopamine and methoxytyramine, testing for *VHL* gene mutations should be performed first, because parasympathetic head and neck PGLs are extremely rare in patients with MEN 2 or NF1 [9••, 10, 25•, 29, 47••, 48••].

Extra-adrenal sympathetic PGLs (in abdominal, thoracic, or pelvic locations) are usually related to *SDHB* (especially solitary, large tumors), less frequently to *SDHD,* rarely to *SDHC*, and very rarely to *SDHA* mutations [9••, 10, 11••, 37•, 41, 47••, 48••]. *SDHB*-related tumors usually overproduce dopamine and norepinephrine (detected by increased plasma levels of methoxytyramine and normetanephrine, respectively). The increased levels of metanephrine (indicating epinephrine hypersecretion) are specific for MEN 2 (or NF1) patients and could distinguish them from those with *VHL* and *SDHx* mutations. *VHL*-related tumors produce only norepinephrine and normetanephrine (Eisenhofer G, Pacak K, et al., unpublished observations).

Conclusions

It has been proved that about 30% or more of PHEOs and PGLs are of inherited origin. So far, nine genes have been established as causing familial PHEOs or PGLs. These tumors may be a part of complex clinical syndromes or can be found alone as apparently sporadic neoplasms. Clinical, histologic, and biochemical evaluation (the "rule of three") may help with decision-making about subsequent gene analysis. Genetic testing for the appropriate germline mutation leads to the correct diagnosis and thus to regular surveillance, early treatment, and better prognosis for patients with PHEOs or PGLs, and similar benefits could extend to other family members.

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Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

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