Published in final edited form as:
J Hum Hypertens. 2013 March ; 27(3): 141–147. doi:10.1038/jhh.2012.20.

An update on the genetics of phaeochromocytoma

David Karasek, M.D., Ph.D.[1], **Urvi Shah, M.D.**[2], **Zdenek Frysak, M.D., C.Sc.**[1], **Constantine Stratakis, M.D., D.Sc.**[2], and **Karel Pacak, M.D., Ph.D., D.Sc.**[3]

[1]3rdDepartment of Internal Medicine, University Hospital Olomouc, I.P. Pavlova 6 Olomouc 77520, Czech Republic

[2]Program on Developmental Endocrinology and Genetics, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892, USA

[3]Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892, USA

Abstract

Phaeochromocytomas and paragangliomas are rare tumors. Nowadays, about 30% or more of them are thought to be of inherited origin due to germ-line mutations in at least ten genes. There is data linking specific genotypes of these tumors to specific locations, typical biochemical phenotypes or future clinical behaviors. Conversely, clinical features, catecholamine production and histological evaluation can help with the proper order of genetic testing for phaeochromocytoma and paraganglioma. The identification of a germ-line mutation can lead to an early diagnosis, appropriate treatment, regular surveillance and better prognosis not only for the patient, but also for their family members. Moreover, the latest discoveries in molecular pathogenesis will probably provide a basis for future personalized therapy.

Keywords

phaeochromocytoma; paraganglioma; genes; catecholamines; metanephrines

Introduction

Phaeochromocytomas (PHEOs) are rare, usually benign and sporadic tumors arising from catecholamine-producing chromaffin cells in the adrenal medulla. Tumors that stem from extra-adrenal chromaffin cells are classified as paragangliomas (PGLs). PGLs can originate in either the sympathetic or parasympathetic paraganglia. Sympathetic paraganglia have a neck to pelvis distribution, parasympathetic paraganglia are mainly found in the head and neck (derived tumors are usually termed head and neck PGLs).

Corresponding author: Karel Pacak, M.D., Ph.D., D.Sc., Chief, Section on Medical Neuroendocrinology, Professor of Medicine, Reproductive and Adult Endocrinology Program, NICHD, NIH, Building 10, CRC, 1-East, Room 1-3140, 10 Center Drive, MSC-1109, Bethesda, Maryland 20892-1109, karel@mail.nih.gov, Fax: 1-301-402-0884, Phone: 1-301-402-4594.

Disclosure summary: The authors have nothing to disclose.

Previously only 10% of cases were identified as being of inherited origin. According to the latest discoveries in genetics of PHEOs/PGLs, nowadays about one third of all these tumors are thought to be caused by germ-line mutations[1] in at least ten genes. These genes include: REarranged during Transfection *(RET*) proto-oncogene, von Hippel-Lindau disease tumor suppressor gene *(VHL)*, neurofibromatosis type 1 tumor suppressor gene *(NF 1)*, genes encoding four succinate dehydrogenase complex (SDH) subunits (*SDHx; i.e. SDHA, SDHB, SDHC*, and *SDHD* genes) (ref. 2–4), gene encoding the enzyme responsible for flavination of the SDHA subunit (*SDHAF2*) (ref. 5,6), and newly described tumor suppressor genes *TMEM 127* (ref. 7) and *MAX* (ref. 8). Furthermore, there is data linking specific genotypes of these tumors to specific locations, typical biochemical phenotypes or future clinical behavior[9–11]. Conversely, clinical features, histological evaluation and catecholamine production can help with the proper order of genetic testing for PHEO/PGL (ref. 12).

Clinical and laboratory features of PHEO/PGL suspected genes (see also table 1)

RET proto-oncogene

An activating germ-line mutation in the *RET* proto-oncogene is responsible for an autosomal-dominant syndrome multiple endocrine neoplasia type 2 (MEN 2) (ref. 2,3). This syndrome is usually divided into three subgroups: MEN 2A is characterized by medullary thyroid carcinoma (MTC) in 95%, PHEO in 50%, and hyperparathyroidism (caused by parathyroid hyperplasia/adenoma) in 15–30% of cases. MEN 2B is characterized by MTC in 100%, PHEO in 50% of cases, marphanoid habitus, and multiple mucosal ganglioneuromas. The third group is represented by familial MTC that occurs alone. Approximately 90% of MEN 2 cases are of the MEN 2A subtype. *RET* proto-oncogene encodes a transmembrane receptor tyrosine kinase involved in the regulation of cell proliferation and apoptosis. Many genotype-phenotype correlations have been found in MEN 2 (ref. 13,14).

In MEN 2 patients the PHEOs are usually adrenal, benign and bilateral in more than 50% of patients[1,2,15,16]. The frequency of malignant transformation is less than 1–5%, but children with MEN 2B-associated PHEOs have a higher risk of malignancy compared to those with MEN 2A or sporadic disease[14]. PHEOs are most commonly diagnosed between the age of 30 to 40 years[1,2,14,15,16]. In most cases, MTC is the first presentation of MEN 2, so PHEOs usually will not present as sporadic non-syndromic tumors. They often overexpress phenylethanolamine N-methyltransferase (the enzyme that converts norepinephrine to epinephrine), thus the biochemical phenotype is consistent with hypersecretion of epinephrine in large amounts. This is connected with the increased plasma and urinary levels of the catecholamine O-methylated metabolite of epinephrine – metanephrine[11,12].

Von Hippel-Lindau disease tumor suppressor gene

Von Hippel-Lindau disease tumor suppressor gene (*VHL*) encodes a VHL protein, which by regulating activity of hypoxia inducible factor - alpha (HIF-α) controls various cellular processes and blood vessel formation. Loss of VHL protein function predisposes the *VHL* carriers to both benign and malignant tumors in multiple organs. Von Hippel-Lindau disease (VHL) is an autosomal-dominant inherited syndrome with PHEOs/PGLs (VHL type 2) or

without PHEOs/PGLs (VHL type 1) caused by germ-line mutations in *VHL* gene. PHEOs develop in 10 to 20% of VHL patients. Approximately 20 % of *VHL* mutations arise *de novo*[2,3,17,18]. VHL type 1 is the most common form, with retinal angiomas, central nervous system hemangioblastomas, clear-cell renal carcinomas and other tumors like islet cell tumors of the pancreas, endolymphatic sac tumors, or cysts and cystadenomas of the kidney, pancreas, epididymis, and broad ligament. VHL type 2 includes PHEOs/PGLs. Type 2A is without renal carcinomas, 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 non-syndromic tumor[19].

PHEOs of VHL patients are most commonly intra-adrenal (up to 50% bilateral, or multiple tumors), although rarely sympathetic PGLs, and parasympathetic head and neck PGLs may be found too. VHL catecholamine-producing tumors are less frequently malignant than sporadic PHEOs (< 5 % of patients) with a mean age of presentation of 30 years[1,3,14–16,20–22]. The biochemical profile of VHL patients differs from those with MEN 2 and NF 1. VHL-associated PHEOs mostly produce only norepinephrine due to a low expression of phenylethanolamine-N-methyltransferase thus, patients usually show solitary increases in plasmatic and urinary normetanephrine levels[11,12].

Neurofibromatosis type 1 tumor suppressor gene

Neurofibromatosis type 1 (NF 1) or von Recklinghausen's disease is an autosomal dominant genetic disorder caused by inactivating mutations of neurofibromatosis type 1 tumor suppressor gene (*NF 1*). This large gene encodes a neurofibromin, which is a GTPase-activating protein involved in the inhibition of RAS signaling cascade and mTOR (formerly mammalian target of rapamycin, now mechanistic target of rapamycin) kinase pathway, which control cellular growth and differentiation[23]. Up to 50% of *NF 1* germ-line mutations occur *de novo*[2,3]. The clinical diagnosis of NF 1 requires at least two of the following 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 sphenoid bone or pseudoarthrosis; and a first degree relative with NF 1 (ref. 24). PHEOs occur in 0.1%–5.7% of patients with NF 1 (ref. 25,26). In addition, other tumors like MTC, carcinoid tumors, parathyroid tumors, peripheral nerve sheath tumors, and chronic myeloid leukemia have been described in NF 1 patients[2,26]. The skin lesions typical for NF 1 usually lead to the diagnosis in childhood, whereas PHEOs are usually diagnosed in adulthood[24].

The mean age of PHEO diagnosis is in the fifth decade (42 years), the same as in the general population. In most cases, the PHEOs are benign and unilateral, although seldom bilateral PHEOs, and rarely extra-adrenal sympathetic PGLs may be seen. Malignant PHEOs have been identified in up to 12% of cases, similar to the frequency of malignancy in the general population[1,2,15,26,27]. Similar to *RET*-associated PHEOs, *NF 1*-related PHEOs produce more epinephrine and less norepinephrine. The increased plasma and urinary levels of metanephrine (indicating epinephrine overproduction) help to discriminate NF 1 patients from those with *VHL* and *SDHx* mutations[11,12].

Genes encoding succinate dehydrogenase complex

Succinate dehydrogenase enzyme complex consists of four subunits encoded by four *SDHx* genes – *SDHA, SDHB, SDHC,* and *SDHD* genes. For correct function of SDHA subunit a cofactor of flavin adenine dinucleotide is necessary. Succinate dehydrogenase complex assembly factor 2 (SDHAF2) encoded by *SDHAF2* gene (or *SDH5,* for its yeast ortholog) plays the main role in flavination of SDHA. All these five genes (*SDHA, SDHB, SDHC, SDHD* and *SDHAF2*) can be involved in development of PHEOs/PGLs[2–6]. Inactivation of SDH is associated with the accumulation of succinate and increase in oxygen free radical production (reactive oxygen species) resulting in the stabilization of HIF-α. Similarly as in VHL, HIF-α is not sufficiently degraded and hypoxia-dependent pathways are activated. HIF-α activates a wide variety of target genes (currently about 100–150 known genes), the products of which are implicated in the regulation of apoptosis, angiogenesis, energy metabolism, proliferation, migration, and invasion of tumor cells[28].

Inactivating mutations of *SDHD* gene are responsible for familial paraganglioma syndrome – 1 (ref. 2,3,29). It is an autosomal-dominant syndrome characterized by familial parasympathetic head and neck PGLs, less commonly by sympathetic extra-adrenal PGLs and rarely by unilateral PHEOs. The head and neck PGLs are often multifocal, bilateral, sometimes recurrent and rarely malignant (< 5%) (ref. 1,3,15,29,30,31). The mean age at diagnosis is about 35 years. Although penetrance of *SDHD* mutations is high, family history in patients is often inconclusive because of maternal genomic imprinting (inactivation) of the target gene[3,32].

Germ-line mutations of *SDHB* gene are causative for familial paraganglioma syndrome – 4. It is an autosomal-dominant syndrome characterized by sympathetic extra-adrenal PGLs, followed by adrenal PHEOs and parasympathetic head and neck PGLs[1,3,15,30,32,33]. An increased risk for renal cell carcinoma, gastrointestinal stromal tumors (GIST), breast and papillary thyroid carcinomas in *SDHB* mutation carriers may be expected[34–36]. The mean age at diagnosis is approximately 30 years, varies from 6 to 77 years. Typically, *SDHB*-related PGLs originate in extra-adrenal locations (abdomen – the organ of Zuckerkandl, thorax – mediastinum, and pelvis). They are often large, mostly solitary and show a greater frequency of malignancy. *SDHB* gene mutations have been implicated as the most common cause in the pathogenesis of malignant PHEOs/PGLs in both children and adults[1,9,10,15,30–32,37,38]. *SDHB* associated tumors have been observed to be malignant in more than 30% of cases[1,21,30,31,39]. All patients with metastatic tumors should be considered for *SDHB* gene mutation testing. Diagnosis of *SDHB*-related PGLs is frequently delayed due to an atypical clinical presentation. Symptoms are caused by tumor mass effect rather than by catecholamine excess[9,31]. No clear genotype-phenotype correlations have been detected for *SDHB* mutations. Due to low penetrance, they are often found in apparently sporadic patients. Identical *SDHB* mutations of family members may result in tumors of variable location, severity and behavior[9,32,38,39].

SDHC gene mutations are causative for familial paraganglioma syndrome – 3. This rare autosomal-dominant syndrome is characterized by benign and seldom multifocal head and neck PGLs[1–3,15,40]. The mean age of onset is the same as in non-familial, sporadic cases.

SDHC mutations were originally believed to be associated with parasympathetic head and neck PGLs only[2,41]. However, extra-adrenal sympathetic PGLs and PHEOs have also been reported[1,30]. These tumors are typically benign, sometimes multiple.

Familial paraganglioma syndrome – 2 is a very rare autosomal-dominant syndrome characterized by familial head and neck PGLs. Most patients have multiple tumors. The mean age at presentation is between 30 to 40 years of age. No cases of PHEOs have been described yet. Hereditary transmission occurs exclusively in children of fathers carrying the gene, pointing to the importance of maternal imprinting[32,41]. *SDHAF2* has been identified as the causative gene[5,6]. Results of recent studies suggest that *SDHAF2* mutation screening should be considered in patients who suffer exclusively from head and neck PGLs, who have familial antecedents (high mutation penetrance) or a very young age of onset, multiple tumors and in whom *SDHB, SDHC,* and *SDHD* genes testing was negative[32].

Initially *SDHA* gene was thought to be associated only with a neurodegenerative disorder known as Leigh syndrome, and not with PHEOs/PGLs[3,18]. However, recently germ-line mutations of *SDHA* have been reported in several patients with PGLs (both sympathetic and parasympathetic) and one patient with PHEO (ref. 4,42). The current significance of *SDHA* mutation testing is minimal, but this may change if additional carriers are indentified[32].

The predominant biochemical phenotype of *SDHx*-related PHEOs/PGLs consists of dopamine hypersecretion alone or hypersecretion of both dopamine and norepinephrine (especially in *SDHB*-related tumors) (ref. 9). Thus, increased plasma levels of methoxytyramine (product of dopamine degradation) could discriminate patients with *SDHx* mutations from those with *VHL*, *RET* or *NF 1* mutations[11,12].

There is also another way to distinguish between *SDHx* and other germ-line mutation carriers. Immunohistochemistry staining for SDHB of removed tumors has been observed as a cost-effective approach for discrimination of *SDHx* related PHEOs/PGLs (negative staining due to the absence of SDHB is seen in *SDHB* and *SDHC; SDHD* mutations maybe weak diffuse or rarely negative) from other forms (positive staining due to the presence of SDHB is seen in *RET*, *VHL* and *NF1*) (ref. 43,44). The sensitivity and specificity of SDHB immunohistochemistry to detect the presence of *SDHx* mutation in prospective series were 100% and 84%, respectively[44]. Recently, immunohistochemistry staining for SDHA of 198 apparently sporadic PHEOs/PGLs found six cases (the negative staining due to absence of SDHA) with *SDHA* germ-line mutation[42].

Other genes related to PHEOs/PGLs

Tumor suppressor gene *TMEM 127* encoding transmembrane protein 127 (TMEM 127) has been identified as a new PHEO susceptibility gene[7]. The function of TMEM 127 is not well defined. It has been linked to mTOR kinase. TMEM 127 dynamically associates with multiple endomembrane organelles, including endosomes, Golgi complex and lysosomes, suggesting a subcompartmental-specific effect. It is possible that TMEM 127 contributes to modulation of mTOR kinase signaling by its association with components of the mTOR pathway within specific endosomal pools[7,32,45]. The association between *TMEM 127* gene and mTOR kinase links it to other genes (*RET, NF 1*) with kinase receptor signaling

pathways (see below). In a large cohort of 990 individuals with PHEOs/PGLs the frequency of *TMEM 127* germ-line mutations was about 2%. Mean age at diagnosis was in the fifth decade (about 43 years). Typically patients had benign (malignancy rate < 5%) adrenal (bilateral in a third of patients) catecholamine secreting PHEOs, with no preferential production of either norepinephrine or epinephrine[46]. However, recently two cases of patients with extra-adrenal abdominal PGL and head and neck PGL have been reported[47]. Other tumors, including papillary thyroid carcinoma and breast cancer have been identified in *TMEM 127* mutation carriers, but a causal relationship remains to be cleared[45].

Protein MAX (also known as MYC-associated factor X) encoded by the *MAX* gene interacts with other transcription factors forming MYC-MAX-MXD1 network that regulates cell proliferation, differentiation and apoptosis[48]. MAX can function as both suppressor and activator of MYC oncoprotein and there is crosstalk between MYC-MAX-MXD1 network and mTOR pathway involved in the development of PHEOs. Recent data suggests that *MAX* germ-line mutations are associated with PHEO susceptibility and that *MAX* behaves as a classic tumor suppressor gene[8]. *MAX* associated adrenal tumors are often bilateral (in 67%) and an association with malignant behaviour was also found (in 25%). The mean age of onset was about 32 years. There is data suggesting paternal transmission of this tumor susceptibility gene similar to *SDHD* or *SDHAF2* (ref. 8).

Very rare causes of patients with PHEO and neuroblastoma were reported in connection with germ-line mutations of kinesin family member 1B gene (*KIF1B*, located on chromosome 1p36.22) (ref. 49). This gene encodes a protein that induces apoptosis. It takes effect downstream of an oxygen-dependent prolyl hydroxylase (EGLN3/PHD3) with an important role in cell response to hypoxia and angiogenesis[49–51].

A germ-line mutation of the egl nine homolog 1gene (*EGLN1*, also termed *PHD2;* located on chromosome 1q42.1) was detected in a family with congenital erythrocytosis and PGLs[52]. The gene product (EGLN1/PHD2) is a prolyl hydroxylase, member of the prolyl hydroxylases family, which has a crucial function in the oxygen-dependent proline hydroxylation of HIF-α and cell response to hypoxia[50,51].

PHEOs/PGLs may also be a part of very rare syndromes (Carney triad syndrome, Carney-Stratakis syndrome) (ref. 35). PHEOs were reported very infrequently as a component of multiple endocrine neoplasia type 1 (ref. 51).

Pathogenesis of hereditary PHEOs/PGLs

There are probably two distinct groups of hereditary PHEOs/PGLs based on their transcription profile. Dahia et al. identified two dominant expression clusters[53]. The first cluster contained *VHL* and *SDHx* mutant tumors, the second contained *RET* and *NF1* mutant tumors. Subsequent studies revealed that the transcription profile of PHEOs/PGLs with germ-line mutations in *TMEM 127* and *MAX* cluster with the *RET/NF1* group[7,8,51].

VHL/SDHx cluster showed a transcription profile associated with angiogenesis, hypoxia and a reduced oxidative response (so-called pseudohypoxic response) by stabilizing HIF-α. HIF-α is a transcription factor that activates several genes leading to dysregulation of apoptosis,

angiogenesis, energy metabolism, proliferation, migration, and invasion of tumor cells[50,51]. *EGLN 1* germ-line mutations seem to belong to this group too, because EGLN 1 proteins take a main part in the degradation of HIF-α[54].

In contrast *RET/NF1* cluster covers genes involved in translation initiation, protein synthesis and kinase signaling. Activation of *RET* oncogene and inactivating mutations of *NF1* are connected with activation of the RAS/RAF/MAPK pathway and the PI3/AKT signaling pathway[51]. Activation of mTOR in *TMEM 127* mutations is a signal downstream of both *RET* and *NF1* mutations via the PI3K/AKT pathway, possibly suggesting a common mechanism for mutations in *RET*, *NF1* and *TMEM127* (ref. 7). Also *MAX* mutations have been counted among this gene cluster, because there is crosstalk between the MYC-MAX-MXD1 network and the mTOR pathway[8,51]. Moreover, activation of the PI3K/AKT/mTOR and RAS/RAF/MAPK signaling cascades may promote the degradation of MXD1, thereby inhibiting it from antagonizing MYC transcription activity. It is also well established that RAS/RAF/MAPK activation promotes MYC stability[51].

Nevertheless, another hypothesis presuming a single common pathway for different genes (*RET, VHL, NF 1, SDHx* and even *KIF1B*) has been proposed[49,55]. According to this model germ-line mutations of the above mentioned genes allow neuronal progenitor cells to escape from c-Jun/EGLN3 dependent apoptosis, which is normally induced by loss of nerve growth factor during early development. These cells may serve as a base for forming PHEOs/PGLs in later life[50,51]. Interaction between c-Jun and MYC may suggest potential role for *MAX* mutation in this model too[56]. However, this hypothesis does not provide an explanation for the two different transcription profiles and no links between *EGLN 1* or *TMEM 127* and neuronal apoptosis has still been found[51]. Despite these controversies in models for PHEO/PGL molecular pathogenesis, discoveries in gene expression and cellular pathways will likely provide a basis for potential personalized therapy in future.

Clinical implication of genetic testing for PHEOs/PGLs

There are three main reasons for genetic testing. 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 clinical follow-up is recommended for better prognosis of the patients[17]. Third, the identification of a germ-line mutation may also lead to early diagnosis, treatment and better prognosis for other family members through regular surveillance.

Personal, family history and clinical examination are starting points for the assessment of an appropriate germ-line mutation. In the case of a positive family history or evidence of specific features of the above mentioned familial syndromes (see table 1), targeted genetic testing should be performed. Germ-line mutations have been found in 100% of syndromic patients[1,57] and in 41% to 64% of non-syndromic patients with positive familial history[1,58]. Overall, about 90% of patients with positive familial history have a specific gene mutation[1].

However, the majority of PHEOs/PGLs are usually sporadic tumors without known family history, or other symptoms of familial syndromes. Previous studies have shown that a

significant number (7.5% to 27.0%) of patients with apparently sporadic PHEOs/PGLs were carriers for germ-line mutations associated with familial syndromes. The frequency of genetic mutations in cases of non-familial PHEOs/PGLs 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[59]. Two large studies have found the frequency of germ-line mutations in non-syndromic patients with negative family history about 18–19% (ref. 47,48). But in the case of multiple or recurrent PHEOs/PGLs, the frequency has been estimated about 39% (ref. 1). With the increasing number of tested genes the frequency will be rising. According to these findings it has been recommended that all patients with apparently sporadic PHEOs should be offered genetic testing. But routine testing for all known genes is too expensive and time consuming. Thus, various predictors for the genetic origin have been suggested. Based on results of several studies, the presence of a germ-line 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), recurrent or malignant disease, positive family history for PHEOs/PGLs[1,15,31,41,50,57–59]. And then the proper order of tested genes can also reduce the financial expenses. Patients with a positive familial history, personal history and/or presenting specific syndromic lesions should be tested for correspondent genes - see table 1. Decision-making for gene testing of non-syndromic patients with apparently sporadic PHEOs/PGLs could be based on histological evaluation, localization and catecholamine production of the tumor – the “rule of three” (ref. 12).

Histological evaluation of PHEOs/PGLs

Malignant PHEOs/PGLs (especially extra-adrenal PGLs) have been associated mostly with *SDHB* germ-line mutations (in more than 30% of cases) (ref. 9,10,30–34,37–39,51). Higher frequency of malignant PHEOs (in 25% of cases) was also detected in *MAX* germ-line mutations[8]. Malignant NF 1-related PHEOs were identified with similar frequency of malignancy (up to 12% of cases) like sporadic PHEOs in the general population[1,2,15,25–27]. Less than 5 % of malignant tumors have been described in carriers of *RET* (ref. 1,2,14–16), *VHL* (ref. 1,2,18–22), *SDHD* (ref. 1,15,29–32), *SDHC* (ref. 1,15,32,40), *SDHAF2* (ref. 32,51) or *TMEM 127* (ref. 46) mutations. Only children with MEN 2B-associated PHEOs have a higher risk of malignancy compared to those with MEN 2A or sporadic disease[14].

Immunohistochemistry staining for SDHB positivity could distinguish *SDHx* related PHEOs/PGLs from other familial syndromes (MEN 2, VHL, NF 1), or true sporadic tumors[43,44]. Immunohistochemistry staining for SDHA may help with detection of carriers with *SDHA* germ-line mutation[42].

Location of tumors

Preferential intra-adrenal location suggests mutation of *RET*, *VHL*, *NF1*, *TMEM 127* or *MAX* gene[1,8,14–16,18–22,25–27,46]. Very rare causes of tumors like *KIF1B* germ-line mutation were also localized intra-adrenally[49]. The frequency of PHEOs caused by *SDHB*-related tumors is about 25% (ref. 1,3,9,10,15,21,30–33,37–39). Intra-adrenal tumors have been less frequently detected in germ-line mutations of *SDHD*, *SDHA* and *SDHC* (ref. 1,15,29–32,40,42,51).

Bilateral PHEOs have been mostly found in carriers with *RET*, *VHL*, *TMEM 127*, and *MAX* mutations[1,8,14–16,18–22,46].

When extra-adrenal tumors are diagnosed, the germ-line mutations are found most commonly in *SDHx* genes[1,3,4,9,10,15,29–33,37–39]. Only PGLs were also detected in a family with rare *EGLN1* mutation[52]. Rarely extra-adrenal tumors have been caused by mutations in *VHL*, *TMEM 127*, *NF1*, and *RET* (ref. 1,14–16,18–22,25–27,46,51).

SDHx-related head and neck parasympathetic PGLs are mostly associated with *SDHD* (especially multiple tumors) and less frequently with *SDHB* or *SDHC* mutations[1,3,4,9,10,15,21,29–33,37–39]. If testing for *SDHD*, *SDHB*, and *SDHC* is negative, then testing for *SDHAF2* mutation should be performed[32]. For other head and neck tumors the testing for *VHL (*and then probably for *TMEM 127)* gene mutations should be made first, because parasympathetic PGLs are extremely rare in patients with MEN 2 or NF 1 (ref. 1,14–16,18–22,25–27,46,51).

Extra-adrenal sympathetic PGLs are usually related to *SDHB* (especially solitary, large tumors), less frequently to *SDHD*, rarely to *SDHC* and *SDHA* mutations[1,3,4,9,10,15,21,29–33,37–39,42]. Rarely these tumors were detected also in carriers of *VHL, TMEM 127, RET, or NF1* mutations[1,14–16,18–22,25–27,46,51].

Biochemical phenotype

Measurements of plasma metanephrine, normetanephrine, and methoxytyramine (the O-methylated metabolites of catecholamines) can help to distinguish between some hereditary forms of PHEOs/PGLs. In contrast to patients with *VHL*, *SDHB*, and *SDHD* mutations, Eisenhofer et al. found all patients with *RET* and *NF1* related tumors characterized by increased plasma concentrations of metanephrine (indicating epinephrine production) (ref. 11). VHL patients usually showed solitary increases in normetanephrine (indicating norepinephrine production), whereas additional or solitary increases in methoxytyramine (indicating dopamine production) characterized 70% of patients with SDHB and SDHD mutations. Patients with NF1 and MEN 2 could be discriminated from those with VHL, SDHB, and SDHD gene mutations in 99% of cases by the combination of normetanephrine and metanephrine. Measurements of plasma methoxytyramine discriminated patients with SDHB and SDHD mutations from those with VHL mutations in an additional 78% of cases[11]

TMEM 127 associated PHEOs were described as catecholamine secreting tumors, with no preferential production of either norepinephrine, or epinephrine[46]. There is data connecting *MAX* germ-line mutations with increased plasma concentrations of metanephrine [unpublished observation].

Conclusion

Nowadays, about 30% or more of PHEOs/PGLs are thought to be of inherited origin. They may be a part of the familial clinical syndromes or could be found alone as apparently sporadic tumors. There are probably two distinct groups of hereditary PHEOs/PGLs based

on their transcription profile, which can explain some common features (catecholamine production, location, etc.). Discoveries in molecular pathogenesis of PHEOs/PGLs will probably provide a basis for future personalized therapy. Today, genetic testing for germline mutation can help with correct and early diagnosis, appropriate treatment and better prognosis not only for the patient, but also for other family members through regular surveillance. Decision-making for the proper order of potential mutations may be based on catecholamine production, location and histological evaluation of the tumor – see figure 1.

Acknowledgments

This work was supported by the Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland.

References

1. Mannelli M, Castellano M, Schiavi F, Filetti S, Giacchè M, Mori L, et al. Italian Pheochromocytoma/Paraganglioma Network. Clinically guided genetic screening in a large cohort of italian patients with pheochromocytomas and/or functional or nonfunctional paragangliomas. J Clin Endocrinol Metab. 2009; 94:1541–1547. [PubMed: 19223516]
2. Bryant J, Farmer J, Kessler LJ, Townsend RR, Nathanson KL. Pheochromocytoma: the expanding genetic differential diagnosis. J Natl Cancer Inst. 2003; 95:1196–1204. [PubMed: 12928344]
3. Benn DE, Robinson BG. Genetic basis of phaeochromocytoma and paraganglioma. Best Pract Res Clin Endocrinol Metab. 2006; 20:435–450. [PubMed: 16980204]
4. Burnichon N, Brière JJ, Libé R, Vescovo L, Rivière J, Tissier F, et al. SDHA is a tumor suppressor gene causing paraganglioma. Hum Mol Genet. 2010; 19:3011–3020. [PubMed: 20484225]
5. Hao HX, Khalimonchuk O, Schraders M, Dephoure N, Bayley JP, Kunst H, et al. SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. Science. 2009; 325:1139–1142. [PubMed: 19628817]
6. Bayley JP, Kunst HP, Cascon A, Sampietro ML, Gaal J, Korpershoek E, et al. SDHAF2 mutations in familial and sporadic paraganglioma and phaeochromocytoma. Lancet Oncol. 2010; 11:366–372. [PubMed: 20071235]
7. Qin Y, Yao L, King EE, Buddavarapu K, Lenci RE, Chocron ES, et al. Germline mutations in TMEM127 confer susceptibility to pheochromocytoma. Nat Genet. 2010; 42:229–233. [PubMed: 20154675]
8. Comino-Méndez I, Gracia-Aznárez FJ, Schiavi F, Landa I, Leandro-García LJ, Letón R, et al. Exome sequencing identifies MAX mutations as a cause of hereditary pheochromocytoma. Nat Genet. 2011; 43:663–667. [PubMed: 21685915]
9. Timmers HJ, Kozupa A, Eisenhofer G, Raygada M, Adams KT, Solis D, et al. Clinical presentations, biochemical phenotypes, and genotype-phenotype correlations in patients with succinate dehydrogenase subunit B-associated pheochromocytomas and paragangliomas. J Clin Endocrinol Metab. 2007; 92:779–786. [PubMed: 17200167]
10. Amar L, Baudin E, Burnichon N, Peyrard S, Silvera S, Bertherat J, et al. Succinate dehydrogenase B gene mutations predict survival in patients with malignant pheochromocytomas or paragangliomas. J Clin Endocrinol Metab. 2007; 92:3822–3828. [PubMed: 17652212]
11. Eisenhofer G, Lenders JW, Timmers H, Mannelli M, Grebe SK, Hofbauer LC, et al. Measurements of plasma methoxytyramine, normetanephrine, and metanephrine as discriminators of different hereditary forms of pheochromocytoma. Clin Chem. 2011; 57:411–420. [PubMed: 21262951]
12. Karasek D, Frysak Z, Pacak K. Genetic testing for pheochromocytoma. Curr Hypertens Rep. 2010; 12:456–464. [PubMed: 20938758]
13. Frank-Raue K, Raue F. Multiple endocrine neoplasia type 2 (MEN 2). Eur J Cancer. 2009; 45 (Suppl 1):267–273. [PubMed: 19775624]
14. Pacak K, Eisenhofer G, Ilias I. Diagnosis of pheochromocytoma with special emphasis on MEN2 syndrome. Hormones (Athens). 2009; 8:111–116. [PubMed: 19570738]

15. Amar L, Bertherat J, Baudin E, Ajzenberg C, Bressac-de Paillerets B, Chabre O, et al. Genetic testing in pheochromocytoma or functional paraganglioma. J Clin Oncol. 2005; 23:8812–8818. [PubMed: 16314641]
16. Lenders JW, Eisenhofer G, Mannelli M, Pacak K. Phaeochromocytoma. Lancet. 2005; 366:665–675. [PubMed: 16112304]
17. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature. 1999; 399:271–275. [PubMed: 10353251]
18. Petri BJ, van Eijck CH, de Herder WW, Wagner A, de Krijger RR. Phaeochromocytomas and sympathetic paragangliomas. Br J Surg. 2009; 96:1381–1392. [PubMed: 19918850]
19. Maher ER, Yates JR, Harries R, Benjamin C, Harris R, Moore AT, et al. Clinical features and natural history of von Hippel-Lindau disease. Q J Med. 1990; 77:1151–1163. [PubMed: 2274658]
20. Hes FJ, Höppener JW, Lips CJ. Clinical review 155: Pheochromocytoma in Von Hippel-Lindau disease. J Clin Endocrinol Metab. 2003; 88:969–974. [PubMed: 12629069]
21. Srirangalingam U, Khoo B, Walker L, MacDonald F, Skelly RH, George E, et al. Contrasting clinical manifestations of SDHB and VHL associated chromaffin tumours. Endocr Relat Cancer. 2009; 16:515–525. [PubMed: 19208735]
22. Boedeker CC, Erlic Z, Richard S, Kontny U, Gimenez-Roqueplo AP, Cascon A, et al. Head and neck paragangliomas in von Hippel-Lindau disease and multiple endocrine neoplasia type 2. J Clin Endocrinol Metab. 2009; 94:1938–1944. [PubMed: 19336503]
23. Johannessen CM, Johnson BW, Williams SM, Chan AW, Reczek EE, Lynch RC, et al. TORC1 is essential for NF1-associated malignancies. Curr Biol. 2008; 18:56–62. [PubMed: 18164202]
24. Boyd KP, Korf BR, Theos A. Neurofibromatosis type 1. J Am Acad Dermatol. 2009; 61:1–14. quiz 15–16. [PubMed: 19539839]
25. Walther MM, Herring J, Enquist E, Keiser HR, Linehan WM. von Recklinghausen's disease and pheochromocytomas. J Urol. 1999; 162:1582–1586. [PubMed: 10524872]
26. Zöller ME, Rembeck B, Odén A, Samuelsson M, Angervall L. Malignant and benign tumors in patients with neurofibromatosis type 1 in a defined Swedish population. Cancer. 1997; 79:2125–2131. [PubMed: 9179058]
27. Bausch B, Borozdin W, Neumann HP. European-American Pheochromocytoma Study Group. Clinical and genetic characteristics of patients with neurofibromatosis type 1 and pheochromocytoma. N Engl J Med. 2006; 354:2729–2731. [PubMed: 16790714]
28. Favier J, Gimenez-Roqueplo AP. Pheochromocytomas: the (pseudo)-hypoxia hypothesis. Best Pract Res Clin Endocrinol Metab. 2010; 24:957–968. [PubMed: 21115164]
29. Baysal BE, Ferrell RE, Willett-Brozick JE, Lawrence EC, Myssiorek D, Bosch A, et al. Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. Science. 2000; 287:848–851. [PubMed: 10657297]
30. Burnichon N, Rohmer V, Amar L, Herman P, Leboulleux S, Darrouzet V, et al. PGL.NET network. The succinate dehydrogenase genetic testing in a large prospective series of patients with paragangliomas. J Clin Endocrinol Metab. 2009; 94:2817–2827. [PubMed: 19454582]
31. Neumann HP, Pawlu C, Peczkowska M, Bausch B, McWhinney SR, Muresan M, et al. European-American Paraganglioma Study Group. Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. JAMA. 2004; 292:943–951. Erratum in: JAMA, 2004, 292–1686. [PubMed: 15328326]
32. Hensen EF, Bayley JP. Recent advances in the genetics of SDH-related paraganglioma and pheochromocytoma. Fam Cancer. 2011; 10:355–363. [PubMed: 21082267]
33. Astuti D, Latif F, Dallol A, Dahia PL, Douglas F, George E, et al. Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. Am J Hum Genet. 2001; 69:49–54. Erratum in: Am J Hum Genet, 2002, 70, 565. [PubMed: 11404820]
34. Ricketts C, Woodward ER, Killick P, Morris MR, Astuti D, Latif F, et al. Germline SDHB mutations and familial renal cell carcinoma. J Natl Cancer Inst. 2008; 100:1260–1262. [PubMed: 18728283]

35. Pasini B, McWhinney SR, Bei T, Matyakhina L, Stergiopoulos S, Muchow M, et al. Clinical and molecular genetics of patients with the Carney-Stratakis syndrome and germline mutations of the genes coding for the succinate dehydrogenase subunits SDHB, SDHC, and SDHD. Eur J Hum Genet. 2008; 16:79–88. [PubMed: 17667967]
36. Lee J, Wang J, Torbenson M, Lu Y, Liu QZ, Li S. Loss of SDHB and NF1 genes in a malignant phyllodes tumor of the breast as detected by oligo-array comparative genomic hybridization. Cancer Genet Cytogenet. 2010; 196:179–183. [PubMed: 20082856]
37. Timmers HJ, Gimenez-Roqueplo AP, Mannelli M, Pacak K. Clinical aspects of SDHx-related pheochromocytoma and paraganglioma. Endocr Relat Cancer. 2009; 16:391–400. [PubMed: 19190077]
38. Brouwers FM, Eisenhofer G, Tao JJ, Kant JA, Adams KT, Linehan WM, et al. High frequency of SDHB germline mutations in patients with malignant catecholamine-producing paragangliomas: implications for genetic testing. J Clin Endocrinol Metab. 2006; 91:4505–4509. [PubMed: 16912137]
39. Ricketts CJ, Forman JR, Rattenberry E, Bradshaw N, Lalloo F, Izatt L, et al. Tumor risks and genotype-phenotype-proteotype analysis in 358 patients with germline mutations in SDHB and SDHD. Hum Mutat. 2010; 31:41–51. [PubMed: 19802898]
40. Müller U, Troidl C, Niemann S. SDHC mutations in hereditary paraganglioma/ pheochromocytoma. Fam Cancer. 2005; 4 :9–12. [PubMed: 15883704]
41. Jiménez C, Cote G, Arnold A, Gagel RF. Review: Should patients with apparently sporadic pheochromocytomas or paragangliomas be screened for hereditary syndromes? J Clin Endocrinol Metab. 2006; 91:2851–2858. [PubMed: 16735498]
42. Korpershoek E, Favier J, Gaal J, Burnichon N, van Gessel B, Oudijk L, et al. SDHA immunohistochemistry detects germline SDHA gene mutations in apparently sporadic paragangliomas and pheochromocytomas. J Clin Endocrinol Metab. 2011; 96:E1472–1476. [PubMed: 21752896]
43. Gill AJ, Benn DE, Chou A, Clarkson A, Muljono A, Meyer-Rochow GY, et al. Immunohistochemistry for SDHB triages genetic testing of SDHB, SDHC, and SDHD in paraganglioma-pheochromocytoma syndromes. Hum Pathol. 2010; 41:805–814. [PubMed: 20236688]
44. van Nederveen FH, Gaal J, Favier J, Korpershoek E, Oldenburg RA, de Bruyn EM, et al. An immunohistochemical procedure to detect patients with paraganglioma and phaeochromocytoma with germline SDHB, SDHC, or SDHD gene mutations: a retrospective and prospective analysis. Lancet Oncol. 2009; 10:764–771. [PubMed: 19576851]
45. Jiang S, Dahia PL. Minireview: the busy road to pheochromocytomas and paragangliomas has a new member, TMEM127. Endocrinology. 2011; 152:2133–2140. [PubMed: 21447639]
46. Yao L, Schiavi F, Cascon A, Qin Y, Inglada-Pérez L, King EE, et al. Spectrum and prevalence of FP/TMEM127 gene mutations in pheochromocytomas and paragangliomas. JAMA. 2010; 304:2611–2619. [PubMed: 21156949]
47. Neumann HP, Sullivan M, Winter A, Malinoc A, Hoffmann MM, Boedeker CC, et al. Germline mutations of the TMEM127 gene in patients with paraganglioma of head and neck and extraadrenal abdominal sites. J Clin Endocrinol Metab. 2011; 96:E1279–1282. [PubMed: 21613359]
48. Grandori C, Cowley SM, James LP, Eisenman RN. The Myc/Max/Mad network and the transcriptional control of cell behavior. Annu Rev Cell Dev Biol. 2000; 16:653–699. [PubMed: 11031250]
49. Schlisio S, Kenchappa RS, Vredeveld LC, George RE, Stewart R, Greulich H, et al. The kinesin KIF1Bbeta acts downstream from EglN3 to induce apoptosis and is a potential 1p36 tumor suppressor. Genes Dev. 2008; 22:884–893. [PubMed: 18334619]
50. Opocher G, Schiavi F. Genetics of pheochromocytomas and paragangliomas. Best Pract Res Clin Endocrinol Metab. 2010; 24:943–956. [PubMed: 21115163]
51. Welander J, Söderkvist P, Gimm O. Genetics and clinical characteristics of hereditary pheochromocytomas and paragangliomas. Endocr Relat Cancer. 2011; 18:R253–R276. [PubMed: 22041710]

52. Ladroue C, Carcenac R, Leporrier M, Gad S, Le Hello C, Galateau-Salle F, et al. PHD2 mutation and congenital erythrocytosis with paraganglioma. N Engl J Med. 2008; 359:2685–2692. [PubMed: 19092153]

53. Dahia PL, Ross KN, Wright ME, Hayashida CY, Santagata S, Barontini M, et al. A HIF1alpha regulatory loop links hypoxia and mitochondrial signals in pheochromocytomas. PLoS Genet. 2005; 1 :72–80. [PubMed: 16103922]

54. Berra E, Benizri E, Ginouvès A, Volmat V, Roux D, Pouysségur J. HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia. EMBO J. 2003; 22:4082–4090. [PubMed: 12912907]

55. Lee S, Nakamura E, Yang H, Wei W, Linggi MS, Sajan MP, et al. Neuronal apoptosis linked to EglN3 prolyl hydroxylase and familial pheochromocytoma genes: developmental culling and cancer. Cancer Cell. 2005; 8:155–167. [PubMed: 16098468]

56. Vaqué JP, Fernández-García B, García-Sanz P, Ferrandiz N, Bretones G, Calvo F, et al. c-Myc inhibits Ras-mediated differentiation of pheochromocytoma cells by blocking c-Jun up-regulation. Mol Cancer Res. 2008; 6:325–339. [PubMed: 18314492]

57. Cascón A, Pita G, Burnichon N, Landa I, López-Jiménez E, Montero-Conde C, et al. Genetics of pheochromocytoma and paraganglioma in Spanish patients. J Clin Endocrinol Metab. 2009; 94:1701–1705. [PubMed: 19258401]

58. Erlic Z, Rybicki L, Peczkowska M, Golcher H, Kann PH, Brauckhoff M, et al. European-American Pheochromocytoma Study Group. Clinical predictors and algorithm for the genetic diagnosis of pheochromocytoma patients. Clin Cancer Res. 2009; 15:6378–6385. [PubMed: 19825962]

59. Pacak, K.; Eisenhofer, G.; Ahlman, H.; Bornstein, SR.; Gimenez-Roqueplo, AP.; Grossman, AB., et al. Nat Clin Pract Endocrinol Metab; International Symposium on Pheochromocytoma. Pheochromocytoma: recommendations for clinical practice from the First International Symposium; October 2005; 2007. p. 92-102.

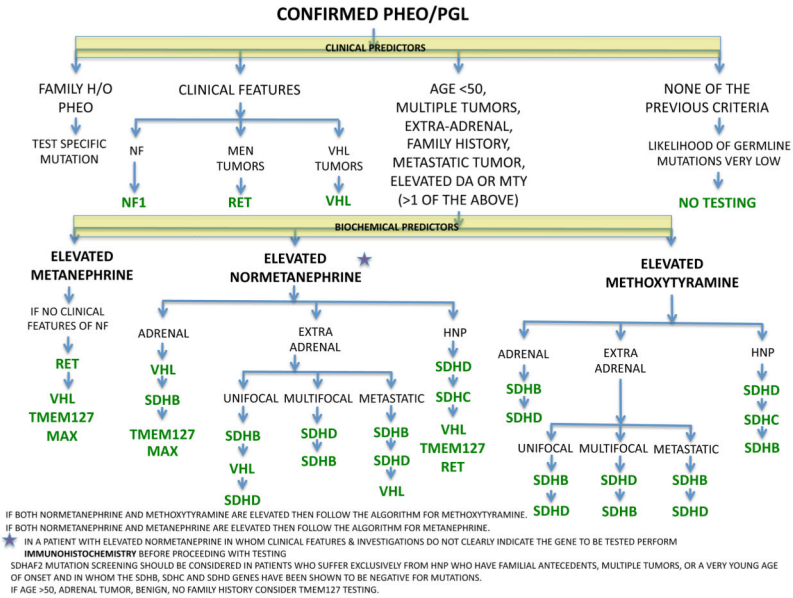

Figure 1.

Table 1

Clinical features of PHEOs/PGLs with a germ-line mutation in predisposing genes

Gene	Localization	Clinical features
RET	10q11.2	mostly benign, adrenal, commonly bilateral PHEOs; accompanied by other MEN 2 features: MEN 2A - MTC, hyperparathyroidism; MEN 2B - marphanoid habitus, mucosal ganglioneuromas
VHL	3p25.5	usually benign, adrenal, often bilateral, or multiple PHEOs, rarely sympathetic or head and neck PGLs; accompanied by other VHL features: retinal angiomas, central nervous system hemagioblastomas, renal cell carcinomas, islet cell tumors of the pancreas, cysts and cystadenoma in the kidney, pancreas, epididymis
NF 1	17q11.2	mostly adrenal PHEOs and very rarely sympathetic PGLs; preceded by other NF1 features: café-au-lait spots, mucosal and cutaneous neurofibromas, inguinal or axillary freckles, benign iris hamartomas (Lisch nodules), optic-nerve glioma, dysplasia of sphenoid bone dysplasia or pseudoarthrosis
SDHD	11q23	mostly benign, often multiple head and neck PGLs, sometimes sympathetic PGLs, very rarely PHEOs; paternal transmission of tumor susceptibility
SDHB	1p36.1-p35	often malignant, solitary sympathetic PGLs, rarely head and neck PGLs, or adrenal PHEOs; accompanied by other malignant tumors: renal cell carcinoma, gastrointestinal stromal tumors, breast and papillary thyroid carcinoma
SDHC	1q23.3	mostly benign and seldom multifocal head and neck PGLs
SDHAF2	11q12.2	so far exclusively head and neck PGLs; paternal transmission of tumor susceptibility
SDHA	5p15	very rarely abdominal extra-adrenal PGLs
TMEM 127	2q11.2	usually benign, adrenal, commonly bilateral PHEOs
MAX	14q23.3	adrenal, often bilateral, not seldom malignant PHEOs; probably paternal transmission of tumor susceptibility

PHEOs = phaeochromocytomas; PGLs = paragangliomas; MEN 2 = multiple endocrine neoplasia type 2; VHL = Von Hippel-Lindau disease; NF 1 = neurofibromatosis type 1; MTC = medullary thyroid carcinoma; *RET* = REarranged during transfection proto-oncogene; *VHL* = Von Hippel-Lindau disease tumor suppressor gene; *NF 1* = neurofibromatosis type 1 tumor suppressor gene; *SDHD* = succinate dehydrogenase subunit D gene; *SDHB* = succinate dehydrogenase subunit B gene; *SDHC* = succinate dehydrogenase subunit C gene; *SDHAF2* = succinate dehydrogenase complex assembly factor 2 gene; *SDHA* = succinate dehydrogenase subunit A gene; *TMEM 127* = TMEM 127 (transmembrane protein 127) gene; *MAX* = MAX (MYC associated factor X) gene