

REVIEWS

Recent Advances in the Genetics of Pheochromocytomas and Paragangliomas

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Abstract

Paragangliomas and pheochromocytomas are rare neuroendocrine tumors, which secrete catecholamines, with the same embryological origin from the neural crest cells. Pheochromocytomas develop from the adrenal medulla, while paragangliomas are extraadrenal tumors, evolving from the autonomic sympathetic and parasympathetic nervous chains. In the last 10 years, molecular medicine has discovered novelties in the understanding of genetics of these tumors. Although the majority of these tumors occur sporadically, recent medical discoveries have indicated that approximately 30-40% of these tumors are associated with an inherited mutation. In addition to this, developments in molecular pathology of pheochromocytomas and paragangliomas will provide the key to target specific cellular markers, assuring a personalised therapy. In this article we have reviewed the current medical literature, in order to summarise the most important aspects of genetics and clinical features of pheochromocytomas and paragangliomas.

Keywords: genetics, pheochromocytomas, paragangliomas

Rezumat

Feocromocitoamele și paraganglioamele sunt tumori neuroendocrine rare secretante de catecolamine, având aceeași origine embriologică din celulele crestei neurale. Feocromocitoamele se dezvoltă din celulele cromafine ale medularei glandei suprarenale, pe când paraganglioamele apar extradrenal la nivelul lanțurilor nervoase autonome simpatice și parasimpatice. În ultimii 10 ani, medicina moleculară a descoperit noutăți în înțelegerea determinismului genetic al acestor tumori. Deși majoritatea acestor tumori apar sporadic, descoperirile medicale recente au indicat că aproximativ 30-40% dintre acestea se asociază cu o mutație genetică moștenită. În plus, progresele în patogeneza moleculară a feocromocitoamelor și paraganglioamelor vor oferi cheia pentru a viza markeri celulari specifici, asigurând o terapie de viitor personalizată. În acest articol am realizat o revizuire aprofundată a literaturii medicale actuale de specialitate, cu scopul de a rezuma cele mai importante aspecte legate de genetică și caracteristicile clinice în feocromocitoame și paraganglioame.

Cuvinte cheie: genetica, feocromocitoame, paraganglioame

INTRODUCTION

Pheochromocytomas (Pheos) and paragangliomas (Pgls) are rare neuroendocrine tumors, with the same embryologic origin. Based on WHO classification, chromaffin tumors of the adrenal medulla are cal-

led Pheos, whereas tumors arising from extra-adrenal chromaffin cells, along the autonomous nervous chains, are named Pgls¹. Furthermore, there are two types of Pgls according to their homologous nervous chain: sympathetic and parasympathetic tumors. Sympathetic Pgls are developing from the pre- and paravertebral

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sympathetic ganglia (ex: Pgl's of the thorax, mediastinum, abdomen) and from the connective tissue within the pelvic organs (ex: urinary bladder Pgl). They release dopamine or norepinephrine (NE) because they lack phenylethanolamine N-methyltransferase (PNMT), the enzyme in charge of transforming NE into epinephrine (E), that is specific to adrenal medulla.

On the other hand, parasympathetic Pgl's originate from the parasympathetic tissue of the head and neck, arising in the orbit, nasal cavity, middle ear (tympanic Pgl), in the internal jugular vein region (jugular Pgl), along with the vagal nerve (base of skull), in the laryngeal region or the most frequent location in the bifurcation of the common carotid arteries (carotid body Pgl). Even though, they are in the vast majority nonsecreting tumors, with a slow growth rate, they can cause symptoms such as cranial nerve palsy, dysphagia and hearing loss due to their mass effect on adjacent neurovascular structures^{2,3}.

Additionally, the clinical presentation of patients with Pheos or sympathetic Pgl's can be very variable due to excessive catecholamine production. Continuous or paroxysmal hypertension is the most common clinical sign and "the classic triad" of symptoms consists in headache, palpitations, and perspirations⁴. These tumors are called 'the great masquerader', thus symptoms such as nausea, vomiting, panic attacks and muscle weakness should attract medical attention. Furthermore, the onset of symptoms can be triggered by physical exercise, surgery, stress, micturition or administration of certain drugs (metoclopramide, corticosteroids)⁵.

The overall incidence of Pheos and Pgl's is approximately 1 in 250,000 persons per year with the highest frequency in the fourth decade with a younger age of occurrence in the case of familial tumors and with no gender differentiation⁶. In hypertensive patients, the prevalence of Pheos/Pgl's ranges from 0.1 to 0.6%⁷, whereas Pheos are found in adrenal incidentalomas in about 5% of cases⁸.

The majority of Pheos/Pgl's are benign, but in 10–15% of cases these tumors are malignant, characterized by the occurrence of metastatic chromaffin cells in non-chromaffin organs such as lungs, liver, bone and lymph nodes⁹. Given the poor prognosis for patients with malignant Pheos/Pgl's, it was of great interest to develop methods for an early diagnosis and to determine the predictive factors.

Currently, there are no histological differentiation criteria between benign and malignant tumors, even though histological features such as size greater than 5 cm, Ki-67 index exceeding 4% and noradrenergic or dopaminergic secretory profile have been identified as

negative predictive factors^{9,10}. In addition to this, studies have reported that malignant Pheos/Pgl's occur frequently in patients which are carriers of SDHB gene mutations and also with high levels of serum chromogranin A and methoxytyramine (metabolite of dopamine) at the diagnosis moment¹¹.

GENETICS

In the past it was thought that 10% of Pheos and Pgl's were hereditary, caused by well-described mutations of three genes, thus resulting three different genetic syndromes: multiple endocrine neoplasia type 2 (MEN2) syndrome involving mutations of the protooncogene RET, von Hippel-Lindau (VHL) disease caused by mutations of VHL gene and neurofibromatosis type 1 (NF1) produced by NF-1 gene mutations. Nowadays, it is known that 30–40% of Pheos/Pgl's are genetically inherited and the recent molecular medicine discoveries describe 16 genes (RET, NF1, VHL, SDHA, SDHAF2, SDHB, SDHC, SDHD, MAX, TMEM127, HIF2A, KIF1B β , H-RAS, EGLN1/PHD2, FH, IDH) which play an important role in the pathogenesis of these tumors^{12,13}. This high percentage of inherited tumors indicate the need for genetic screening for all the patients with Pheos/Pgl's. Besides, these genes have been classified into two main tumorigenesis clusters, depending on the molecular pathways: 1) cluster 1, a pseudohypoxic cluster, represented by VHL, SDHx, HIF2A, FH, PHD2/EGLN1 genes and 2) cluster 2, rich in kinase receptor-signaling cluster, composed of NF1, RET, MAX, TMEM127, KIF1B genes^{14,15}. These clusters indicate two different pathways of tumor growth, emphasizes the need for further researches, in order to contribute to the discoveries of new molecular targeted treatments.

A. Familial syndromes associated with Pheos/Pgl's

Multiple endocrine neoplasia type 2 (MEN2) syndrome

MEN2 syndrome is caused by activating germline mutations in the REarranged during Transfection (RET) proto-oncogene located on chromosome 10. RET gene encodes for a transmembrane receptor of the tyrosine kinase family, which plays an important role in the cell growth, migration, differentiation and apoptosis. According to the latest classification, MEN 2 syndrome is divided into two categories: MEN2A and MEN2B. The former is further divided in four subtypes: classical MEN2A (the most common), MEN 2A associated with cutaneous lichen planus amyloido-

sis, MEN 2A associated with Hirschprung disease and familial medullary thyroid cancer (FMTC)¹⁶. Classically, MEN2A is represented by the clinical manifestation of Pheo in 50% of cases, hyperparathyroidism in 15–30% of patients and medullary thyroid carcinoma, usually the first and most common clinical feature¹⁷. In MEN 2B, a more aggressive subtype, the hyperparathyroidism is absent, while medullary thyroid carcinoma is found in all the patients and Pheo in 50% of cases. MEN2B patients can present with mucosal neuromas, intestinal ganglioneuromas and a specific marfanoid habitus¹⁸. FMTC is represented by the medullary thyroid cancer as the single clinical manifestation.

Most of the genetic mutations found in MEN2A syndrome affect the cysteine residues, encoded in RET exons 10 (codons 609, 611, 618, and 620), 11 (codon 630,634) and also exons 8,13,14,15,16. Nevertheless, the most frequent mutation found in MEN2A patients is at codon 634 in exon 11¹⁹. The majority of MEN2B mutations affect exon 16 (codon 918), but genetic defects in exon 15 (codon 883) have also been described²⁰.

Furthermore, genetic defects which increase the risk for developing Pheos, have been classified in three risk classes: highest-risk category refers to RET (codon 918), SDHB and SDHD mutations, high-risk class includes RET (codons 609, 611, 618, 620,630, 634) and VHL gene missense mutations and lowest risk class comprises RET (codons 768, 790, 791, 804,891) and VHL truncating defects^{16,21}.

It is very important to notice that MEN2 patients who present Pheos are usually identified at a younger age than patients with sporadic Pheos, have multifocal epinephrine secreting tumors and present a low risk of malignancy²².

Von Hippel-Lindau (VHL) disease

Genetic defects of the von Hippel-Lindau (VHL) gene, found on chromosome 3, lead to VHL disease development. The VHL protein encoded by the above mentioned gene, modulates the actions of hypoxia inducible factor - alpha (HIF- α), thus controlling collagen IV synthesis and blood vessels development. VHL disease is characterized by a predisposition to develop multiple tumors such as: retinal, spinal and cerebellar hemangioblastomas, pancreatic tumors, clear cell renal carcinomas, endolymphatic sac tumors, cystadenomas of the kidney, epididymis and broad ligament. The disorder is subdivided into two categories according to the risk of Pheos/Pgls: VHL disease type 1 without Pheos/Pgls and VHL disease type 2 characterized by the presence of these tumors. Type 2 VHL disease is further divided into three classifications according to

the presence of clear cell renal carcinomas: 2A without clear cell renal carcinoma, 2B with clear cell renal carcinoma and 2C characterized exclusively by the presence of Pheos/Pgls²³.

Bilateral Pheos occur in approximately 20–40% of patients, mostly carriers of missense mutations of VHL gene at codon 167 (exon 3), presenting a norepinephrine biochemical phenotype²⁴. Pheos develop in the childhood period in approximately 40% of cases²⁵. Furthermore, rare sympathetic Pgls cases were described and the malignant potential of Pheos/Pgls within this disorder is very low²⁶.

Neurofibromatosis type 1 (NF1) syndrome

Neurofibromatosis type 1 (NF 1) syndrome also known as von Recklinghausen's disease is an autosomal dominant pathology produced by genetic defects of NF1 gene localised on the long of chromosome¹⁷, which encodes for a protein called neurofibromin. The diagnosis of NF1 is made on at least two of the next criteria: six or more café-au-lait spots, two or more cutaneous neurofibromas, two or more iris hamartomas (Lisch nodules), optic-nerve glioma, intertriginous freckling, pseudoarthrosis or dysplasia of the sphenoid bone and a first-degree relative with NF1. Secondly, NF1 patients could present with sarcomas, chronic myeloid leukemias of childhood, seizures, macrocephaly, short stature and Pheos/Pgls^{17,22,27}. Pheos within this syndrome, which are very rare described (2–5% of cases), develop unilateral or bilateral and have an epinephrine or norepinephrine secretory profile²⁷.

Familial paragangliomas syndromes (SDHx and SDHAF2)

Inherited paragangliomas syndromes are determined by mutations in the genes encoding for the succinate dehydrogenase (SDH) mitochondrial complex. SDHx genes mutations lead to inactivation of SDH, increasing succinate and oxygen free radicals, stabilization of hypoxia-inducible factor 1 alpha (HIF-1 α) and consequently expose tissues to chronic hypoxia and anaerobic cellular proliferation, specific for tumoral cells. Even if mutations in the SDHx genes affect the same complex, their clinical manifestations can be very different^{22,28,29}.

Mutations of the SDHA gene, located on chromosome 5, that encode for subunit A of the SDH complex were first described in Leigh syndrome, a neurodegenerative disorder³⁰. More recently, heterozygous SDHA gene mutation carriers, have been reported with Pheos and Pgls³¹. However, further studies are required in order to establish phenotype-genotype correlations and risk of malignant transformation.

Besides, SDHAF2 or SDH5 gene localised on chromosome 11, encodes for a protein, which is responsible for flavination of the SDHA subunit. Its genetic defects presenting a paternal inheritance can cause exclusively multifocal benign head and neck PGLs, defining familial PGL type 2^{32,33}.

Mutations in the SDHB gene localised on chromosome 1 define familial PGL syndrome type 4. They are the most common gene mutations found in Pheos/PGLs and they are inherited by autosomal dominant transmission. Their clinical picture consists of multiple aggressive Pheos/PGLs with younger ages at diagnosis time and with a high risk of malignancy (30-70%)^{34,35}. In addition to this, SDHB gene have been also linked with gastrointestinal stromal tumors (GIST), papillary thyroid neoplasm, breast cancer and clear cell renal cancer³⁶. It is worth noticing that numerous studies recommend screening for SDHB gene mutations for all the patients with Pheos/PGL, especially for those with malignant tumours or with noradrenergic or dopaminergic biochemical phenotype^{37,38}.

Furthermore, SDHC gene mutations, localised on chromosome 1, have been associated with familial PGL type 3, which is an autosomal dominant disorder, characterized classically by the clinical occurrence of head and neck benign PGLs³⁹.

Moreover, SDHD gene mutations found on chromosome 11, have been described in familial PGL type 1, presenting a paternal transmission. Patients with PGL type 1 syndrome present frequently with multifocal benign parasympathetic head and neck PGLs, whereas sympathetic PGLs and Pheos cases have also been described^{37,40}.

Furthermore, rare tumor syndromes, such as Carney-Stratakis dyad and Carney triad have also been described in association with SDHx genes. Carney-Stratakis dyad is characterized by Pheos/PGLs and gastrointestinal stromal tumors (GIST), while Carney triad consists in GIST, Pheos/PGLs and pulmonary chondromas⁴¹.

B. New genes related to PHEOs/PGLs

Recently, new susceptibility genes for Pheos/PGLs have been identified, increasing the level of knowledge regarding the pathogenesis of these tumors. The KIF1Bβ (Kinesin Family Member 1B) gene is located on chromosome 1p36.22. and encodes for a transport protein involved in neuronal cells apoptosis. Different studies have reported its implication in cases of Pheos/PGLs and neuroblastoma^{42,43}. The transmembrane protein 127 (TMEM127), a tumor suppressor gene, located on 2q11.2 chromosome has been pointed as a cause for frequently benign unilateral or bilateral Pheos/PGLs^{44,45}.

Another tumor suppressor gene, MYC-associated factor X (MAX), located on 14q23.3 chromosome, implicated in the modulation of cell development, has been linked to malignant Pheos/PGLs⁴⁶. Furthermore, hypoxia-inducible factor 2-alpha (HIF2A) gene defects located on 2p21 chromosome, have been associated with the development of Pheos/PGLs⁴⁷. Its mutations were reported in patients with multiple Pheos/PGLs, polycythemia and somatostatinomas, the authors defining a new medical entity, Pacak-Zhuang syndrome. Somatic mutations in the HRAS gene have been reported in few cases of Pheos/PGLs⁴⁸. Moreover isocitrate dehydrogenase (IDH) gene somatic mutation has been reported in a carotid PGL case⁴⁹. Furthermore, germline mutations in fumarate hydratase (FH) and egg-laying-defective nine 1 (EGLN1/PHD2) genes were described in cases of malignant Pheo, respectively multiple PGLs^{50,51}.

Patient management

The initial work-up of the patients should start with a thorough clinical examination, looking for clinical clues: skin neurofibromas, intertriginous freckling or café-au-lait spots indicating a NF1 syndrome or a thyroid nodule found in medullary thyroid carcinoma in MEN2 syndrome. The next step should be the patient's family and past medical history. It is important to remember that even in the absence of any known family history of Pheos/PGLs, the existence of sudden death at younger ages due to strokes in family members could indicate the possibility of a familial disease. Furthermore, modified paraclinical examinations should draw attention: positive ocular fundoscopic examination for retinal haemangioblastomas suggestive for VHL disease, positive computer tomography scans for renal or pancreatic tumors could indicate also VHL disease, elevated catecholamines, metoxytyramine, chromogranin A and calcitonin could orient to neuroendocrine tumors. Genetic testing should be proposed to patients with age below 45 years, with multifocal tumors or presenting distant metastases, with a positive family or personal medical history of Pheos/PGLs^{13-15,17,26}.

Given the high prices and lack of availability of the genetic tests, studies concluded that the decision to test for a specific gene should be done based on tumor location, malignant potential, secretory profile or specific clinical or biochemical features. In case of positive family history or specific clinical manifestations, RET and VHL genes should be tested firstly. The mutational analysis technique is difficult due to the large size of the NF1 gene and the existence of many different mutations. Therefore, the diagnosis is based on the clinical examination.

Furthermore, if the patient presents with bilateral tumors, RET, VHL, SDHB and SDHD genes mutations should be searched^{22,28,29,52}. In case of malignant Pheos/Pgls, the genetic screening should begin with SDHB gene, followed by MAX, SDHD and VHL genes^{9,10,11,37}. In case of Pgls the first genes that need to be tested are SDHB,SDHC,SDHD and SDHAF2^{13,14,26,28}. If particular biochemical feature such as polycythemia occurs, HIF2A gene should be tested⁴⁷. According to the secretory behaviour, a dominant epinephrine profile should indicate RET and NF1 genes testing, an elevated norepinephrine secretion should suggest VHL and SDHB genes screening, while an increased dopamine or its metabolite metoxytyramine should lead to SDHB and SDHD genes screening^{53,54}. Presented data were summarized in Figure 1.

CONCLUSIONS

There have been numerous recent discoveries regarding the genetics of Pheos and Pgls in the last 10 years. Currently, it is recognised that approximately 30-40% of Pheos/Pgls have a genetic pathogenesis. They can compose the clinical picture of a genetic syndrome or may occur apparently sporadic. Thus, 16 gene mutations (RET, NF1, VHL, SDHA, SDHB, SDHC, SDHD, SDHAF2, MAX, TMEM127, HIF2A, KIF1B, H-

RAS, PHD2/EGLN1, IDH, FH) were indicated as being involved in the development of these tumors. Essentially, patients diagnosed with Pheos/Pgls and with one of the following clinical criteria should undergo genetic testing: a younger age (below 45 years), multiple, recurrent or malignant tumors, a positive genetic familial syndrome or a personal medical history of head and neck Pgls. Furthermore, in order to identify the causative genes, it is important to continue the genetic screening relying on an algorithm which include: the secretory profile, localization of the tumor, malignant character and previous family history. The detection of a gene mutation in a patient with Pheo/Pgl is of great interest, particularly because it can assure an early diagnosis and an adequate treatment with a regular follow-up and consequently it can lead to a better prognosis for patients and their relatives. The future clinical studies based on next-generation sequencing methods will provide valuable information for defining the genotype-phenotype correlations and will offer new options in the clinical management of Pheos/Pgls. Moreover, recent and future discoveries regarding molecular pathogenesis of Pheos/Pgls will assure a starting point for the future personalized treatment. In this article we have reported the most significant aspects regarding the clinical characteristics and recent advances in the genetics of Pheos/Pgls.

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