Chapter 8
Hereditary Diseases Predisposing to Pheochromocytoma (VHL, NF-1, Paraganglioma Syndromes, and Novel Genes)

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Abstract Pheochromocytomas (Pheo) and paragangliomas (PGL) are rare tumors originating from catecholamine-producing chromaffin cells. They occur approximately in 0.1% of patients affected with hypertonia. Pheo/PGL may manifest itself at any age; in 10% of the patients, the disease is bilateral, and also in 10% it occurs outside of the adrenal medulla. From a genetic aspect, a considerable proportion of these tumors represents a prototype for an autosomal dominantly inherited syndrome with incomplete penetrance. In addition, to date more than 15 genes have been identified representing genetic susceptibility for Pheo/PGL and accounting for 40% of all cases. In general, in familiar cases, the tumor manifests at younger age, and they are often occurring as multiplex tumors. Permanent recovery can be achieved with an early diagnosis and with a successful surgical removal of the tumor tissue. On the other hand, undiagnosed, hormonally active Pheos may lead to severe, or even lethal, consequences. This chapter will summarize our recent knowledge about the genetics of Pheo/PGL, focusing on tumor syndromes where Pheo/PGLs are among the main manifestations.

Keywords Pheochromocytoma · Paraganglioma · Hereditary tumor syndromes

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List of Abbreviations

CT	Computed tomography
FuH	Fumarate hydratase
GAP	GTPase-activating protein
GLUT1	Glucose transporter 1
HIF-1	Hypoxia-inducible factor type 1
HNPGL	Head and neck paraganglioma
KIF1	Kinesin-like protein 1
LDH	Lactate dehydrogenase
MAX	MYC-associated factor X
MDH2	Malate dehydrogenase type 2
MIBG	Meta-iodobenzylguanidine
MLPA	Multiple ligation probe amplification
MRI	Magnetic resonance imaging
NF1	Neurofibromatosis type 1
PDGF	Platelet-derived growth factor
PGL	Paraganglioma
PHD1,2	Prolyl-hydroxylases 1 and 2
Pheo	Pheochromocytoma
RT-PCR	Reverse transcription polymerase chain reaction
SDH	Succinate dehydrogenase
SLC25A11	Solute carrier family 25 member 11
TCA	Tricarboxylic acid cycle
TMEM127	Transmembrane protein 127
VEGF	Vascular endothelial growth factor
VHL	von Hippel-Lindau

8.1 Introduction

Pheochromocytomas (Pheo) and paragangliomas (PGL) are tumors arising from neural crest cells. There are some discrepancies in terminology, but in this chapter we use the term “Pheo” for tumors located in the adrenal gland (adrenal medulla), while the term “PGL” is used for tumors located both at the head and neck (HNPGL) or intra-abdominal but extra-adrenal PGL. For PGLs, the most prominent location is the carotid body, but tumors in the vagal, jugular, and tympanic glomus are also observed. Usually, both Pheos and PGLs are benign, slowly growing tumors. Hormonal activity is related to their catecholamine synthesis and release, and major clinical manifestations and symptoms are also caused by catecholamines. Pheo are more hormonally active compared to head and neck PGL. For evaluation of hormone activity, collected urine for determination of catecholamine and catecholamine metabolites as well as serum chromogranin A assay are recommended (Lenders et al. 2014).

Hormonally inactive tumors are usually detected incidentally through imaging studies of the abdomen, thorax, or skull. On the other hand, in patients with suspicion for Pheo and PGL, imaging studies are important part of the diagnosis. Computed tomography (CT) and magnetic resonance imaging (MRI) as well as carotid ultrasound for head and neck PGLs, while for intra-abdominal tumors abdominal ultrasound, CT and MRI, and meta-iodobenzylguanidine (MIBG) scan are used. In many cases, most often in the case of extra-adrenal tumors, PET is the most effective diagnostic tool. In familial cases, tumors may be multifocal, and their localization is best identified by MIBG. The definitive diagnosis of PGL is achievable by histological examination. It must be noted that malignancy of Pheo/PGL cannot be established by histological diagnosis, and malignancy can only be based on clinical criteria, i.e., presence of metastases. Metastases can appear several years after tumor removal. Thus, although malignancy is rare, all Pheo/PGLs should be considered potentially malignant and should be followed up regularly (Plouin et al. 2016).

In general, these tumors are rare; an annual incidence is approximately 1 per 100,000. HNPGL represents less than 0.5% of all head and neck tumors. 20% of Pheo and PGLs are diagnosed in children, where usually symptomatic, bilateral, and extra-adrenal tumors are typical. Of note, many Pheos were unidentified during life and were diagnosed during autopsies. The prevalence of Pheo is about 1:2000 in autopsy series. Therefore, clinical diagnosis in many cases is missed. The main reason behind this phenomenon is the lack of specific clinical symptoms or lack of biochemical evidence. Genetics and developments of diagnostic procedures from the past decade might help in improving the diagnostics.

Genetic background is well established in Pheo/PGLs. Some of these tumors develop within a classical tumor syndrome including multiple endocrine neoplasia type 2 (MEN2), von Hippel-Lindau disease, neurofibromatosis type 1, and hereditary PGL syndromes (PGL1-5). Patients with hereditary Pheo have a lifelong risk of second primary tumors and relapse. The recent findings about their pathogenesis and phenotypic characteristics help to reduce their morbidity and mortality. In many cases gene- or even codon/mutation-specific preventive medical management has been described (Frank-Raue et al. 2011; Bausch et al. 2017).

Early onset of disease, bilateral, multifocal, extra-adrenal, and malignant tumors are clinical hallmarks of hereditary disease. Pheo/PGLs are the most genetically determined tumors. Except for *RET* proto-oncogene, other genes involved in genetic susceptibility for Pheo/PGLs are classical tumor suppressor genes. The tumorigenesis mechanism in these cases follows the Knudson described mechanism. The first mutation (first hit) appears germline, but for the development of the tumor, an additional somatic mutation or loss of wild type allele (second hit) is necessary. Tumor suppressor genes associated to date with development of Pheo/PGLs are the following: *SDHD*, *SDHC*, *SDHB*, *SDHAF2*, *SDHA*, *VHL*, *HIF2*, *FuH*, *EGLN1*, *EGLN2*, *KIF1ß*, *NF1*, *MAX*, *TMEM127*, *GOT2*, *MDH2*, and *SLC25A11* (Bausch et al. 2017; Buffet et al. 2018). *RET* mutations cause multiple endocrine neoplasia type 2. This syndrome was discussed in the previous chapter (Chap. 7), but all other genes will be presented in detail in this current chapter.

8.2 von Hippel-Lindau Syndrome (VHL) (OMIM NM_000551.3)

8.2.1 Prevalence, Phenotype

The VHL syndrome was first described by Eugen von Hippel and Arvid Lindau. Generally it manifests with central nervous system's vessel-related tumors (hemangioblastomas of the cerebellum, spinal cord, and retina), renal cell carcinoma, endolymphatic cancer and Pheo. Moreover, it is often accompanied by cystic lesions of the kidney, the pancreas, and the epididymis. Pancreatic neuroendocrine tumor also occurs, and it represents significant morbidity and mortality (Neumann et al. 2004).

Prevalence is estimated at 1/53,000 and annual birth incidence at 1/36,000. Pheo is estimated to develop in 10–20% of VHL patients, but depending on the mutation, major phenotype differences can be observed between VHL families.

Classification of VHL subtypes is based on their specific genotype-phenotype correlations, depending on the presence of Pheo. Patients with VHL type 1 have very low risk for Pheo, while type 2 patients often develop Pheo. The mortality due to Pheo in VHL patients is estimated to be 5%. Pheo appears earlier in VHL patients compared to sporadic cases (generally at age 30 years), and the manifestation is often bilateral and multiplex, but malignant cases are rare (Chen et al. 1995; Eisenhofer et al. 2001). Tumor can be extra-adrenal and intra-abdominal, and even extra-abdominal.

Hemangioblastomas are the most frequent tumors in VHL syndrome, developing in 60–80% of VHL patients, generally around age 30 years. The most frequently affected regions are the spinal cord (50%), the cerebellum (37%), and the brainstem (10%). The clinical symptoms are influenced by the location, size, associated edema, and cysts as well. Tumors of the cerebellum are accompanied by headaches, dizziness, nausea, and imbalance and ataxia; brain stem location is associated with hyperesthesia, headaches, dysphagia, and hyperreflexia. Hyperesthesia and pain could also point to spinal cord localization. Although the hemangioblastomas are slowly growing, benign tumors, the subsequent increased pressure in the central nervous system is accountable for major cases of VHL morbidity and mortality.

Angiomas of the retina occur in 50% of the cases. These benign tumors are often recurrent, bilateral, and multiplex. Retinal detachment and blindness regularly develop without definitive therapy (laser coagulation, cryotherapy); therefore, consultations with an ophthalmologist should take place yearly.

Renal cell carcinoma is the second most common lesion in VHL, occurring approximately in 24–70% of the patients. Renal cell carcinoma is often multiplex, bilateral, rapidly progressing tumor with a potential to form metastases early to the regional lymph nodes, the liver, and the brain. Despite these severe manifestations, the disease is usually silent for years, and symptoms (hematuria and pain) appear in advanced stages only.

Pancreas lesions (usually cysts) accompany VHL in 25% of the cases, but autopsy confirms this number is around 70%. Islet cell tumors also appear in 5–10% of the patients. Clinical symptoms are related to the size of the cyst (abdominal discomfort or pain, rarely pancreatitis) (Neumann et al. 1991). Diagnosis can be achieved with ultrasound or CT. The treatment depends on the extension of the cyst. In solitary cases surgical excision is recommended, but if there are no clinical symptoms or signs of malignancy, even conservative therapy is possible with continuous control.

8.2.2 *Genetic Background: Function of the VHL Tumor Suppressor Gene*

Located in the third chromosome's short arm (3p25 locus), *VHL* tumor suppressor gene's germline mutations are responsible for the von Hippel-Lindau syndrome. According to Knudson's hypothesis (Chap. 1), tumors develop in patients where the wild allele becomes mutated or deleted. *VHL* gene encodes the VHL protein, which forms a stable ubiquitin-ligase complex with Rbx1, Cullin2, and elongin B and C proteins. This complex mediates the function of RNA polymerase II and participates in the ubiquitin-mediated degradation of hypoxia-inducible factor 1 (HIF-1). The prolyl-hydroxylase enzyme serves as a molecular sensor in the presence of oxygen and through hydroxylation degrades the alfa subunit of HIF-1. Under hypoxic conditions or when *VHL* mutated, HIF-1α stabilizes and attaches to HIF-1ß and then translocates to the nucleus where it promotes the transcription of its target genes. The activated genes (VEGF, PDGF, erythropoietin, LDH, and GLUT1 transport) are responsible for the accommodation to hypoxic conditions. This mechanism is called pseudo-hypoxia, and it has also been described in tumors associating with mutations of succinate dehydrogenase subunits encoding genes (*SDHx, discussed later)*. In addition, VHL participates in the regulatory functions of the mitochondria and the cell cycle. According to novel data, VHL is also engaged in the organization of microtubules and in the formation of cilium due to the interaction with the PAR proteins which determine the membrane domains of the polarized epithelial cells. Loss of VHL function results in the false organization of microtubes with the inhibition if ciliogenesis, which results in the disturbance of the cytoskeleton and in the loss of polarization of the epithelial cells (Kaelin Jr 2002).

From genotype-phenotype associations should be highlighted that truncating mutations and large deletions associate with type 1 disease, while missense mutations with type 2 disease. Mutations which disrupt the VHL-HIF protein interaction more often cause renal cell cancer, while mutations in other parts of the gene lead to Pheo. In addition, missense mutations located at the surface of the protein have been found more commonly in Pheo compared to missense mutations located in the deeper protein region (Ong et al. 2007).

8.2.3 Genetic Diagnosis and Genetic Counseling

VHL syndrome is an autosomal dominant disease; therefore, there is 50% chance of an offspring to inherit the disease-related gene alteration from their parents. The whole *VHL* gene needs to be screened in case of VHL syndrome. This requires the bidirectional sequencing of the coding regions and the utilization of the quantitative molecular biological technologies which can detect the heterozygote loss of certain exons. Routine examination for large germline deletions of the *VHL* gene is mandatory, because so-called large deletion can be observed in 15–20% of the VHL patients. Exceptionally sensitive methods are necessary for the detection of germline heterozygote deletions, for example, southern blot hybridization, quantitative real-time PCR, and multiplex ligation probe amplification (Gergics et al. 2009). The clinical manifestation of VHL syndrome varies even between patients with the same mutation, therefore strict clinical and laboratory observation of the pathogenic mutation carriers is highly recommended. The comprehensive and regularly updated VHL mutation database can be found at http://www.umd.be/VHL/.

Pheo is present in 10–20% of VHL patients, but differences in the phenotype can be observed depending on the mutations (Chen et al. 1995). Pheo can be the earliest manifestation of the disease, and it accounts for at least half of the Pheo developed in childhood (Bausch et al. 2014). Malignancy is rare in VHL-associated Pheos, but clinically symptomatic tumors are often developing, and rarely HNPGLs can also be detected. Clinical screening for Pheo in VHL-associated families starts at age 5 years, and annual plasma-free metanephrines or urinary catecholamine and catecholamine metabolites are indicated.

8.3 Neurofibromatosis Type 1 (OMIM 613675)

8.3.1 Prevalence, Phenotype

Neurofibromatosis type 1 (NF-1) or von Recklinghausen disease is an autosomal dominantly inherited hereditary genetic disorder with neurocutaneous abnormalities. Its prevalence is approximately 1: 3–4000 (Bausch et al. 2007).

NF-1 is characterized by neurofibromas on the skin, pigmented “cafe au lait” spots, and the hamartomas of the iris (Lisch nodules) (Otsuka et al. 2001). The disease may be accompanied by nerve optic glioma, Pheo, and carcinoid tumor. Pheo is relatively rare (about 1% of cases) and is expected to occur in older age, but in patients with hypertensive neurofibromatosis, the frequency of Pheo can be as high as 50%. Neurofibromas are benign tumors of the peripheral nerve sheath; they may manifest as cutaneous, subcutaneous, or plexiform lesions. Compression of peripheral nerves, spinal nerves, or spinal cord can cause neurological symptoms. NF1 patients have about 7–12% chance of developing malignant neoplasmic tumors, which often develop from existing subcutaneous or plexiform neurofibromas

(in contrast, cutaneous neurofibromas do not transform into malignant form). Café au lait spots can be confirmed in 95% of patients, usually before the age of 30 years. The lesions appear most often in the intertriginous areas first. Occasionally, hypopigmented macules may also appear. Malignant central nervous system tumors, cutaneous hemangiomas, and NF-1-related vasculopathy might also occur.

In terms of differential diagnostics, other syndromes with skin manifestations should be considered (phacomatosis syndromes including Cowden disease, Carney complex, tuberous sclerosis, *discussed in Chaps. 7 and 9 of this book*).

Management of the disease depends on the tumors. Treatment of benign, non-symptomatic neurofibromas is not required. In more severe cases, when neurofibromas develop in the central nervous system and affecting the optic nerve, surgical removal is recommended. Treatment of Pheos is always surgical. The prognosis of NF-1 is good; morbidity and mortality depend on the number of neurofibromas and their subtype. In most cases, regular (yearly) physical examination and ocular examination of patients is sufficient. Rarely, severe malignant tumors can occur (<10% of cases), and NF1 vasculopathy may develop, which includes stenosis of the kidney artery, possibly coarctation of the aorta. In these cases, the morbidity and mortality rates are worse (Bausch et al. 2007).

8.3.2 Genetic Background: Function of the NF1 Protein

NF1 is caused by mutations of the *NF1* tumor suppressor gene, located on the short arm of chromosome 17 (17q11.2). The product of the *NF1* gene is the cytoplasmic-derived neurofibromin protein, which is expressed in large numbers of cells in the central nervous system. Neurofibromin has a role in the signal transduction pathway of the mitogenetic regulatory Ras system. Connecting to the Ras/GTPase-activating protein (GAP), neurofibromin catalyzes the hydrolysis of the activated Ras and thus promotes the formation of inactive GDP-bound Ras, which inhibits cell proliferation. When the neurofibromin function is impaired, MAPKs are activated (Barker et al. 1987).

8.3.3 Genetic Diagnosis and Genetic Counseling

In contrast to other autosomal dominant hereditary syndromes, molecular genetic screening of *NF1* gene was not part of the routine diagnostics before next-generation sequencing era. Nowadays, using comprehensive cancer panels or Pheo/PGL-specific targeted sequencing, the molecular analysis of *NF1* is widely available. Genotype-phenotype correlations are unknown.

The disease has an autosomal dominant hereditary pathway; hence, the offsprings have a 50% chance to inherit the disease-causing mutation from the affected parent. Prenatal testing is also available.

8.4 Hereditary Pheo/PGL Syndromes

This subgroup of hereditary Pheo/PGLs is caused by mutations of the genes encoding the subunits of succinate dehydrogenase (SDH). SDH is composed of four subunits, of which subunits A and B are catalytic subunits and participate in succinate-fumarate hydroxylation. Subunits C and D anchor SDHA and SDHB to the inner mitochondrial membrane. Besides participating in the tricarboxylic acid cycle (TCA), SDH is involved in mitochondrial electron transport chain too. Since the first report showing that the germline mutation of the *SDHD* associated with Pheo/PGLs during the past 18 years, mutations of genes encoding other subunits of *SDH* or mutations of genes encoding other enzymes of TCA have been identified as pathogenetic factors of Pheo/PGL.

The common genetic feature for SDH subunits is that all these genes are nucleus-encoded genes; hence, the disease follows the classical autosomal dominant pattern. Chromosomal localizations and associated phenotypes are listed in Table 8.1.

In familial cases, germline mutations in *SDHB*, *SDHC*, and *SDHD* can be confirmed in about 50–70% of cases. Besides the familial cases, the apparently sporadic cases carry a mutation in one of the *SDHx* genes in 25–30% of the affected patients. Several genotype-phenotype relationships can be observed based on the available literature; however, overlaps between these syndromes are also known.

8.4.1 Paraganglioma Syndrome Type 1 (OMIM 168000)

It was reported in 2000 for the first time that germline mutation of *SDHD* is associated with Pheo/PGL (Baysal et al. 2000). From that time, several reports confirmed that *SDHD* mutations contribute to both intra-abdominal and HNPGLs. The gene is a relatively small gene, encoded by 4 exons. Disease-causing mutations spread all over the gene. Nonsense, frameshift, and missense mutations have been described, but there are more mutations leading to protein breakage. For precise genotype-phenotype associations and for every day clinical genetics practice an online tool, the SDH mutation database (https://databases.lovd.nl/shared/genes/) is available. *SDHD* gene is maternally imprinted which means that only patients inheriting the mutation from the paternal side will be clinically affected (Fig. 8.1).

Based on data obtained in the largest cohort of independent patients with Pheo/PGLs showed that multiple, benign tumors occurred in 74% of *SDHD* mutation carriers. Adrenal Pheos occurred in 53%, extra-adrenal PGLs in 21%, and HNPGLs in 79% of *SDHD* carriers. Age-related penetrance reached 86% by age 50 years for *SDHD* carriers (Neumann et al. 2002).

Table 8.1 Genes and syndromes presenting genetic susceptibility for pheochromocytoma and paraganglioma

Gene (Ref seq.)	Chromosomal localization	Syndrome	Year of gene discovery	Predominant localization of Pheo/PGL tumors	Other manifestations
NF1 NM_000267.3	17q11.2	Neurofibromatosis type 1	1990	Adrenal	Cafe au lait spots Neurofibromas
RET NM_020975.4	10q11.21	Multiple endocrine Neoplasia type 2	1994	Adrenal, freq. bilateral	Medullary thyroid cancer Hyperparathyroidism Neurinoma (MEN2B)
VHL NM_000551.3	3p25.3	von Hippel-Lindau disease	1993	Adrenal, freq. bilateral, extra-adrenal Pheo	Retina angioma Hemangioblastoma Renal cell cancer
SDHD NM_003002.3	11q23.1	PGL1	2000	Head and neck PGL Intra-abdominal Pheo and PGL	C cell hyperplasia and hypercalcemia
SDHC NM_003001.3	1q23.3	PGL3	2001	Head and neck PGL	Gastrointestinal stromal tumor
SDHB NM_003000.2	1p36.13	PGL4	2000	PGL intra-abdominal, malignant	Renal carcinoma, gastrointestinal stromal tumor, leiomyomas
PHD2	1q42.2		2008	PGL	Familial erythrocytosis type 3
KIF1B	1p36.22		2008	Adrenal Pheo	Charcot-Marie-Tooth Disease, Axonal, Type 2a1
SDHAF2/SDH5 NM_017841.2	11q12.2	PGL2	2010	PGL	
TMEM127 NM_017849.3	2q11.2		2010	Adrenal Pheo	
SDHA NM_004168.3	5p15.33	PGL5	2011	PGL	Leigh's syndrome
MAX NM_002382.4	14q23.3		2011	Adrenal Pheo, PGL	
MDH2	7q21.12		2015	Adrenal Pheo, PGL	Epileptic encephalopathy

(continued)

Table 8.1 (continued)

Gene (Ref seq.)	Chromosomal localization	Syndrome	Year of gene discovery	Predominant localization of Pheo/PGL tumors	Other manifestations
FuH	1q43		2014	PGL	Renal cell cancer, leiomyomas
GOT2	16q21		2017	Adrenal Pheo, PGL	
SLC25A11	17p13.2		2018	PGL	

NF1 neurofibromatosis type 1, *RET* rearranged during transfection, *SDHD* succinate dehydrogenase subunit D, *SDHB* succinate dehydrogenase subunit B, *SDHC* succinate dehydrogenase subunit C, *SDHA* succinate dehydrogenase subunit A, *SDHAF2* succinate dehydrogenase assembly factor 2, *PHD2* prolyl-hydroxylase type 2, *KIF1B* kinesin family member 1B, *TMEM127* transmembrane protein 127, *MAX* Myc-associated factor X, *MDH2* malate dehydrogenase type 2, *FH* fumarate hydratase, *GOT2* glutamic-oxaloacetic transaminase 2, *SLC25A11* solute carrier family 25 member 11 (oxoglutarate/malate carrier 2), *VHL* von Hippel-Lindau, *PGL* paraganglioma, *Pheo* pheochromocytoma (adrenal localization), *PGL1-5* paraganglioma syndrome type 1-type 5, *MEN2B* multiple endocrine neoplasia type 2B

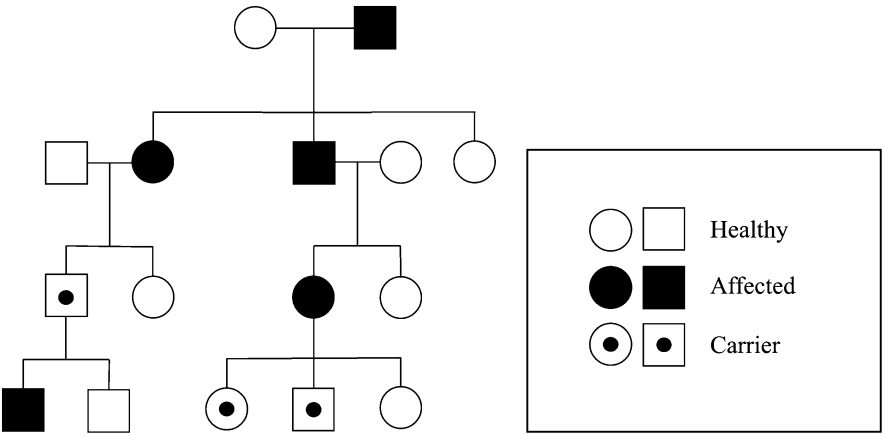

Fig. 8.1 Schematic pedigree showing maternal imprinting in a family with SDHD mutation

8.4.2 Paraganglioma Syndrome Type 2 (OMIM 601650)

The gene responsible for PGL2 is *SDHAF2* (also known as *SDH5)* which encodes a factor necessary for flavination of the SDHA protein. The gene was identified in 2009 (Hao et al. 2009). It represents an extremely rare (<1%) cause for HNPGLs. The inheritance is similar to those described for *SDHD* (autosomal dominant with paternal transmission). Very few genotype-phenotype associations were reported. Based on data obtained on the largest kindred (Kunst et al. 2011), the average age of onset was 33 years, while the penetrance by age 50 years reached 100%. No malignancy was reported. Genetic analysis, therefore, should be offered for patients with HNPGLs who were negative for *SDHD* and *SDHC* mutations, but it should also highlight that the chance to detect any pathogenic variant in *SDHAF2* is low.

8.4.3 Paraganglioma Syndrome Type 3 (OMIM 605373)

SDHC mutation is a rare cause of HNPGLs. It was identified in 2000 (Niemann and Muller 2000), and from then it was reported to account for less than 1% of all HNPGLs and extremely rarely in Pheos or intra-abdominal PGLs. Clinically, the *SDHC*-mutated tumors show benign behavior (Schiavi et al. 2005).

8.4.4 Paraganglioma Syndrome Type 4 (OMIM: 115310)

Germline mutations in the *SDHB* gene can be found in approximately 10% of PHEOs/PGLs (Bjorklund et al. 2016). The overall penetrance is around 50%, but it should be noticed that there are data showing both smaller (21–30%) (Hes et al. 2010; Schiavi et al. 2010; Rijken et al. 2016) and larger (65–100%) (Neumann et al. 2004; Benn et al. 2006; Solis et al. 2009; Ricketts et al. 2010) intervals. This discrepancy can be expected either from the calculation methods used in these studies (inclusion of index cases or just screened individuals, statistical method, etc.) or from populations studied. In many regions, founder mutations have been described which may cause bias in correct determination of the real penetrance (Hensen et al. 2012).

The most important phenotype associated with *SDHB* mutation is malignancy (defined as Pheo/PGL tissues in non-chromaffin tissues) (Gimenez-Roqueplo et al. 2003). Of the 16 Pheo/PGL genes, malignancy was found to associate with *SDHB*, *MAX*, and *FuH* mutations. A recent, multivariate model showed that the presence of metastasis correlated with *SDHB* mutation (OR 5.68 [95% CI 1.79–18.06]) but not with the primary location of the tumor. In *SDHB* carriers, tumors can be found intra-abdominal, intra-adrenal, and also at head and neck regions with an approximately 60%, 20%, and 20%. Malignancy tends to be associated with intra-abdominal, extra-adrenal located tumors.

SDHB mutations have been also found in patients with clear cell renal carcinoma, breast adenocarcinoma, prostate cancer, gastrointestinal stromal tumors, papillary thyroid cancer, and pituitary tumors (Neumann et al. 2004; Ni et al. 2008; Ricketts et al. 2008). Of these tumors, both gastrointestinal stromal tumor and clear cell renal carcinoma are present in the Carney triad caused also by germline *SDHB* mutations (Carney triad consists of gastric leiomyosarcoma, pulmonary chondroma and extra-adrenal PGL). Other associations reported (i.e., pituitary tumors, breast carcinoma, colon tumors) the need for further studies for confirmation and to place these data in clinical practice.

For finding *SDHB* mutation-associated tumors, immunostaining for SDHB protein has been successfully introduced and validated in clinical practice. *SDHB*-associated tumors show completely negative staining. Pheos, PGLs, and renal cell carcinoma specimens confirmed the accuracy of this screening method (Gill et al. 2011; Patocs et al. 2016).

8.4.5 Paraganglioma Syndrome Type 5 (OMIM 600857)

Mutation of SDHA gene causes PGL syndrome type 5. The causative role of this gene was identified in a patient with intra-abdominal PGL (Burnichon et al. 2010). SDHA is a rare cause of Pheo/PGLs; it accounts for $<1\%$ of all cases. The penetrance is also low. In addition, the molecular genetic testing of SDHA gene is

difficult; this is the largest gene of SDHx genes, and it has three pseudogenes. Based on our experience and also suggested by a recent guideline (Lenders et al. 2014), genetic analysis of *SDHA* is recommended after testing of *SDHB*, *SDHD*, *TMEM127*, and *MAX* for nonsyndromic Pheo and *SDHB*, *SDHD*, and *SDHC* for HNPGLs. Recent practice using next-generation sequencing allows the comprehensive mutation analysis for all cases (Sarkadi et al. 2018; Ben Aim et al. 2019).

Of note, SDHA is a genetic cause of the autosomal recessive inherited mitochondrial complex II deficiency presenting as Leigh syndrome (OMIM: 256000) (Bourgeron et al. 1995).

8.5 Other Genes Associated with Pheo/PGL

In the past decade, another 10 genes were identified by whole exome sequencing as genetic predisposition for Pheo/PGL. Based on data published about the genotype-phenotype association found between genes *SDHA*, *TMEM127*, *MAX*, and *SDHAF2*, it is clear that even their prevalence is rare. Their mutation frequencies were 3.0% for *SDHA*, 2.1% for *TMEM127*, 0,8% for *MAX*, and 0,1 for *SDHAF2* among patients with apparently sporadic Pheo/PGL (Bausch et al. 2017). In addition, the prevalence of the most novel genes (*KIF1B*, *FuH*, *PHD1*, *PHD2*, *HIF2*, *MDH2*, *GOT2*, *SLC25A11*) is largely unknown, but it is expected to be extremely low. Based on their rarity, we do not have special genotype-phenotype correlations. All patients with mutations in one of the *SDHA*, *TMEM127*, *MAX,* and *SDHAF2* genes could have Pheo/PGL all over the body. The earliest age at onset in *SDHA* mutation carriers was 8 years, and extra-adrenal tumors, especially HNPGLs, were found in two third of patients harboring *SDHA* mutations. *MAX* and *TMEM127* mutations were associated mainly with bilateral adrenal Pheos, but HNPGLs were also described. Malignancy occurred in approximately 10% of patients harboring one of the *SDHA*, *MAX*, and *TMEM127* mutations (Bausch et al. 2017). Interestingly, FuH mutation carriers also presented with metastatic Pheo/PGL and with multiple tumors including multiple cutaneous and uterine leiomyomatosis (Castro-Vega et al. 2014). *MDH2* mutation identified with WES associated also with malignant PGL (Cascon et al. 2015). In summary, our knowledge about the genetics of Pheo/PGL extensively increased with the start of using next-generation sequencing in everyday clinical practice. However, it should be noted that the recently described mutations (FuH, MDH2, SLC25A11) were found through systematic screening of large patient cohorts, and their cumulative prevalence is less than 2%, suggesting that these genes are not representing major genetic susceptibility loci for Pheo/PGL. However, these genes also cause autosomal dominantly inherited tumors which should be followed clinically similarly to those associated with earlier identified genes. Therefore, molecular genetic analysis for patients with Pheo/PGL should include these novel genes too (Sarkadi et al. 2018; Ben Aim et al. 2019).

8.6 The Pathomechanism Associated with *SDHx* Mutations

The pathomechanism associated with the abnormal functioning of the SDH protein is unclear. Due to the reduced function of the succinate dehydrogenase enzyme, succinate is accumulated and then translocated into the cytoplasm, where it inhibits the prolyl-hydroxylase enzyme. As a consequence, HIF1 is stabilized and transcription of several genes (hypoxia-sensitive genes: VEGF, PDGF, GLUT1) is enhanced (pathomechanism of pseudo-hypoxia) (Fig. 8.2). This pathomechanism is responsible for tumorigenesis in VHL-associated tumors too. Furthermore, the role of DNA damage due to increased production of reactive oxygen radicals has been implicated with the development of the disease. The other possible pathogenetic processes associating with SDH failure are the activation of ATP synthesis, the increased activation of cellular survival pathways as a result of abnormal mitochondrial function and the impairment of apoptosis. Taken together all biochemical, molecular, and cell biological data, it can be concluded that SDH inactivation leads to the accumulation of its substrate, succinate, which acts as an oncometabolite by inhibiting 2-oxoglutarate-dependent dioxygenases. Of this class of enzymes, inhibition of HIF prolyl-hydroxylases and TET enzymes lead to tumorigenesis through pseudo-hypoxic signaling and DNA hypermethylation (Tretter et al. 2016). Increased succinate to fumarate ratio was demonstrated in *SDHx*-mutated tumors compared to *SDHx* wild type tumors (Lendvai et al. 2014). This mechanism is common for *SDHx* and *VHL*-associated tumors, and it can be differentiated from the second cluster which comprises *RET*-, *NF1*-, *MAX*-, and *TMEM127*-associated tumors. In this latter group, the main pathomechanism is the activation of MAP kinase/AKT/mTOR pathways (Dahia et al. 2005).

8.7 Genetic Diagnosis and Genetic Counseling

The differential diagnosis of the disease aims to exclude the hereditary tumor syndromes. The recent guideline (Lenders et al. 2014) suggests clinical feature-driven molecular genetic testing in centers where NGS-based gene panel or WES analysis is not available. The most challenging situations are cases with bilateral Pheos at young age. In these patients, clinical screening for VHL syndrome is always recommended. By serum calcitonin test, medullary thyroid carcinoma can be excluded. If there is no clinical sign of MEN2 or VHL syndrome and the *VHL* gene mutation screening is negative, genetic testing of *SDHx*, *TMEM127* and *MAX* genes is recommended. In cases of intra-abdominal paragangliomas, the *SDHB* and *SDHD* genes are to be screened for mutation first by direct PCR reaction followed by direct bidirectional DNA sequencing. In the case of head-neck paragangliomas, the *SDHD*, *SDHB*, and *SDHC* genes are to be analyzed first. For comprehensive molecular genetic testing, investigation of heterozygous gene deletion (hemizygosity is common in *SDHD* and *VHL* genes, but can also be found in

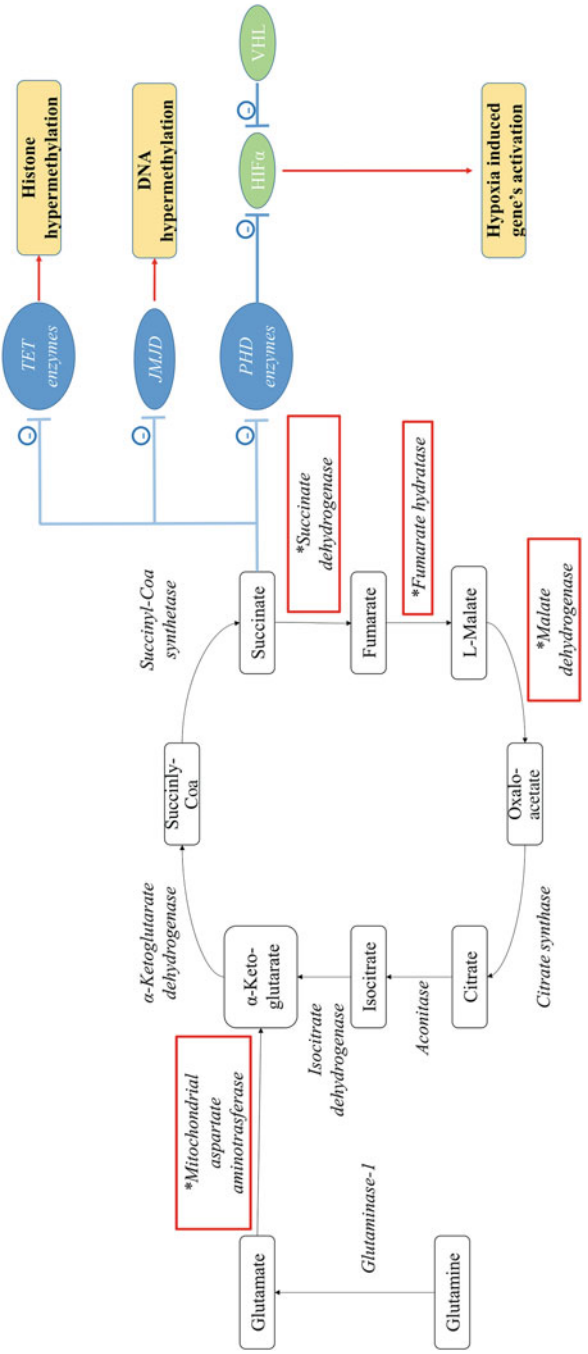

Fig. 8.2 Schematic illustration of the tricarboxylic acid (TCA) cycle and the pathomechanism associated with succinate accumulation. Enzymes are marked with bold letters. Enzymes associated with Pheo/PGL are marked with∗. *PHD* prolyl hydroxylases, *TET* ten-eleven translocation (TET) family of 5-methylcytosine (5mC) hydroxylases, *JMJD* Jumonji C domain-containing histone lysine demethylases, *HIF* hypoxia-induced factor, *VHL* von-Hippel-Lindau protein

SDHB and *SDHC* genes too) should also be carried out using copy number analysis tools in NGS-based techniques, quantitative RT-PCR, and MLPA (further details can be found in Chap. 4).

Followed mutation analysis, gene-specific recommendation and clinical and biochemical surveillance should be started in order to prevent and/or early detect any lesions. In many cases regular systematic whole-body imaging investigation is required. Disease treatment consists of surgical removal of tumors. In the case of hormonally inactive, very slowly growing and non-symptomatic tumors (most often in case of head and neck tumors), conservative treatment is recommended, which consists mainly of monitoring the growth of the tumor. Intra-abdominal paragangliomas should be removed because of the greater risk of malignancy. Malignancy is most at risk for tumors associated with intra-abdominal and *SDHB* mutations. In the case of large tumors causing compression symptoms, when surgical removal is dangerous, irradiation as well as radio-nucleotide treatments can be used.

8.8 Other Rare Disease Accompanied with Pheochromocytoma

The extremely rare disease described below is among the disease group called hamartomatosis or phacomatosis syndromes. These syndromes are characterized by skin malformations. Many of these syndromes, Carney complex, Cowden disease, and Peutz-Jeghers syndrome, associate with various endocrine tumors or endocrine malignancies. These syndromes will be presented in this book in other chapters (Chaps. 8 and 11); only the Sturge-Weber syndrome will be detailed here.

8.8.1 Sturge-Weber Syndrome (OMIM 185300)

The prevalence of Sturge-Weber's syndrome in Europe is estimated to be 1:20,000 to 1:50,000. Symptoms include one-sided naevus flammeus on the face, leptomeningeal angiomatosis with calcification of affected areas, and eye lesions (often causing glaucoma). Neurological symptoms consist of epilepsy and mental retardation. Hemiparesis can also occur. Cranial X-ray and CT scans illustrate the extent of calcification and associated cortical atrophy. In case of drug-resistant epilepsy surgery is required. Genetic background is unknown, the role of somatic mosaicism is assumed (Shirley et al. 2013).

8.9 Conclusions

Pheo/PGLs represent a disease entity with strong genetic background. At least 40% of cases are genetically determined by approximately 16 genes identified to date. This genetic diversity has a major impact on clinical follow-up of the affected patients. Our goals are to provide personalized and adequate clinical follow-up for these patients and their relatives. The post-diagnostic clinical, biochemical and imaging follow-up are determined by the syndrome or gene identified. These syndromes represent a lifelong risk for development of novel tumors. Depending on the gene and even on the mutation detected, specific recommendations have been published and specific recommendations are available. Malignancy, recurrences are the most intruding clinical questions. In these perspectives, *SDHB* is the most important genetic factor for malignant tumors, while *VHL* and *SDHD* mutations prone for development of recurrent tumors. It is also important to note that recurrent tumors are not restricted to the primary localization of the first tumor. In VHL syndrome non-endocrine organs are affected at most, while *SDHx* mutations-associated recurrent tumors affect the contralateral adrenals or extra-adrenal PGLs.

Genetic counseling is mandatory in these syndromes. Pre- and post-testing counseling should be included in the clinical practice. It is important to point out the type of the molecular genetic analysis performed in order to elucidate which genes were tested and potentially other method will be also needed. Nowadays, comprehensive testing using NGS-based technologies is available in many centers, but not in all countries and endocrine centers. In addition, the number of genes identified during the past decade highlights the need for biobanking of DNA samples previously analyzed and showing no mutation. Reanalyzing these samples would increase the number of cases with mutation. However, the usage of these samples requires specific consent form signed by patients. Having in hands a genetic report containing the detected mutation, genetic counseling must also include counseling for risks of both adrenal Pheos and extra-adrenal and HNPGLs.

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