Chapter 2
Molecular Genetics of Pheochromocytoma and Paraganglioma

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2.1 Inherited Pheochromocytomas and Paragangliomas

Pheochromocytomas (PCCs) and paragangliomas (PGLs), together abbreviated as PPGL, are neural crest-derived catecholamine-secreting tumors arising from the adrenal medulla and sympathetic/parasympathetic paraganglia, respectively. These tumors can develop in an apparently sporadic manner or as part of one of several tumoral syndromes associated with alterations in distinct genes. Particularly in the latter case, PPGLs can present with other pathologies within a family and even in the same individual. This variable clinical phenotype is reflection of the genetic complexity that underlies the development of this disease (Fig. 2.1).

While initially it was thought that only 10 % of cases were caused by germline mutations, the discovery of mutations in several additional susceptibility genes during the last 15 years has brought the percentage of hereditary cases up to approximately 40 %. Genes such as *VHL*, *RET*, *NF1*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *MEN1*, *KIF1Bβ*, *EGLN1*, *EGLN2*, *TMEM127*, *MAX*, *EPAS1* (*HIF2A*), *FH*, and *MDH2* are involved in PPGL susceptibility [1–3]. Recent findings have uncovered new candidate genes involved in chromatin remodeling [4], and the contribution of this genetic pathway to disease etiology requires further exploration.

Approximately 40 % of hereditary PPGLs develop primarily in the context of three familial tumor syndromes: von Hippel–Lindau disease (VHL), multiple endocrine neoplasia type 2 (MEN2), and familial PPGL. Patients diagnosed with multiple endocrine neoplasia type 1 (MEN1) and those with neurofibromatosis type 1 (NF1) can also develop PCC, but do so less frequently. PGLs present almost

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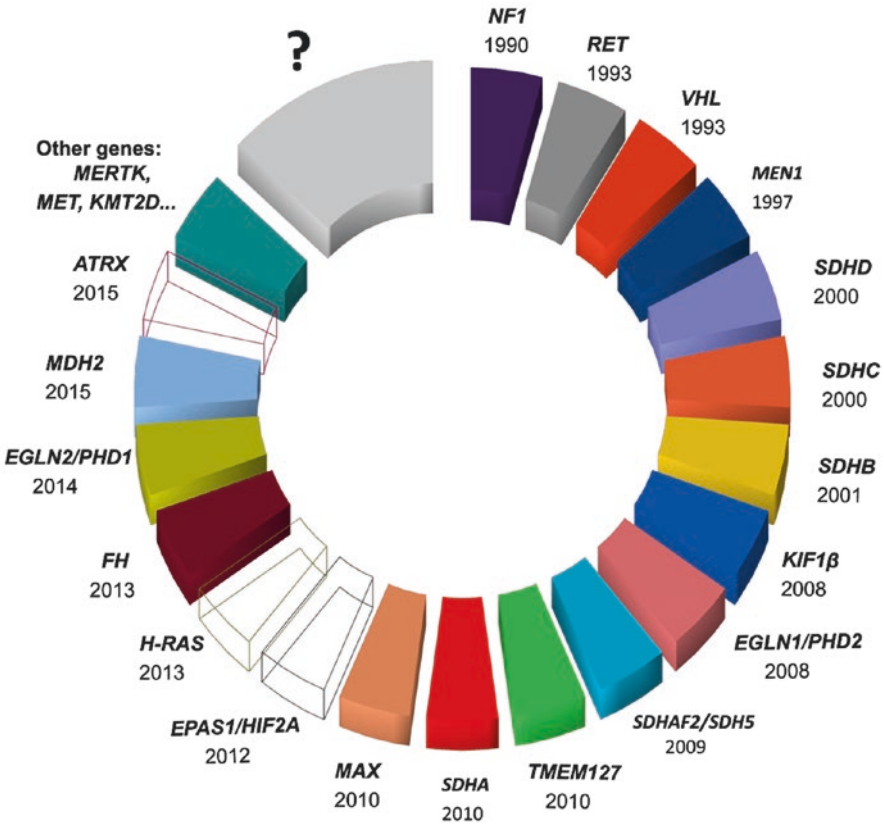

Fig. 2.1 Representation of all reported pheochromocytoma/paraganglioma susceptibility genes, specifying the year of publication, with special attention to the genes involved in the hereditary predisposition to the disease (*opaque colored boxes*). Genes recently reported or lacking further demonstration of their involvement are grouped in "other genes." *Question mark* indicates that other unknown genes are pending to be uncovered

exclusively as part of familial PPGL, although there are very rare tumor syndromes in which these tumors are part of the clinical features (Table 2.1).

Between 10 and 20 % of patients with PPGL are diagnosed during childhood or adolescence [5–7], and PCC is the most frequently diagnosed endocrine tumor in children [8]. The proportion of pediatric patients with a germline mutation in one of the known susceptibility PPGL genes is higher than that found in adults. Recent studies have reported that up to 70–80 % of children with PCC are mutation carriers, regardless of their family history [5, 6, 9]. In addition, a proportion of patients with clinical characteristics indicative of hereditary disease (bilateral PCC, multiple PGLs, family history, and/or early-onset disease) do not carry mutations in any of the known genes, suggesting that other loci remain to be discovered (Fig. 2.1). The

Table 2.1 Genetic and clinical characteristics of syndromes associated with the development of PCC/PGL

Syndrome	Gene	Inheritance	Biochemical phenotype	Associated pathology
MEN2	*RET*	Autosomal dominant	E	MTC, PCC, infrequently PGL Type 2A: PHPT, cutaneous lichen amyloidosis Type 2B: Marfanoid body habitus, mucocutaneous neuromas, intestinal ganglioneuromatosis
VHL	*VHL*	Autosomal dominant	NE, D	HB (CNS and retina), ccRCC, neuroendocrine pancreatic tumors, cysts and cystadenoma of the pancreas, kidney epididymis, or broad ligament, renal cysts, endolymphatic sac tumors, PCC, PGL, etc.
PGL1	*SDHD*	Autosomal dominant (paternal transmission)	NS or NE	PGL (head and neck, thoracic and abdominal), PCC, infrequently GIST, pituitary adenoma, infrequently ccRCC
PGL3	*SDHC*	Autosomal dominant	NS or NE	PGL (head and neck), infrequently PCC or GIST, infrequently pituitary adenoma
PGL4	*SDHB*	Autosomal dominant	NE, D	PGL, PCC, infrequently ccRCC, GIST, pituitary adenoma or PTC
NF1	*NF1*	Autosomal dominant	E	Neurofibromas, cafe au lait spots, axillary freckling, optic gliomas, pigmented hamartomas of the iri*s*, PCC
MEN1	*MEN1*	Autosomal dominant	E	PHPT, pituitary adenoma, neuroendocrine gastroenteropancreatic tumors, PCC
PGL2	*SDHAF2*	Autosomal dominant (paternal transmission)	NS	PGL (head and neck)
PGL5/FPCC1	*TMEM127*	Autosomal dominant	E, NE	PCC, infrequently PGL (head and neck); ccRCC
PGL6	*SDHA*	Autosomal dominant	NE	PGL (head and neck, thoracic and abdominal), PCC; GIST, infrequently ccRCC and pituitary adenoma

(continued)

Table 2.1 (continued)

Syndrome	Gene	Inheritance	Biochemical phenotype	Associated pathology
PGL7/FPCC2	*MAX*	Autosomal dominant (paternal transmission)	NE, E	PCC (single, bilateral, multiple); up to 20 % of patients also develop PGL (thoracic and abdominal)
PGL8	*FH*	Autosomal dominant	NE	PGL (head and neck, thoracic and abdominal), PCC; cutaneous and uterine leiomyoma, type 2 papillary renal carcinoma
KIF1B	*KIF1B*	Autosomal dominant	NE	PCC (bilateral); neuroblastoma
PHD1/2	*PHD1/2*	Autosomal dominant	NE	PGL (multiple), PCC (bilateral); polycythemia
MDH2	*MDH2*	Autosomal dominant	NE	PGL, PCC; no other associated tumors
Pacak–Zhuang	*EPAS1*	Somatic mosaicism	NE	Polycythemia, somatostatinoma, CNS HB, PGL (multiple), PCC (single)

MTC medullary thyroid carcinoma, *PCC* pheochromocytoma, *PGL* paraganglioma, *PHPT* primary hyperparathyroidism, *HB* hemangioblastomas, *CNS* central nervous system, *ccRCC* clear cell renal cell carcinoma, *GIST* gastrointestinal stromal tumor, *PTC* papillary thyroid carcinoma, *FPCC* familial PCC, *E* epinephrine, *NE* norepinephrine, *D* dopamine, *NS* non-secreting

task of identifying new susceptibility genes is complicated by the fact that this disease can follow an autosomal dominant model, with or without preferential paternal transmission [10–14], and that post-zygotic somatic events have also been observed [4, 15]. It is probable that other models of inheritance, such as recessive, occur in some families with the disease. While uncovering this unexplained heritability of PPGL remains a substantial challenge, new approaches based on next-generation sequencing have begun to shed new light on the comprehensive biology of this tumor. It is paramount to correctly genetically classify each patient in order to be able to offer them the most appropriate clinical follow-up.

2.1.1 Syndromic PCC

Some patients develop PCC or PGL as part of a hereditary tumor syndrome; they present with other clinical signs that can help identify the gene most likely to be involved and therefore be used to prioritize genetic testing. Such patients have often developed other neoplasms or have a family history indicative of a strong genetic etiology, as is the case for PCC associated with multiple endocrine neoplasia type 2 (MEN2), von Hippel–Lindau (VHL), or neurofibromatosis type 1 (NF1) and to a lesser extent other syndromes such as Carney triad, Carney–Stratakis syndrome, and neoplasia endocrine multiple type 1 (MEN1). Patients with a germline mutation

in *RET* have more often been previously diagnosed with medullary thyroid carcinoma (MTC), while those from MEN1 families tend to have had primary hyperparathyroidism (PHPT) and those from NF1 families cafe au lait spots. As described in detail below, one exception to this tendency to have particular comorbidities are patients with specific germline mutations in *VHL*, who tend to develop PCC as the sole manifestation of their disease.

2.1.1.1 MEN2-Associated PCC

MEN2 (OMIM 171400) has an estimated annual incidence of 5 per 10,000,000 persons and a prevalence of 1 in 30,000. MEN2 follows an autosomal dominant mode of inheritance. MEN2 patients can develop MTC (medullary thyroid carcinoma), PCC, and/or PHPT (primary hyperparathyroidism), the latter resulting from hyperplasia or from parathyroid adenomas. The syndrome is classified into three subtypes, MEN2A, MEN2B, and familial MTC (FMTC), each defined according to the combination of pathologies developed by the individuals affected (Table 2.1). MEN2A patients may develop all three pathologies. They are also more likely to develop "cutaneous lichen amyloidosis," a pruritic skin lesion in the upper area of the back caused by the uncontrolled deposition of amyloid protein between the dermis and epidermis. In addition, these patients may occasionally develop Hirschsprung disease (HSCR). Patients are classified as MEN2B if they develop, in the absence of parathyroid disease, MTC; PCC; multiple mucocutaneous neuromas involving the lips, tongue, and eyelids; corneal nerve myelination; intestinal ganglioneuromas (hyperganglionic megacolon); or *marfanoid habitus*, including skeletal deformities and hypermobility of joints. Finally, families in which affected members have developed only MTC or C-cell hyperplasia (CCH) are considered to have the third subtype, FMTC, but only if more than ten members have MTC. A less conservative definition has been proposed by [16], based on the presence in at least four family members of MTC without other manifestations of MEN2A. Defining and distinguishing FMTC from MEN2A is challenging, and an exhaustive clinical follow-up of these families is required to rule out the presence of other tumors characteristic of MEN2, especially in older family members.

In the early 1990s, activating mutations in the rearranged during transfection (*RET*) proto-oncogene were identified as the genetic basis for MEN2A, MEN2B, and FMTC [17–20]. Since then, germline *RET* mutations have been identified in 98 % of patients with MEN2A, 95 % of patients with MEN2B, and 88 % of patients with FMTC [21].

RET is located on chromosomal band 10q11.2 and encodes a tyrosine kinase receptor that is mainly expressed in cells derived from the neural crest (C cells, parafollicular thyroid cells, and adrenal medulla cells, among others) and in urogenital system precursor cells [22]. The genetic testing of *RET* is relatively simple, since the mutations associated with the development of MEN2 are mainly located on exons 10, 11 and 13–16. Additional, less frequent mutations on exon 5 and 8 have been also reported in MEN2 patients. Sequencing of the entire coding region

of *RET* is recommended only for those who meet clinical criteria for MEN2 but in whom initial sequencing of selected exons gives negative results [23] or if there is a discrepancy between the MEN2 phenotype and the expected genotype [24]. If a *RET* variant is detected at a noncanonical position, it is important to consult different databases for evidences that the variant may be a polymorphism with no clinical significance [25]. A sequence change in *RET* is considered to be a causative MEN2 mutation if it segregates with the clinical expression of disease within a family including at least two affected individuals having the MEN2 phenotype.

The established genotype–phenotype relationships for MEN2 syndrome are based on the classification of individual mutations according to their transforming ability, and therefore the expected aggressiveness with which the disease they cause will develop [24]. The impact of *RET* mutation testing on the management of MEN2 patients is without doubt one of the most robust examples of the utility of genetic diagnosis in personalizing clinical follow-up [24].

Approximately 50 % of MEN2 patients develop PCC in their lifetime, and the mean age at diagnosis is 35 years. A PCC is the first manifestation of MEN2 in only 12–15 % of cases, and so *RET* explains relatively few cases of non-syndromic disease (around 5 %), compared to other syndromes [26, 27]. *RET* mutations are very rarely found in patients diagnosed with PCC before age 20 [5–7]. Thus, *RET* is not a priority in the genetic testing of pediatric patients, although it should still be included in genetic diagnosis algorithms [5]. PCCs developed by *RET* mutation carriers are bilateral in 50–80 % of patients, they tend to be epinephrine-secreting tumors, and very few (no more than 5 %) are malignant.

It is recommended that screening for PCC begins between ages 5 and 16 for carriers of highest-, high-, and moderate-risk mutations [24, 27].

2.1.1.2 VHL

VHL (OMIM 193300), with an incidence of 1 in 36,000 live births, is a dominantly inherited familial cancer syndrome caused by germline mutations in the *VHL* tumor suppressor gene [28, 29]. This gene encodes three gene products: a protein comprising 213 amino acids and two shorter isoforms, one produced by alternative splicing (excluding exon 2) and the other by alternative initiation. While the protein (pVHL) is involved in multiple processes, its best characterized role is the regulation of the proteasomal degradation of hypoxia-inducible factors (HIFs) [30]. Under normal oxygen tension, the α subunits of HIF (HIF-1α, HIF-2α, and HIF-3α) are hydroxylated by the dioxygenases PHD (PHD1, PHD2, and PHD3). The hydroxylated HIF-α are then targeted by pVHL for proteasomal degradation [31, 32]. Under hypoxia conditions, HIF-α is stabilized and binds to the HIF-β subunit to form an active transcription factor that regulates expression of a large repertory of genes involved in angiogenesis, cell survival, erythropoiesis, and tumor progression [30]. This explains the highly vascularized nature of the tumors associated with VHL syndrome [33].

Table 2.2 Clinical classification of VHL patients

	Subtype				
Clinical features	VHL-1A	VHL-1B	VHL-2A	VHL-2B	VHL-2C
HB (CNS, retina)					
ccRCC			Low risk	High risk	
Cysts and cystadenoma of the pancreas					
PCC					

HB hemangioblastoma, *CNS* central nervous system, *ccRCC* clear cell renal cell carcinoma, *PCC* pheochromocytoma

The penetrance of causal mutations is age dependent, and the disease demonstrates marked phenotypic variability. Patients with VHL are at a higher risk of developing hemangioblastomas (HBs) of the retina and central nervous system (CNS), PCC and/or PGL, clear cell renal cell carcinoma (ccRCC), renal and pancreatic cysts (serous cystadenoma), neuroendocrine pancreas tumors, endolymphatic sac tumors, pancreatic serous cystadenomas, and papillary cystadenomas of the epididymis in men and of the broad ligament in women (Table 2.1) [27, 33, 34]. After identifying *VHL* in 1993, the phenotype associated with *VHL* gene mutations was expanded to include VHL disease, dominantly inherited familial PCC, and, in cases with particular mutations, autosomal recessive congenital polycythemia (also known as familial erythrocytosis-2; MIM# 263400) [35, 36]. It has been suggested that VHL accounts for approximately a third of patients with a CNS HBs, >50 % of patients with a retinal angioma, 1 % of patients with RCC, 50 % of patients with apparently isolated familial PCC, and 11 % of patients with an apparently sporadic PCC [37].

The disease is classified into four subtypes (1, 2A–2C) based on the clinical phenotype. VHL type 1 families have a relatively low risk of developing PCC, but may present with any of the other tumors associated with the disease. VHL type 2 is subdivided into three categories corresponding to a low (2A) or high risk (2B) of developing ccRCC or to a higher risk of PCC and PGLs as the only clinical sign of the disease (2C) (Table 2.2).

Systematic characterization of germline *VHL* mutations has led to the identification of genotype–phenotype correlations such that germline mutations causing amino acid changes on the surface of pVHL are associated with a higher risk of PCC [34]. *VHL* germline deletions, mutations predicted to cause a truncated protein, and missense mutations that disrupt the structural integrity of the VHL protein are associated with a lower risk of PCC (and therefore associated with type 1 VHL syndrome). However, phenotypes related to *VHL* gene deletions appear to be influenced by the retention of some genes surrounding *VHL*. In particular, *VHL* deletions associated with the loss of the actin regulator *HSPC300* gene (also known as *BRICK1*) are associated with protection against ccRCC [38–40]. These clinical findings led McNeill et al. (2009) to suggest that the subclassification of VHL syndrome should take into account not only clinical phenotype but also *VHL* mutation data [40] (Table 2.2).

PCCs/PGLs Associated with VHL

Approximately 20 % of patients with VHL develop PCC or PGL (sympathetic and parasympathetic), although the latter is much less frequent. Tumors are exclusively norepinephrine-secreting, related to a low, or no, expression of phenylethanolamine N-methyltransferase (PNMT), multifocal or bilateral in 43–45 % of cases and malignant in less than 5 % [41–43]. The median age at diagnosis of PCC/PGL is 29 years, which is lower than for other syndromes and particularly relevant for genetic testing since between 12 and 32 % of patients with PCC diagnosed during childhood are found to carry a germline mutation in *VHL* [44]. Also of note is that PCC (principally) or PGL (occasionally) is the first manifestation for 30–50 % of patients with VHL [41]. For these reasons, *VHL* mutation screening is essential in patients diagnosed before age 18. Furthermore, *VHL* has a high mutation rate (20–21 %) [45, 46], and so germline mutation testing of this gene is recommended specifically for patients with single tumors and non-syndromic cases. As we will review in this chapter, the role of *VHL* is also relevant in sporadic presentation, as a notable proportion of tumors develop as consequence of somatic *VHL* mutations [47].

The development of VHL-related tumors has been linked to the alteration of interactions between pVHL and other proteins with which it forms complexes, specifically pVHL-ElonginC-ElonginB complexes (CBC^{VHL}). The most accepted hypothesis in this regard is that the development of PCC in the context of VHL is associated with a partial retention in the function of pVHL to assemble at least to some extent into CBC^{VHL} [48, 49], which protects them from rapid intracellular degradation [50–52]. A hot spot in *VHL* that is associated with the development of PCC affects residue 167, located in the alpha domain. This domain has the role of interacting with other proteins, so that mutations giving rise to amino acid changes in this region do not result in loss of function of pVHL. The finding that 23 % (7/30) of patients with PCC who carry deleterious germline variants in *VHL*, but have no signs of either VHL or MEN2, have a mutation that affects this residue is consistent with this hypothesis [53]. On the basis of the above findings, it has been proposed that the measurement of change in pVHL stability could be used as an additional tool to understand the clinical features developed by a VHL patient [52]. Indeed, the use of this tool led to the identification of an association between ccRCC and missense mutations that significantly alter pVHL stability. A subsequent study classified these mutations as "surface" or "deep," depending on the location of the affected residue in the protein structure, and found a clear difference between them in the associated risk of PCC [34].

Based on the earliest described age at PCC diagnosis, it is recommended that screening be initiated at age 5 years [27, 44].

2.1.1.3 Neurofibromatosis Type 1

Neurofibromatosis type 1 (NF1), formerly known as von Recklinghausen disease, is a common hereditary disease with an incidence of 1 per 2,500–3,300 newborns that primarily involves the skin and nervous system. The condition is usually diagnosed in

early childhood, when cutaneous manifestations are apparent. It is characterized by the appearance of multiple neurofibromas, cafe au lait spots, freckling in the armpits and groin, iris hamartomas (Lisch nodules), bone lesions such as scoliosis, sphenoid dysplasia or pseudoarthrosis, macrocephaly, learning disorders, cognitive deficits, predisposition to optic and CNS glioma, and leukemia [27, 54]. Other malignancies occur less frequently in patients with NF1, including PCC, rhabdomyosarcoma, leukemia, and brain tumors other than optic gliomas [55]. Some features of neurofibromatosis 1 are present at birth, and others are age-related manifestations, which means that periodic monitoring is required to address ongoing health and developmental problems and to minimize the risk of serious medical complications [56].

The gene responsible for NF1, *NF1* (17q11.2), encodes the protein neurofibromin, which is expressed primarily in the nervous system and has the role of suppressing cell proliferation by inactivating RAS proteins. Loss-of-function mutations in *NF1* lead to the activation of RAS and the PI3K/AKT/mTOR pathway, which depends on RAS [54].

The detection of mutations in *NF1* by DNA analysis has proven to be challenging because of the gene's large size (it has 58 exons), the lack of mutation hot spots, and the existence of pseudogenes. *NF1* mutations are predominantly truncating and often accompanied by loss of the wild-type allele in the tumor. Although molecular technology is now available to detect most mutations in *NF1* [57], it is typically not required because in 95 % of cases a diagnosis of NF1 can be made by age 11 years on the basis of clinical findings alone. *NF1* has one of the highest rates of spontaneous mutation of any gene in the human genome. This in part explains why between 30 and 50 % of patients have de novo mutations, which if they occur post-zygotically, can give rise to mosaic phenotypes [58]. Events of germline mosaicism are very rare in this condition [59].

PCCs Associated with NF1 Disease

An estimated 0.1–5.7 % of NF1 patients develop PCC, although this figure is 3.3–13 % based on autopsy studies. Therefore, NF1 is not a common diagnosis in PPGL patients [60]. These tumors are more prevalent among NF1 patients with hypertension (20–50 %) [61]. NF1-associated PCCs tend to develop at a later age (mean 41 years), can be unilateral or bilateral, and are rarely extra-adrenal and slightly more often malignant (up to 12 %) than those in VHL and MEN2 cases [62]. The earliest recorded age at diagnosis of PCC is 7 years, but given the low penetrance of *NF1* mutations for this tumor development, screening of the gene is only recommended in cases of hypertension or symptoms suggestive of disease [27].

2.2 Non-syndromic PCC/PGL

In addition to the syndromic forms, many genes have been described over the last few years related to susceptibility to develop PCC or PGL as the only disease manifestation. Associations with other tumors have been reported, but only in a limited

number of patients. We will outline the functions of the SDH genes, as well as *FH*, *MDH2*, *TMEM127* and *MAX*, and detail the clinical manifestations associated with mutations in each of these. Other genes will be also reviewed.

2.2.1 *Non-syndromic PCC/PGL Associated with Mutations in the SDH Genes,* FH*, and* MDH2

2.2.1.1 SDH Gene Function

The connection between the SDH genes and the development of neuroendocrine tumors was established in 2000 when germline mutations in *SDHD* were first described in patients with familial PGL [14]. The SDH genes encode complex II of the mitochondrial respiratory chain, also known as succinate dehydrogenase (SDH), which plays a key role in both the electron transport chain and the oxidation of succinate to fumarate at the tricarboxylic acid (TCA) cycle. SDH is a heterotetramer composed of four proteins: two catalytic (SDHA and SDHB) and two structural (SDHC and SDHD). The latter are responsible for attaching the SDH complex to the inner mitochondrial wall (reviewed on [63]).

An associated protein, SDHAF2, is a highly conserved cofactor of flavin adenine dinucleotide (FAD) which is implicated in the flavination of SDHA and is essential for SDH function. *SDHAF2* mutations have been reported to be associated with the development of PPGLs, confirming the importance of this complex for the disease [12, 13].

Heterozygous mutations in the *SDHA*, *SDHB*, *SDHC*, and *SDHD* genes cause complex II destabilization affecting the ability of cells to detect oxygen. SDH dysfunction results in the accumulation of succinate [64], its TCA cycle substrate, which acts as a competitive inhibitor of the 2-oxoglutarate (2-OG)-dependent HIF prolyl hydroxylases [65, 66]. This stabilizes HIF-alpha and, mediated by pVHL, activates genes that facilitate angiogenesis and anaerobic metabolism [66]. This link between mutations in the SDH genes and the HIF-1α pathway is also corroborated by results from tumor expression profiling studies of PPGLs [67]. Mutations in the SDH genes, both catalytic and structural, cause defects in the enzymatic activity of the complex, which lead to accumulation of succinate [68], along with the absence of SDHB [69]. Thus, negative SDHB immunostaining indicates the likely involvement of these genes in disease etiology; these findings represent robust tools that can be used to select patients for genetic testing, if paraffin-embedded tumor material is available.

Global DNA hypermethylation has been described as a hallmark of tumors with TCA cycle abnormalities resulting from SDH genes and *FH* and *MDH2* mutations [1, 70, 71]. This CpG island methylator phenotype (CIMP) has revealed that succinate acts as an oncometabolite, inhibiting 2-oxoglutarate-dependent dioxygenases, such as hypoxia-inducible factor prolyl hydroxylases and histone and DNA demethylases.

Prognosis associated with high CIMP is cell-type dependent. For instance, in glioblastoma high CIMP is associated with a more favorable prognosis, whereas poor prognosis has been reported for neuroblastoma and PPGL [70, 72]. SDH gene-related, and particularly *SDHB*-related, PPGLs have a higher risk of progressing to metastatic disease [73]. It has been found that although all SDH gene-mutated tumors displayed CIMP, the level of hypermethylation is significantly higher, and the expression of target genes significantly lower, in *SDHB*-mutated tumors. As target genes include genes implicated in neuroendocrine differentiation and epithelial-to-mesenchymal transition (EMT), this could explain the particular metastasis-prone nature of *SDHB*-mutated tumors [70].

Loss-of-function mutations in the four SDH complex subunits and SDHAF2 have been demonstrated to cause PPGL, though the frequency of mutations and associated tumor types vary by genes. In addition to PPGL, *SDHB* and *SDHD* mutations have been associated with ccRCC [74, 75] and thyroid carcinoma [76, 77]. In addition, mutations in *SDHB*, *SDHC*, and *SDHD* can give rise to Carney–Stratakis syndrome [78], characterized by the dyad of PGL and gastrointestinal stromal tumors (GISTs). These findings revealed a novel molecular mechanism underlying the development of GISTs, which are usually related to gain-of-function mutations in *KIT* or *PDGFRA* [79, 80]. It has more recently been recognized that SDH gene mutations are associated with the development of pituitary adenomas (PA) ([81–83] and reviewed in [84]).

2.2.1.2 Mutations in the SDH/FH/MDH2 Genes: Genotype–Phenotype Relationship

Clinical Presentation Associated with Mutations in SDHD

The hereditary syndrome PGL1 (OMIM ID: 168000) is caused by mutations in the *SDHD* gene. The estimated penetrance of germline mutations in *SDHD* (11q23.1) is 86 % to age 50 years, and carriers normally present with multiple PGLs at a mean age of 35 years. *SDHD* carriers primarily develop head and neck PGL (84 % of cases), although up to 22 % also develop thoracic and abdominal PGL and 12–24 % PCC, the latter rarely being bilateral [76, 85, 86] (Table 2.1). Although PCCs and extra-adrenal PGLs are relatively rare in patients with *SDHD* germline mutations, it has been described that the type of mutation influences the phenotype. *SDHD* mutations predicted to result in an absent or unstable SDHD protein were associated with an increased risk of PCCs and PGLs, compared to missense mutations or in-frame deletions, which were not predicted to impair protein stability [76].

SDHD-related disease follows an autosomal dominant mode of inheritance, with preferential paternal transmission [87]. That is, a mutation carrier will only develop the disease if their mutation came from their father; if it came from their mother, they will not be affected, although they will still be able to pass on the mutation to their children. While this pattern of inheritance suggests the existence of maternal genomic imprinting of this gene, the observed bi-allelic expression of *SDHD* in

different normal tissues and in neural crest-derived tumors does not support this hypothesis [88–90]. In order to explain this *SDHD*-linked parental effect, it has been proposed that the loss of the entire maternal copy of chromosome 11, a hallmark of *SDHD*-linked tumors, leads to the simultaneous deletion of the *SDHD* gene and an exclusively maternally expressed gene [91, 92].

Regardless of the mechanism underlying this preferential paternal transmission, the hereditary nature of disease is masked in families in which by chance the mutation has been transmitted from generation to generation only from mother to child. The disease skips generations and these can only be identified in genetic counseling centers that collect information from second- and third-degree relatives.

A key issue in clinical follow-up is the fact that 3–10 % of carriers of a germline mutation in *SDHD* develop metastasis [76, 77, 93–95]. In the case of pediatric patients, despite the possible lack of family history, it has been suggested that a diagnosis of at least one head and neck PGL is sufficient to justify genetic testing; in fact, 8–16 % of patients under age 20 years carry a germline alteration in *SDHD* [5, 6].

In relation to the development of other tumors, it should be noted that there has been some controversy around two variants in *SDHD*, p.H50R, and p.G12S. Both were initially reported to be associated with the development of Merkel cell carcinoma and familial CCH and even Cowden-like syndrome. However, they were subsequently classified as polymorphisms, present in several healthy populations (http://www.lovd.nl/3.0/home), and their associations with the proposed diseases have therefore been ruled out [96].

Even though the earliest reported age at diagnosis is 5 years, screening is recommended from the age of 10 years [27].

Clinical Presentation Associated with Mutations in SDHB

The PGL4 syndrome (OMIM ID: 115310) is due to mutations in the *SDHB* gene (1p36.13). Overall, the *SDHB* gene is the most commonly mutated of all the SDH-related genes [97]. An estimated 67 % of patients carrying mutations in *SDHB* develop primarily thoracic and abdominal PGL, 27 % develop head and neck PGL, and 17–29 % develop adrenal PCC, which is rarely bilateral [69, 85, 86].

Although, to date, a clear genotype–phenotype relationship does not exist for *SDHB* mutations, an association between *SDHB* missense mutations and an increased risk of head and neck PGL have been described, compared to truncating mutations [76]. Large deletions seem to lead to similar phenotypes and penetrance to those patients with point mutations. Several large germline founder deletions in *SDHB* have been reported in multiple unrelated subjects from the Netherlands [98] and Spain [86, 99]. In these populations, the proportion of carriers of these founder mutations could be sufficiently high for the testing of large deletion in *SDHB* to be the first step in genetic screening.

At least 40 % of *SDHB* mutation carriers develop metastatic disease [97]. Thus, it is widely accepted that the identification of a mutation in *SDHB* is a marker of poor prognosis and more close clinically monitoring of the patient is required.

Of all the known susceptibility genes for hereditary PCC/PGL, *SDHB* constitutes a paradigm of heterogeneity in and of itself. Mutations in this gene are usually associated with the presence at diagnosis of a single retroperitoneal tumor [100]. In fact, only 25–40 % of all carriers will ever develop a tumor [101, 102]. This explains why most patients have no family history of disease at the time of their diagnosis. This fact, along with the frequent appearance of a single tumor in affected individuals, makes it very difficult to identify potentially hereditary cases. Although the underlying cause of reduced penetrance is unknown, possible genetic explanations include inhibition of cell proliferation due to secondary loss of vital genetic material in the proximity of the remaining normal allele or that additional loss of chromosome 11 is required (Hensen model) [63]. For all these reasons, and principally because of the high risk of developing metastases, all the algorithms currently used to guide genetic diagnoses include the testing of *SDHB* in patients with PPGL.

Although the earliest reported age at diagnosis is 6 years, screening is recommended from age 5 years [27].

Clinical Presentation Associated with Mutations in SDHC

Mutations in the *SDHC* gene (1q23.3) cause the PGL3 syndrome (OMIM #605373) [103]. Since relatively few mutations in *SDHC* have been described worldwide, the associated clinical manifestations have not been clearly defined; nevertheless, it is known that mutation carriers tend to develop PGL (93 % parasympathetic and 7 % sympathetic) and infrequently PCC or GIST. Up to 23 % of affected individuals have multiple PGLs and 25 % have a family history, suggesting that mutations have incomplete penetrance [85, 86, 104]. Thus, the clinical features of *SDHC*-associated cases are similar to those found in patients with sporadic head and neck PGLs. Mutation carriers typically present with solitary head and neck PGLs and a very low tendency to be malignant [105]. The mediastinum is the second most common location for *SDHC*-related PGL (10 % of all tumors), occurring in up to 13 % of patients [106]. The mean age at diagnosis is 38 years [62], and very little is known about the involvement of this gene in pediatric disease.

Epigenetic inactivation of *SDHC* is a recently discovered phenomenon in GISTs and PPGLs from patients with Carney triad syndrome [107, 108]. This event has been reported as the genetic cause of a patient that presented with two abdominal PGLs and an adrenocortical adenoma, providing evidence that *SDHC* promoter methylation can cause PGLs due to *SDHC* inactivation [109].

Clinical Presentation Associated with Mutations in SDHA

Mutations in the *SDHA* gene (5p15.33) cause the rare familial PGL5 syndrome (OMIM #614165) [110]. Based on currently available information, *SDHA* (5p15.33) appears to contribute little to PCC/PGL. Korpershoek and colleagues reported that 3 % of their series of 198 patients with apparently sporadic PCC or PGL were found to carry mutations in *SDHA* [69]. These carriers developed PCC, head and neck PGL, or thoracic and abdominal PGL (Table 2.1), but rarely metastatic disease. Although it has been established through biochemical analysis that nonsense *SDHA* mutations are associated with disease, these same mutations have been found in unaffected population controls, suggesting that these mutations have very low penetrance; these findings add additional complexity to the genetic counseling offered to carriers, most of which will not develop clinical symptoms [63].

Nevertheless, *SDHA* should be considered in genetic testing for patients presenting with clinical evidence of familial PPGL who test negative for the other known susceptibility genes. As previously mentioned, mutations in any of the SDH genes have the effect of suppressing the enzymatic activity of complex II and a key indicator that this has occurred is negative immunostaining for SDHB. Furthermore, it is now known that mutations in *SDHA* also give rise to negative immunostaining for SDHA [69]. This relatively easily implemented clinical screening tool should be incorporated into molecular diagnostic protocols to ensure that appropriate mutation testing is carried out in the most efficient and cost-effective manner.

Clinical Presentation Associated with Mutations in SDHAF2

SDHAF2, also known as *SDH5*, was identified as the susceptibility gene for the PGL2 syndrome (OMIM ID: 601650) [13]. *SDHAF2* (11q12.2) is similar to *SDHD* in that it has an autosomal dominant mode of inheritance, with a preferential paternal transmission. To date only head and neck PGLs have been reported in *SDHAF2* mutation carriers, most diagnosed at an early age and all with a family history of the disease ([12] and references contain therein). Few distinct *SDHAF2* mutations have been described [12, 111, 112]. While available data suggest that mutations in *SDHAF2* do not explain a substantial portion of cases (<1 %), further studies in different populations are required to determine their relevance. Nevertheless, genetic testing of *SDHAF2* should be offered to patients with head and neck PGLs with negative tumor staining for SDHB and who test negative for mutations in *SDHD*, *SDHC*, and *SDHB*. While currently too few data are available to draw clear conclusions, no affected mutation carriers developed PGL before age 20 years, suggesting that mutations are not relevant to the development of pediatric tumors.

FH: Clinical Presentation Associated with Mutations in FH

FH is the TCA cycle enzyme involved in the reversible hydration/dehydration of fumarate to malate. It is known that germline mutations in *FH* (1q43) predispose to leiomyomas and papillary RCC in an autosomal dominant hereditary syndrome

named hereditary leiomyomatosis and renal cell cancer (HLRCC) [113]. Inactivation of FH leads to accumulation of its substrate, fumarate, and inhibits α-ketoglutarate-dependent HIF prolyl hydroxylases, leading to HIF activation [64, 66]. Other dioxygenases, including histone demethylases and the TET (ten–eleven translocation) family of 5-methylcytosine (5 mC) hydroxylases, are also inhibited by succinate and fumarate accumulation [114]. Very recently, Letouze et al. [70] identified a germline mutation in *FH* by whole-exome sequencing applied to blood and tumor DNA obtained from a 63-year-old female presenting with one PCC. The patient was selected to be sequenced because the tumor showed a methylome- and transcriptome-based profile very similar to that found in tumors carrying mutations in the SDH genes. The subsequent screening of almost 600 patients with PPGL but no mutations in the major susceptibility genes revealed that five carried pathogenic germline *FH* mutations, providing further evidence of the involvement of this gene in the development of PPGL [115]. Clinically, a metastatic phenotype and multiple tumors were significantly more frequent in patients with *FH* mutations than those without such mutations. Recently, as previously mentioned for succinate, fumarate has been reported as an epigenetic modifier that elicits epithelial-to-mesenchymal transition [164]. *FH* should thus be added to the list of PPGL susceptibility genes and should be considered in mutation screening, to assess the risk of metastatic disease.

MDH2: Clinical Presentation Associated with Mutations in MDH2

MDH2, which encodes another TCA cycle enzyme implicated in the reversible conversion of malate to oxaloacetate with the concurrent reduction of NAD to NADH, has been recently described as a new PPGL susceptibility gene [1]. The causal mutation was identified by whole-exome sequencing, which revealed a germline mutation in the mitochondrial malate dehydrogenase gene (*MDH2*) in a patient with multiple malignant PGLs.

As explained above, the accumulation of succinate and fumarate leads to the enzymatic inhibition of multiple alpha-KG-dependent dioxygenases. This inhibition causes impaired histone demethylation and 5-mC hydroxylation (5-hmC) and, consequently, a characteristic CIMP [70]. Expression profiling analysis focused on hypermethylated and downregulated genes in SDH gene-mutated and non-SDH gene-mutated tumors revealed that the *MDH2*-mutated tumor clustered with SDH gene-mutated tumors, suggesting a similar CIMP (CIMP-like) profile. Findings from additional immunohistochemical studies evaluating 5-hmC and trimethylation of histone H3 lysine 27 (H3K27me3) were also consistent with the *MDH2*-mutated tumor exhibiting a CIMP-like profile [1]. Apart from this study, no other PPGL patient has been reported to carry a *MDH2* mutation. An international consortium has undertaken an initiative to establish the prevalence of *MDH2* mutations among PPGL patients with no mutations in known susceptibility genes. Nevertheless, the contribution of *MDH2* mutations to disease seems to be low (<0.5 %; unpublished data), and the associated clinical features associated and penetrance are yet to be established.

Nevertheless, these findings once again link the disruption of the TCA cycle to PPGL development and indicate that other alterations of this major metabolic pathway may explain additional cases of this disease.

2.2.2 *Non-syndromic PPGL Associated with Mutations in* TMEM127

2.2.2.1 The TMEM127 Gene

TMEM127 (2q11) was identified as a new PCC susceptibility gene in 2010, via an integrated analysis of results from studies using several genomic platforms, including linkage analysis, gene expression profiling, and mapping of chromosomal gains and losses [116]. Loss of heterozygosity (LOH) of the wild-type allele was observed in all available tumors from carriers of *TMEM127* mutations, suggesting that the gene acts as a classic tumor suppressor.

TMEM127 encodes a transmembrane protein with no known functional domains. Functional studies suggest that the protein (TMEM127) localizes to the plasmatic membrane and cytoplasm and is associated with a subpopulation of vesicular organelles, including the Golgi and lysosomes. TMEM127 is dynamically distributed at the subcellular level in response to nutrient signals [116]. It has also been demonstrated that TMEM127 modulates mTOR complex 1 (mTORC1). The mTOR kinase is a common component of two complexes, mTORC1 and mTORC2, which control some relevant aspects of cell metabolism, growth, proliferation, survival, and differentiation [117, 118]. *TMEM127* downregulation leads to hyperphosphorylation of mTORC1 targets 4EBP1 (eukaryotic translation initiation factor 4E-binding protein 1) and S6K (ribosomal protein S6 kinase), as well as to the increase of cell size and proliferation [116], indicating that TMEM127 is associated with mTORC1 downregulation.

Subsequent analysis of the global expression profile of *TMEM127* tumors grouped them with those associated with *RET* and *NF1* mutations [47, 119].

2.2.2.2 Clinical Presentation Associated with Mutations in TMEM127

Few studies have been published to date based on patient series genetically tested for mutations in *TMEM127*. The most relevant of these reported the genetic findings in 990 patients with PPGL who tested negative for mutations in *RET*, *VHL*, and *SDHB/C/D* [120]; 2% carried germline *TMEM127* mutations and presented with disease at a mean age of 43 years. Subsequent reports have described two mutation carriers with PGL, one thoracic and abdominal and the other with multiple head and neck tumors [121]. Globally, more than 30 mutations have been identified in *TMEM127*. Most (60%) result in a truncated protein or predominantly target one of the transmembrane regions of the protein (reviewed in [2]). Although all variants are germline in nature, less than 20% of patients carrying a *TMEM127* mutation report a family history of PPGL [120]. In addition, germline *TMEM127* mutations have also been detected in rare cases of ccRCC patients without PPGL [122].

As for other susceptibility genes, the findings published to date suggest that mutations in *TMEM127* have incomplete penetrance, which would tend to mask the underlying hereditary disease and in many cases mean that patients may not meet the selection criteria for genetic testing. Given the mean age at disease onset for mutation carriers studied to date, genetic testing of *TMEM127* is not recommended in pediatric patients with PPGL.

2.2.3 *Non-syndromic PPGL Associated with Mutations in* MAX

2.2.3.1 The MAX Gene and Its Protein

MAX (14q23.3) encodes a transcription factor that plays an important role in the regulation of cell proliferation, cell differentiation, and apoptosis, as part of the MYC/MAX/MXD1 axis. These proteins form dimers to bind to DNA; in fact, MYC forms a heterodimer with MAX to bind to specific DNA sequences called "E-boxes," which are located in MYC target genes, and this entire complex acts as a transcription activator. Both the lethal character demonstrated in *Max* knockout mice and the fact that *MAX* is constitutively expressed in many cell types make it difficult to understand how MYC can carry out its function without the presence of MAX. However, the PC12 cell-line, derived from PCC in rat, carries a homozygous *Max* mutation [123], which points to the existence of an additional unknown factor that is able to regulate the function of MYC ([124] and reviewed in [10]).

The identification of *MAX* as a PCC susceptibility gene was the result of a study of the entire exomes of three unrelated patients with PCC and a family history of the disease [10]. These patients had been selected because their tumors had a common transcription profile that differentiated them from tumors related to other known susceptibility genes [67]. LOH in the tumors of germline *MAX* mutation carriers, along with the absence of MAX protein shown by an immunohistochemical analysis, suggested that *MAX* acts as a tumor suppressor gene.

2.2.3.2 Clinical Presentation Associated with Mutations in MAX

Following the identification of pathogenic mutations in *MAX* in the three initial families, the genetic study was extended to 59 patients that had tested negative for the key known susceptibility genes. These 59 patients were chosen because they were diagnosed with PCC before age 30 years, had bilateral disease, or had a family history of the disease. *MAX* mutations were found in 8.5 % of them; 67 % of mutation carriers had bilateral disease and 25 % had developed metastases. The malignant behavior of tumors with mutations in *MAX* seemed to be consistent with what was known about neuroblastoma, the other tumor derived from neural crest and developed mainly from the adrenal medulla. Up to 22 % of neuroblastomas show MYC amplification; they are strongly associated with advanced disease stages and rapid tumor progression [125], which would support the idea that MAX loss of function was related to metastatic potential, since MAX is the main regulator of MYC. An additional striking finding was that the mutated allele had to have been inherited paternally in order for the carrier to develop the disease, as is the case for *SDHD* and *SDHAF2*, although the mechanism behind this remains unknown.

A subsequent study screening for mutations in *MAX* in a series of 1,694 patients and 245 tumors was undertaken in order to establish the prevalence of *MAX* mutations and the associated clinical presentation. This study was made possible through the collaboration of 17 reference centers from around the world [11]. The interpretation

of findings took into account only the pathogenic germline mutations identified, excluding all those classified as variants of unknown significance. The study reported pathogenic mutations in 1.3 % of patients; 21 % of them had developed thoracic and abdominal PGL in addition to PCC, although none of the patients diagnosed only with PGL carried a *MAX* mutation. Thirty-seven percent had a family history of the disease and 10 % had metastases. Thus, the association with metastatic disease described in the first study was not confirmed. The mean age at diagnosis for mutation carriers was 32 years and 21 % were diagnosed at or before age 18. These findings suggest that *MAX* should be included along with *VHL* and *SDHB* in genetic testing protocols for pediatric cases [5].

It was also established that the frequency of somatic mutations was 1.65 % and that the associated biochemical-secretor profile was characterized by elevated levels of normetanephrine and associated with normal or slightly increased levels of metanephrine [11]. It should be noted that, though the overall prevalence of *MAX* mutations in the entire series only slightly exceeded 1 %, this increased to 12 % in patients with isolated tumors and to 66 % in cases with bilateral PCC, when considering only cases with PCC and family history [11]. Thus, this second study elucidated the importance of *MAX* mutations in PPGL susceptibility, pointing to the need for the inclusion of this gene in the genetic workup of affected patients, particularly those with PCC (bilateral or multifocal), and/or with family history. These conclusions have been confirmed in more recent studies, which have also identified additional *MAX* germline mutations [126].

2.2.4 Rare PPGL Susceptibility Genes

In addition to the twelve PPGL susceptibility genes already discussed, there are others such as *MEN1*, *KIF1B*, *PDH1* (also called *EGLN2*, egl nine homolog 2), *PDH2* (also called *EGLN1*, egl nine homolog1), *MERTK*, and *MET* [4, 127] that have recently been added to the list of genes related to PPGL susceptibility.

It is known that mutations in the *MEN1* gene (11q13) are responsible for multiple endocrine neoplasia type 1 development. This syndrome is characterized by tumors of the pancreatic islet cells, anterior pituitary, and parathyroid gland [128]. Some patients may also develop adrenal cortical tumors, carcinoid tumors, facial angiofibromas, collagenomas, and lipomas. PCC is observed in less than 1 % of MEN1 germline mutation carriers [129].

A germline mutation in *EGLN1* (1q42.1) was reported in a patient with PGL and congenital erythrocytosis [130]. Germline mutations in *EGLN1* had previously been reported in patients with erythrocytosis, but not in association with tumors [131]. The detected mutation affected EGLN1 function and stabilized HIF-1α and HIF-2α in HEK-293 cells. LOH was detected in the tumors, suggesting that *EGLN1* may act as a tumor suppressor gene. The first germline mutation in *EGLN2* (19q13.2) was also found in one patient suffering from multiple PGLs and congenital polycythemia [3]. A novel germline *EGLN1* was described in a second patient. Both mutant

tumors exhibited reduced protein stability with substantial quantitative protein loss and thus compromised catalytic activities [3].

KIF1B is a large gene located at 1p36.22 that is frequently deleted in neural crest-derived tumors. The gene has two splice variants, *KIF1Bα* and *KIF1Bβ*. The beta isoform functions as a tumor suppressor that is necessary for neuronal apoptosis [132]. *KIF1Bβ* has been found mutated in one sporadic PCC, and in the germline of one apparently sporadic patient and a single family affected by PCC and neuroblastoma [132–134]. No metastases were reported in these studies. Other tumors such as ganglioneuroma, leiomyosarcoma, and lung adenocarcinoma have also been reported in a family with *KIF1Bβ* mutations [133]. It is likely that this gene has a more relevant role in PPGL development than expected, but the large size of its coding sequence makes screening for additional deleterious mutations a difficult task. The use of next-generation sequencing in routine genetic screening will likely help to determine the role of *KIF1Bβ* in the disease.

2.3 Sporadic PPGL

Only few years ago, it was accepted that the proportion of PPGLs explained by somatic events was very low, these mainly affecting *VHL*, *RET*, *SDHB*, and *SDHD* [135–137]. The scenario changed completely after it was reported that 14 % of PPGLs were explained by somatic *RET* and *VHL* mutations [47] and the *NF1* somatic events were found in 21–24 % of PPGLs [138, 139]. It is now clear that somatic mutations play a role in PPGLs as they have been described in up to 40 % of tumors [9, 47, 138–141]. These mutations involve not only the genes involved in heritable susceptibility but also others that have emerged as new predisposition genes, thereby giving insights into the mechanisms and pathways implicated in the disease (Fig. 2.2). Furthermore, these findings highlight the importance of working with germline and tumor DNA from the same patients in order to provide a comprehensive genetic diagnosis [9].

Other genes not previously mentioned in this chapter will be reviewed in the following sections.

2.3.1 PPGLs with Mutations in EPAS1

The HIF family of transcription factors (HIF-1α, HIF-2α [EPAS1], and HIF-3α) plays a key role in the regulation of response to hypoxia to counteract the lack of oxygen in normal homeostasis. It has been suggested that HIF-1α preferentially drives genes implicated in apoptosis and glycolysis and HIF-2α is involved in cell proliferation and angiogenesis [142, 143]. Recently, a new and direct link has been found between HIF proteins and PPGL development [15]; post-zygotic somatic mutations in *EPAS1* (located at 2p21) were found in two unrelated patients with

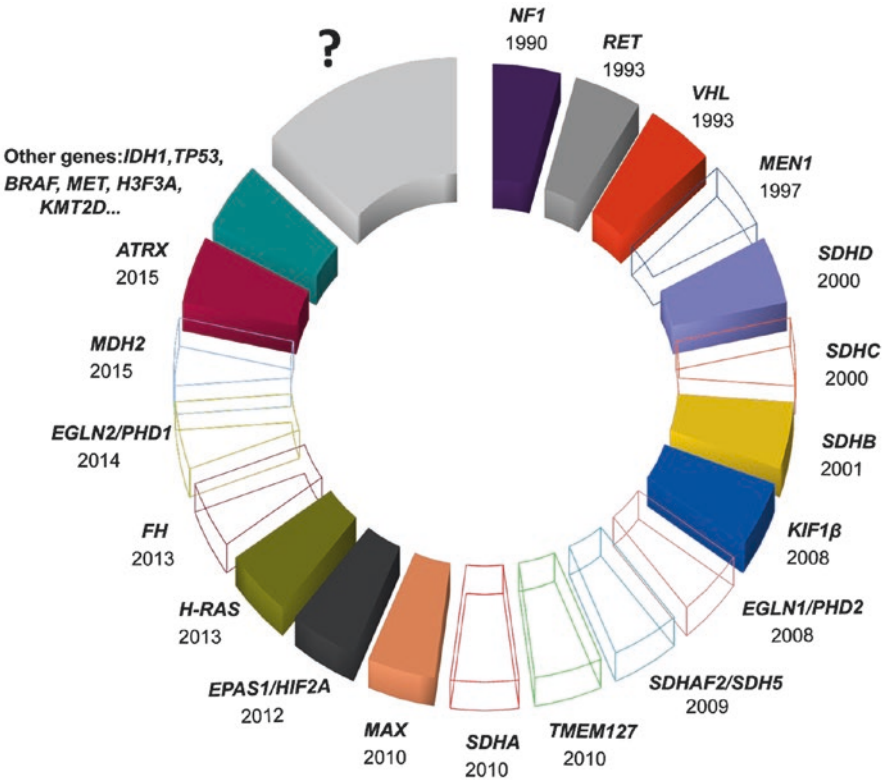

Fig. 2.2 Representation of all reported pheochromocytoma/paraganglioma susceptibility genes, specifying the year of publication, with special attention to the genes involved in the sporadic presentation of the disease (*opaque colored boxes*). Genes recently reported or mutated in only one sporadic tumor are grouped in "other genes." *Question mark* indicates that other unknown genes are pending to be uncovered

multiple PGLs, somatostatinomas, and polycythemia. The mutations were found in the residues located close to the prolyl hydroxylation site of the protein (proline 531) which was shown to disrupt the recognition of EPAS1 by members of the PHD family, as well as its hydroxylation and the consequent degradation by VHL [144, 145]. Thus, mutations affecting the *EPAS1* gene stabilize the protein, causing the aforementioned pseudohypoxia, indicating that *EPAS1* behaves as an oncogene. Later, somatic mutations in *EPAS1* were found in sporadic PPGL cases, demonstrating that the mutations in *EPAS1* are involved in a considerable proportion (~6 %) [9] of the sporadic presentation of the disease [146]. A germline alteration affecting *EPAS1* was also found in a patient with multiple PGLs and polycythemia. Although it was demonstrated that this latter variant stabilized the protein, its location outside the prolyl hydroxylation sites, and the absence of segregation with the disease in the family of the variant carrier, made this result somewhat controversial [147].

Moreover, the clinical implications of mutations affecting residues located in other prolyl hydroxylation sites remain unclear [148].

2.3.2 *PPGLs with Mutations in* HRAS

The members of the RAS family of oncoproteins (e.g., *HRAS*, *NRAS*, and *KRAS*) are small GTP-binding proteins that affect multiple downstream pathways related to cell growth and homeostasis. They were first linked to cancer in 1982 (reviewed in [149]), and it is now known that together they represent around 30 % of all oncogene-activating mutations found in many different cancers [149, 150]. Somatic mutations in the *Harvey rat sarcoma viral oncogene homolog* (*HRAS*) gene (11p15.5) was first described in one PCC by Yoshimoto *et al.* [151]. Crona et al. [141] applied whole-exome sequencing to 58 PCCs and found that four harbored somatic mutations in *HRAS*. The subsequent study of a larger series of tumors determined that 10–15 % of sporadic PCCs have mutations in *HRAS* and ruled out the involvement of *NRAS* and *KRAS* in the disease [9, 152]. The presence of mutations in one of the isoforms of RAS was not a new discovery in the development of endocrine tumors since they were known to be present in around 10–20 % of follicular cell-derived thyroid cancers and in 18 % of *RET*-negative sporadic MTCs [153–155]. A very recent gene expression analysis including seven *HRAS*-mutated tumors grouped all mutated tumors within the so-called transcriptional cluster 2 and confirmed that *HRAS* mutations and alterations in the known PPGL susceptibility genes are mutually exclusive [119].

2.3.3 *PPGLs with Mutations in* ATRX

Recently *ATRX* has been reported to be recurrently mutated in PPGL tumors [156]. The authors reported that 12.6 % of PPGLs analyzed had somatic *ATRX* mutations, one-third truncating mutations, and two-thirds missense mutations affecting a known functional domain and classified as deleterious by three in silico prediction algorithm. Considering only the tumors that had been genetically characterized, it seemed that *ATRX* mutations coexisted with other known PPGL driver mutations, mainly in the SDH genes "although ATRX has been recently described as a driver gene in PCC" [156, 164].

ATRX is a large gene located on the X chromosome (Xq21.1) that encodes a member of the SWI/SNF family of chromatin remodeling proteins. Mutations in this gene are associated with an X-linked mental retardation syndrome most often accompanied by alpha-thalassemia (ATR-X syndrome) [157]. *ATRX* plays a role in telomere maintenance, chromosomal segregation in mitosis, and transcriptional regulation [158, 159]. It is frequently lost in tumor cells that use ALT (alternative

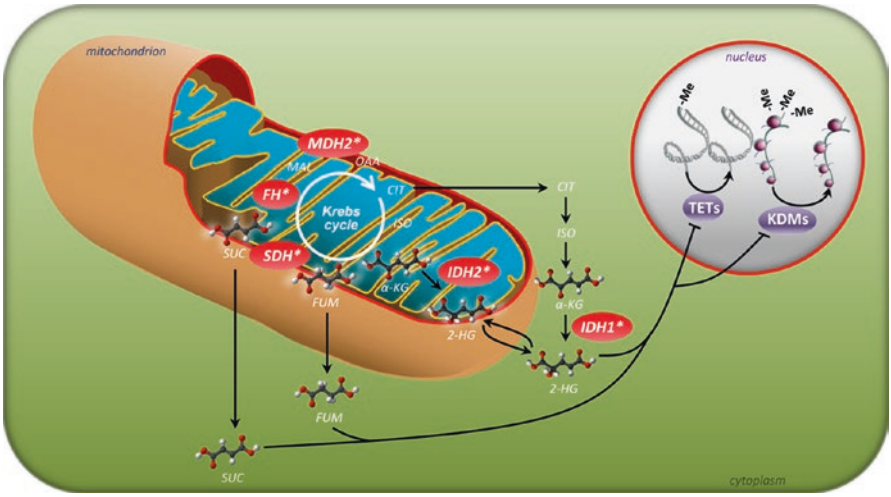

Fig. 2.3 Mutations (*) in genes belonging to the Krebs cycle (*IDH1/2*, SDH genes, *FH*, and now *MDH2*) lead to the accumulation of metabolites (2-hidroxyglutarate [2-*HG*], succinate [*SUC*], and fumarate [*FUM*], respectively) structurally similar to α-ketoglutarate (α-*KG*), a co-substrate pivotal for several dioxygenases. These "oncometabolites" act as competitive inhibitors of demethylases of DNA (*TETs*) and histone lysines (*KDMs*), causing important alterations in gene expression

lengthening of telomeres) for telomere maintenance [160, 161], which is associated with poor prognosis [160, 162].

2.3.4 Other Somatically Mutated PPGL-Related Genes

The knowledge of the genetic landscape of PPGL has dramatically increased since the application of new technologies to interrogate genetic alterations across the exome. Some of the previously mentioned susceptibility genes were in fact discovered using these platforms, and other genes have been reported, although their relative contribution to disease incidence is not yet well established. Among this latter group are somatic mutations in *TP53*, *BRAF*, *IDH1*, *FGFR1*, *H3F3A*, *MET*, *KMT2D*, *SETD2*, *JMJD1C*, *KMT2B*, or *EZH2* [4] (Fig. 2.2). It is particularly interesting that part of these genes is involved in chromatin-mediated gene regulation, suggesting it is likely other members of this and related pathways also contribute to PPGL pathogenesis.

Epigenetic changes leading to enhanced DNA and histone methylation have been linked to loss of function of TCA cycle-related genes. These mutations lead to elevated levels of intermediates that act as oncometabolites. The accumulation of these metabolites is postulated to cause hypermethylation by inhibiting 2-oxoglutarate-dependent histone and DNA demethylase enzymes [1, 70, 163] (Fig. 2.3). New findings described by Toledo *et al*. point to mutations of chromatin

remodeling genes that could represent another mechanism underlying chromatin architecture defects, something which is a feature of a considerable proportion of PPGLs [4].

2.4 Conclusions

Faced with the complex genetic etiology of PPGL described in this chapter, it is essential to collect comprehensive clinical information as well as germline and tumor DNA from patients in order to perform efficient genetic testing and to offer appropriate genetic counseling. The detection of a germline mutation in one of the genes related to the development of these tumors has clear implications for the clinical follow-up of the patient. The identification of a somatic mutation avoids additional germline genetic screening as new susceptibility genes are discovered and represents a valuable source of knowledge for future therapeutic opportunities.

New insights are emerging with the use of next-generation sequencing-based approaches, and it is probable that findings reviewed herein are only the tip of the iceberg in terms of the genetic landscape underlying PPGL.

References

1. Cascon A, Comino-Mendez I, Curras-Freixes M, de Cubas AA, Contreras L, Richter S, et al. Whole-exome sequencing identifies MDH2 as a new familial paraganglioma gene. J Natl Cancer Inst. 2015;107(5).
2. Dahia PL. Pheochromocytoma and paraganglioma pathogenesis: learning from genetic heterogeneity. Nat Rev Cancer. 2014;14(2):108–19.
3. Yang C, Zhuang Z, Fliedner SM, Shankavaram U, Sun MG, Bullova P, et al. Germ-line PHD1 and PHD2 mutations detected in patients with pheochromocytoma/paraganglioma-polycythemia. J Mol Med (Berl). 2015;93(1):93–104.
4. Toledo RA, Qin Y, Cheng ZM, Gao Q, Iwata S, Silva G, et al. Recurrent mutations of chromatin remodeling genes and kinase receptors in pheochromocytomas and paragangliomas. Clin Cancer Res. 2016;22:2301–10.
5. Cascon A, Inglada-Perez L, Comino-Mendez I, de Cubas AA, Leton R, Mora J, et al. Genetics of pheochromocytoma and paraganglioma in Spanish pediatric patients. Endocr Relat Cancer. 2013;20:L1–6.
6. King KS, Prodanov T, Kantorovich V, Fojo T, Hewitt JK, Zacharin M, et al. Metastatic pheochromocytoma/paraganglioma related to primary tumour development in childhood or adolescence: significant link to SDHB mutations. J Clin Oncol. 2011;29(31):4137–42.
7. Barontini M, Levin G, Sanso G. Characteristics of pheochromocytoma in a 4- to 20-year-old population. Ann N Y Acad Sci. 2006;1073:30–7.
8. Pacak K, Lenders JWM, Eisenhofer G. Pheochromocytoma: diagnosis, localization, and treatment. Malden: Blackwell Pub.; 2007.
9. Curras-Freixes M, Inglada-Perez L, Mancikova V, Montero-Conde C, Leton R, Comino-Mendez I, et al. Recommendations for somatic and germline genetic testing of single pheochromocytoma and paraganglioma based on findings from a series of 329 patients. J Med Genet. 2015;52:647–56.

10. Comino-Mendez I, Gracia-Aznarez FJ, Schiavi F, Landa I, Leandro-Garcia LJ, Leton R, et al. Exome sequencing identifies MAX mutations as a cause of hereditary pheochromocytoma. Nat Genet. 2011;43(7):663–7.
11. Burnichon N, Cascon A, Schiavi F, Morales NP, Comino-Mendez I, Abermil N, et al. MAX mutations cause hereditary and sporadic pheochromocytoma and paraganglioma. Clin Cancer Res. 2012;18(10):2828–37.
12. 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(4):366–72.
13. 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(5944):1139–42.
14. 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(5454):848–51.
15. Zhuang Z, Yang C, Lorenzo F, Merino M, Fojo T, Kebebew E, et al. Somatic HIF2A gain-of-function mutations in paraganglioma with polycythemia. N Engl J Med. 2012;367(10):922–30.
16. Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, Gagel RF, et al. The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis. JAMA. 1996;276(19):1575–9.
17. Donis-Keller H, Dou S, Chi D, Carlson KM, Toshima K, Lairmore TC, et al. Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. Hum Mol Genet. 1993;2(7):851–6.
18. Eng C, Smith DP, Mulligan LM, Nagai MA, Healey CS, Ponder MA, et al. Point mutation within the tyrosine kinase domain of the RET proto-oncogene in multiple endocrine neoplasia type 2B and related sporadic tumours. Hum Mol Genet. 1994;3(2):237–41.
19. Hofstra RM, Landsvater RM, Ceccherini I, Stulp RP, Stelwagen T, Luo Y, et al. A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. Nature. 1994;367(6461):375–6.
20. Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardner E, et al. Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature. 1993;363(6428):458–60.
21. Figlioli G, Landi S, Romei C, Elisei R, Gemignani F. Medullary thyroid carcinoma (MTC) and RET proto-oncogene: mutation spectrum in the familial cases and a meta-analysis of studies on the sporadic form. Mutat Res. 2013;752(1):36–44.
22. de Groot JW, Links TP, Plukker JT, Lips CJ, Hofstra RM. RET as a diagnostic and therapeutic target in sporadic and hereditary endocrine tumours. Endocr Rev. 2006;27(5):535–60.
23. Kloos RT, Eng C, Evans DB, Francis GL, Gagel RF, Gharib H, et al. Medullary thyroid cancer: management guidelines of the American Thyroid Association. Thyroid. 2009;19(6):565–612.
24. Wells Jr SA, Asa SL, Dralle H, Elisei R, Evans DB, Gagel RF, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. Thyroid. 2015;25(6):567–610.
25. Chernock RD, Hagemann IS. Molecular pathology of hereditary and sporadic medullary thyroid carcinomas. Am J Clin Pathol. 2015;143(6):768–77.
26. Milos IN, Frank-Raue K, Wohllk N, Maia AL, Pusiol E, Patocs A, et al. Age-related neoplastic risk profiles and penetrance estimations in multiple endocrine neoplasia type 2A caused by germ line RET Cys634Trp (TGC>TGG) mutation. Endocr Relat Cancer. 2008;15(4):1035–41.
27. Waguespack SG, Rich T, Grubbs E, Ying AK, Perrier ND, Ayala-Ramirez M, et al. A current review of the etiology, diagnosis, and treatment of pediatric pheochromocytoma and paraganglioma. J Clin Endocrinol Metab. 2010;95(5):2023–37.

28. Latif F, Tory K, Gnarra J, Yao M, Duh FM, Orcutt ML, et al. Identification of the von Hippel-Lindau disease tumour suppressor gene. Science. 1993;260(5112):1317–20.
29. Gnarra JR, Tory K, Weng Y, Schmidt L, Wei MH, Li H, et al. Mutations of the VHL tumour suppressor gene in renal carcinoma. Nat Genet. 1994;7(1):85–90.
30. Kaelin Jr WG. The von Hippel-Lindau tumour suppressor protein: O2 sensing and cancer. Nat Rev Cancer. 2008;8(11):865–73.
31. 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(6733):271–5.
32. Stebbins CE, Kaelin Jr WG, Pavletich NP. Structure of the VHL-ElonginC-ElonginB complex: implications for VHL tumour suppressor function. Science. 1999;284(5413):455–61.
33. Lonser RR, Glenn GM, Walther M, Chew EY, Libutti SK, Linehan WM, et al. Von Hippel-Lindau disease. Lancet. 2003;361(9374):2059–67.
34. Ong KR, Woodward ER, Killick P, Lim C, Macdonald F, Maher ER. Genotype-phenotype correlations in von Hippel-Lindau disease. Hum Mutat. 2007;28(2):143–9.
35. Ang SO, Chen H, Hirota K, Gordeuk VR, Jelinek J, Guan Y, et al. Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia. Nat Genet. 2002;32(4):614–21.
36. Pastore YD, Jelinek J, Ang S, Guan Y, Liu E, Jedlickova K, et al. Mutations in the VHL gene in sporadic apparently congenital polycythemia. Blood. 2003;101(4):1591–5.
37. Maher ER, Neumann HP, Richard S. Von Hippel-Lindau disease: a clinical and scientific review. Eur J Hum Genet. 2011;19(6):617–23.
38. Maranchie JK, Afonso A, Albert PS, Kalyandrug S, Phillips JL, Zhou S, et al. Solid renal tumour severity in von Hippel Lindau disease is related to germline deletion length and location. Hum Mutat. 2004;23(1):40–6.
39. Cascon A, Escobar B, Montero-Conde C, Rodriguez-Antona C, Ruiz-Llorente S, Osorio A, et al. Loss of the actin regulator HSPC300 results in clear cell renal cell carcinoma protection in Von Hippel-Lindau patients. Hum Mutat. 2007;28(6):613–21.
40. McNeill A, Rattenberry E, Barber R, Killick P, MacDonald F, Maher ER. Genotype-phenotype correlations in VHL exon deletions. Am J Med Genet A. 2009;149A(10):2147–51.
41. Gimm O, Koch CA, Januszewicz A, Opocher G, Neumann HP. The genetic basis of pheochromocytoma. Front Horm Res. 2004;31:45–60.
42. Eisenhofer G, Huynh TT, Pacak K, Brouwers FM, Walther MM, Linehan WM, et al. Distinct gene expression profiles in norepinephrine- and epinephrine-producing hereditary and sporadic pheochromocytomas: activation of hypoxia-driven angiogenic pathways in von Hippel-Lindau syndrome. Endocr Relat Cancer. 2004;11(4):897–911.
43. 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(3):411–20.
44. Aufforth RD, Ramakant P, Sadowski SM, Mehta A, Trebska-McGowan K, Nilubol N, et al. Pheochromocytoma screening initiation and frequency in von Hippel-Lindau syndrome. J Clin Endocrinol Metab. 2015;100(12):4498–504.
45. Sgambati MT, Stolle C, Choyke PL, Walther MM, Zbar B, Linehan WM, et al. Mosaicism in von Hippel-Lindau disease: lessons from kindreds with germline mutations identified in offspring with mosaic parents. Am J Hum Genet. 2000;66(1):84–91.
46. Evans DG, Howard E, Giblin C, Clancy T, Spencer H, Huson SM, et al. Birth incidence and prevalence of tumour-prone syndromes: estimates from a UK family genetic register service. Am J Med Genet A. 2010;152A(2):327–32.
47. Burnichon N, Vescovo L, Amar L, Libe R, de Reynies A, Venisse A, et al. Integrative genomic analysis reveals somatic mutations in pheochromocytoma and paraganglioma. Hum Mol Genet. 2011;20(20):3974–85.
48. Knauth K, Bex C, Jemth P, Buchberger A. Renal cell carcinoma risk in type 2 von Hippel-Lindau disease correlates with defects in pVHL stability and HIF-1alpha interactions. Oncogene. 2006;25(3):370–7.

49. Knauth K, Cartwright E, Freund S, Bycroft M, Buchberger A. VHL mutations linked to type 2C von Hippel-Lindau disease cause extensive structural perturbations in pVHL. J Biol Chem. 2009;284(16):10514–22.
50. Schoenfeld AR, Davidowitz EJ, Burk RD. Elongin BC complex prevents degradation of von Hippel-Lindau tumour suppressor gene products. Proc Natl Acad Sci U S A. 2000; 97(15):8507–12.
51. Kamura T, Brower CS, Conaway RC, Conaway JW. A molecular basis for stabilization of the von Hippel-Lindau (VHL) tumour suppressor protein by components of the VHL ubiquitin ligase. J Biol Chem. 2002;277(33):30388–93.
52. Ruiz-Llorente S, Bravo J, Cebrian A, Cascon A, Pollan M, Telleria D, et al. Genetic characterization and structural analysis of VHL Spanish families to define genotype-phenotype correlations. Hum Mutat. 2004;23(2):160–9.
53. Neumann HP, Bausch B, McWhinney SR, Bender BU, Gimm O, Franke G, et al. Germ-line mutations in nonsyndromic pheochromocytoma. N Engl J Med. 2002;346(19):1459–66.
54. Boyd KP, Korf BR, Theos A. Neurofibromatosis type 1. J Am Acad Dermatol. 2009;61(1):1–14; quiz 5–6.
55. Cassidy SB, Allanson JE. Management of genetic syndromes. 2nd ed. Hoboken: Wiley-Liss; 2005.
56. Hersh JH. Health supervision for children with neurofibromatosis. Pediatrics. 2008; 121(3):633–42.
57. Pasmant E, Parfait B, Luscan A, Goussard P, Briand-Suleau A, Laurendeau I, et al. Neurofibromatosis type 1 molecular diagnosis: what can NGS do for you when you have a large gene with loss of function mutations? Eur J Hum Genet. 2015;23(5):596–601.
58. Kehrer-Sawatzki H, Cooper DN. Mosaicism in sporadic neurofibromatosis type 1: variations on a theme common to other hereditary cancer syndromes? J Med Genet. 2008;45(10):622–31.
59. Trevisson E, Forzan M, Salviati L, Clementi M. Neurofibromatosis type 1 in two siblings due to maternal germline mosaicism. Clin Genet. 2014;85(4):386–9.
60. Bausch B, Koschker AC, Fassnacht M, Stoevesandt J, Hoffmann MM, Eng C, et al. Comprehensive mutation scanning of NF1 in apparently sporadic cases of pheochromocytoma. J Clin Endocrinol Metab. 2006;91(9):3478–81.
61. Jimenez 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(8):2851–8.
62. Fishbein L, Nathanson KL. Pheochromocytoma and paraganglioma: understanding the complexities of the genetic background. Cancer Genet. 2012;205(1–2):1–11.
63. Hoekstra AS, Bayley JP. The role of complex II in disease. Biochim Biophys Acta. 2013;1827(5):543–51.
64. Pollard PJ, Briere JJ, Alam NA, Barwell J, Barclay E, Wortham NC, et al. Accumulation of Krebs cycle intermediates and over-expression of HIF1alpha in tumours which result from germline FH and SDH mutations. Hum Mol Genet. 2005;14(15):2231–9.
65. Briere JJ, Favier J, Benit P, El Ghouzzi V, Lorenzato A, Rabier D, et al. Mitochondrial succinate is instrumental for HIF1alpha nuclear translocation in SDHA-mutant fibroblasts under normoxic conditions. Hum Mol Genet. 2005;14(21):3263–9.
66. Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, et al. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. Cancer Cell. 2005;7(1):77–85.
67. Lopez-Jimenez E, Gomez-Lopez G, Leandro-Garcia LJ, Munoz I, Schiavi F, Montero-Conde C, et al. Research resource: transcriptional profiling reveals different pseudohypoxic signatures in SDHB and VHL-related pheochromocytomas. Mol Endocrinol. 2010;24(12): 2382–91.
68. King A, Selak MA, Gottlieb E. Succinate dehydrogenase and fumarate hydratase: linking mitochondrial dysfunction and cancer. Oncogene. 2006;25(34):4675–82.

69. 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(9):E1472–6.
70. Letouze E, Martinelli C, Loriot C, Burnichon N, Abermil N, Ottolenghi C, et al. SDH mutations establish a hypermethylator phenotype in paraganglioma. Cancer Cell. 2013;23(6):739–52.
71. de Cubas AA, Korpershoek E, Inglada-Perez L, Letouze E, Curras-Freixes M, Fernandez AF, et al. DNA methylation profiling in pheochromocytoma and paraganglioma reveals diagnostic and prognostic markers. Clin Cancer Res. 2015;21(13):3020–30.
72. Abe M, Ohira M, Kaneda A, Yagi Y, Yamamoto S, Kitano Y, et al. CpG island methylator phenotype is a strong determinant of poor prognosis in neuroblastomas. Cancer Res. 2005;65(3):828–34.
73. 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(10):3822–8.
74. Vanharanta S, Buchta M, McWhinney SR, Virta SK, Peczkowska M, Morrison CD, et al. Early-onset renal cell carcinoma as a novel extraparaganglial component of SDHB-associated heritable paraganglioma. Am J Hum Genet. 2004;74(1):153–9.
75. 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(17):1260–2.
76. Ricketts CJ, Forman JR, Rattenberry E, Bradshaw N, Lalloo F, Izatt L, et al. Tumour risks and genotype-phenotype-proteotype analysis in 358 patients with germline mutations in SDHB and SDHD. Hum Mutat. 2010;31(1):41–51.
77. Neumann HP, Pawlu C, Peczkowska M, Bausch B, McWhinney SR, Muresan M, et al. Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. JAMA. 2004;292(8):943–51.
78. Stratakis CA, Carney JA. The triad of paragangliomas, gastric stromal tumours and pulmonary chondromas (Carney triad), and the dyad of paragangliomas and gastric stromal sarcomas (Carney-Stratakis syndrome): molecular genetics and clinical implications. J Intern Med. 2009;266(1):43–52.
79. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumours. Science. 1998;279(5350):577–80.
80. Hirota S, Ohashi A, Nishida T, Isozaki K, Kinoshita K, Shinomura Y, et al. Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumours. Gastroenterology. 2003;125(3):660–7.
81. Xekouki P, Szarek E, Bullova P, Giubellino A, Quezado M, Mastroyannis SA, et al. Pituitary adenoma with paraganglioma/pheochromocytoma (3PAs) and succinate dehydrogenase defects in humans and mice. J Clin Endocrinol Metab. 2015;100(5):E710–9.
82. Papathomas TG, Gaal J, Corssmit EP, Oudijk L, Korpershoek E, Heimdal K, et al. Non-pheochromocytoma (PCC)/paraganglioma (PGL) tumours in patients with succinate dehydrogenase-related PCC-PGL syndromes: a clinicopathological and molecular analysis. Eur J Endocrinol. 2014;170(1):1–12.
83. Denes J, Swords F, Rattenberry E, Stals K, Owens M, Cranston T, et al. Heterogeneous genetic background of the association of pheochromocytoma/paraganglioma and pituitary adenoma: results from a large patient cohort. J Clin Endocrinol Metab. 2015;100(3):E531–41.
84. O'Toole SM, Denes J, Robledo M, Stratakis CA, Korbonits M. 15 YEARS OF PARAGANGLIOMA: the association of pituitary adenomas and phaeochromocytomas or paragangliomas. Endocr Relat Cancer. 2015;22(4):T105–22.
85. Mannelli M, Castellano M, Schiavi F, Filetti S, Giacche M, Mori L, et al. 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(5):1541–7.

86. Cascon A, Pita G, Burnichon N, Landa I, Lopez-Jimenez E, Montero-Conde C, et al. Genetics of pheochromocytoma and paraganglioma in Spanish patients. J Clin Endocrinol Metab. 2009;94(5):1701–5.
87. Baysal BE, Willett-Brozick JE, Lawrence EC, Drovdlic CM, Savul SA, McLeod DR, et al. Prevalence of SDHB, SDHC, and SDHD germline mutations in clinic patients with head and neck paragangliomas. J Med Genet. 2002;39(3):178–83.
88. Cascon A, Ruiz-Llorente S, Fraga MF, Leton R, Telleria D, Sastre J, et al. Genetic and epigenetic profile of sporadic pheochromocytomas. J Med Genet. 2004;41(3):e30.
89. Yamashita R, Usui T, Hashimoto S, Suzuki H, Takahashi M, Honkura K, et al. Predominant expression of mutated allele of the succunate dehydrogenase D (SDHD) gene in the SDHD-related paragangliomas. Endocr J. 2009;56(9):1129–35.
90. Baysal BE, McKay SE, Kim YJ, Zhang Z, Alila L, Willett-Brozick JE, et al. Genomic imprinting at a boundary element flanking the SDHD locus. Hum Mol Genet. 2011;20(22):4452–61.
91. Hensen EF, Jordanova ES, van Minderhout IJ, Hogendoorn PC, Taschner PE, van der Mey AG, et al. Somatic loss of maternal chromosome 11 causes parent-of-origin-dependent inheritance in SDHD-linked paraganglioma and phaeochromocytoma families. Oncogene. 2004;23(23):4076–83.
92. Pigny P, Vincent A, Cardot Bauters C, Bertrand M, de Montpreville VT, Crepin M, et al. Paraganglioma after maternal transmission of a succinate dehydrogenase gene mutation. J Clin Endocrinol Metab. 2008;93(5):1609–15.
93. Burnichon N, Rohmer V, Amar L, Herman P, Leboulleux S, Darrouzet V, et al. The succinate dehydrogenase genetic testing in a large prospective series of patients with paragangliomas. J Clin Endocrinol Metab. 2009;94(8):2817–27.
94. Havekes B, Corssmit EP, Jansen JC, van der Mey AG, Vriends AH, Romijn JA. Malignant paragangliomas associated with mutations in the succinate dehydrogenase D gene. J Clin Endocrinol Metab. 2007;92(4):1245–8.
95. 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(34):8812–8.
96. Cascon A, Ruiz-Llorente S, Cebrian A, Leton R, Telleria D, Benitez J, et al. G12S and H50R variations are polymorphisms in the SDHD gene. Genes Chromosomes Cancer. 2003;37(2):220–1.
97. Lenders JW, Duh QY, Eisenhofer G, Gimenez-Roqueplo AP, Grebe SK, Murad MH, et al. Pheochromocytoma and paraganglioma: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2014;99(6):1915–42.
98. Bayley JP, Grimbergen AE, van Bunderen PA, van der Wielen M, Kunst HP, Lenders JW, et al. The first Dutch SDHB founder deletion in paraganglioma-pheochromocytoma patients. BMC Med Genet. 2009;10:34.
99. Cascon A, Landa I, Lopez-Jimenez E, Diez-Hernandez A, Buchta M, Montero-Conde C, et al. Molecular characterisation of a common SDHB deletion in paraganglioma patients. J Med Genet. 2008;45(4):233–8.
100. Cascon A, Lopez-Jimenez E, Landa I, Leskela S, Leandro-Garcia LJ, Maliszewska A, et al. Rationalization of genetic testing in patients with apparently sporadic pheochromocytoma/paraganglioma. Horm Metab Res. 2009;41(9):672–5.
101. Hes FJ, Weiss MM, Woortman SA, de Miranda NF, van Bunderen PA, Bonsing BA, et al. Low penetrance of a SDHB mutation in a large Dutch paraganglioma family. BMC Med Genet. 2010;11:92.
102. Schiavi F, Milne RL, Anda E, Blay P, Castellano M, Opocher G, et al. Are we overestimating the penetrance of mutations in SDHB? Hum Mutat. 2010;31(6):761–2.
103. Niemann S, Muller U. Mutations in SDHC cause autosomal dominant paraganglioma, type 3. Nat Genet. 2000;26(3):268–70.
104. Welander J, Soderkvist P, Gimm O. Genetics and clinical characteristics of hereditary pheochromocytomas and paragangliomas. Endocr Relat Cancer. 2011;18(6):R253–76.

105. Schiavi F, Boedeker CC, Bausch B, Peczkowska M, Gomez CF, Strassburg T, et al. Predictors and prevalence of paraganglioma syndrome associated with mutations of the SDHC gene. JAMA. 2005;294(16):2057–63.
106. Else T, Marvin ML, Everett JN, Gruber SB, Arts HA, Stoffel EM, et al. The clinical phenotype of SDHC-associated hereditary paraganglioma syndrome (PGL3). J Clin Endocrinol Metab. 2014;99(8):E1482–6.
107. Haller F, Moskalev EA, Faucz FR, Barthelmess S, Wiemann S, Bieg M, et al. Aberrant DNA hypermethylation of SDHC: a novel mechanism of tumour development in Carney triad. Endocr Relat Cancer. 2014;21(4):567–77.
108. Killian JK, Miettinen M, Walker RL, Wang Y, Zhu YJ, Waterfall JJ, et al. Recurrent epimutation of SDHC in gastrointestinal stromal tumours. Sci Transl Med. 2014;6(268):268ra177.
109. Richter S, Klink B, Nacke B, deCubas AA, Mangelis A, Rapizzi E, et al. Epigenetic mutation of the succinate dehydrogenase C promoter in a patient with two paragangliomas. J Clin Endocrinol Metab. 2015;101:359–63
110. Burnichon N, Briere JJ, Libe R, Vescovo L, Riviere J, Tissier F, et al. SDHA is a tumour suppressor gene causing paraganglioma. Hum Mol Genet. 2010;19(15):3011–20.
111. Piccini V, Rapizzi E, Bacca A, Di Trapani G, Pulli R, Giache V, et al. Head and neck paragangliomas: genetic spectrum and clinical variability in 79 consecutive patients. Endocr Relat Cancer. 2012;19(2):149–55.
112. Kunst HP, Rutten MH, de Monnink JP, Hoefsloot LH, Timmers HJ, Marres HA, et al. SDHAF2 (PGL2-SDH5) and hereditary head and neck paraganglioma. Clin Cancer Res. 2011;17(2):247–54.
113. Schmidt LS, Linehan WM. Hereditary leiomyomatosis and renal cell carcinoma. Int J Nephrol Renov Dis. 2014;7:253–60.
114. Xiao M, Yang H, Xu W, Ma S, Lin H, Zhu H, et al. Inhibition of alpha-KG-dependent histone and DNA demethylases by fumarate and succinate that are accumulated in mutations of FH and SDH tumour suppressors. Genes Dev. 2012;26(12):1326–38.
115. Castro-Vega LJ, Buffet A, De Cubas AA, Cascon A, Menara M, Khalifa E, et al. Germline mutations in FH confer predisposition to malignant pheochromocytomas and paragangliomas. Hum Mol Genet. 2014;23(9):2440–6.
116. 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(3):229–33.
117. Bhaskar PT, Hay N. The two TORCs and Akt. Dev Cell. 2007;12(4):487–502.
118. Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. Cancer Cell. 2007;12(1):9–22.
119. Stenman A, Welander J, Gustavsson I, Brunaud L, Backdahl M, Soderkvist P, et al. HRAS mutation prevalence and associated expression patterns in pheochromocytoma. Genes Chromosomes Cancer. 2016;55:452–9.
120. Yao L, Schiavi F, Cascon A, Qin Y, Inglada-Perez L, King EE, et al. Spectrum and prevalence of FP/TMEM127 gene mutations in pheochromocytomas and paragangliomas. JAMA. 2010;304(23):2611–9.
121. 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(8):E1279–82.
122. Qin Y, Deng Y, Ricketts CJ, Srikantan S, Wang E, Maher ER, et al. The tumour susceptibility gene TMEM127 is mutated in renal cell carcinomas and modulates endolysosomal function. Hum Mol Genet. 2014;23:2428–39.
123. Hopewell R, Ziff EB. The nerve growth factor-responsive PC12 cell line does not express the Myc dimerization partner Max. Mol Cell Biol. 1995;15(7):3470–8.
124. Ribon V, Leff T, Saltiel AR. c-Myc does not require max for transcriptional activity in PC-12 cells. Mol Cell Neurosci. 1994;5(3):277–82.
125. Seeger RC, Brodeur GM, Sather H, Dalton A, Siegel SE, Wong KY, et al. Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. N Engl J Med. 1985;313(18):1111–6.

126. Peczkowska M, Kowalska A, Sygut J, Waligorski D, Malinoc A, Janaszek-Sitkowska H, et al. Testing new susceptibility genes in the cohort of apparently sporadic phaeochromocytoma/paraganglioma patients with clinical characteristics of hereditary syndromes. Clin Endocrinol (Oxf). 2013;79(6):817–23.
127. Castro-Vega LJ, Letouze E, Burnichon N, Buffet A, Disderot PH, Khalifa E, et al. Multi-omics analysis defines core genomic alterations in pheochromocytomas and paragangliomas. Nat Commun. 2015;6:6044.
128. Raue F, Zink A. Clinical features of multiple endocrine neoplasia type 1 and type 2. Horm Res. 1992;38 Suppl 2:31–5.
129. Brandi ML, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordi C, et al. Guidelines for diagnosis and therapy of MEN type 1 and type 2. J Clin Endocrinol Metab. 2001; 86(12):5658–71.
130. 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(25): 2685–92.
131. Percy MJ, Zhao Q, Flores A, Harrison C, Lappin TR, Maxwell PH, et al. A family with erythrocytosis establishes a role for prolyl hydroxylase domain protein 2 in oxygen homeostasis. Proc Natl Acad Sci U S A. 2006;103(3):654–9.
132. 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 tumour suppressor. Genes Dev. 2008;22(7):884–93.
133. Yeh IT, Lenci RE, Qin Y, Buddavarapu K, Ligon AH, Leteurtre E, et al. A germline mutation of the KIF1B beta gene on 1p36 in a family with neural and nonneural tumours. Hum Genet. 2008;124(3):279–85.
134. Welander J, Andreasson A, Juhlin CC, Wiseman RW, Backdahl M, Hoog A, et al. Rare germline mutations identified by targeted next-generation sequencing of susceptibility genes in pheochromocytoma and paraganglioma. J Clin Endocrinol Metab. 2014;99(7): E1352–60.
135. Dannenberg H, De Krijger RR, van der Harst E, Abbou M, IJzendoorn Y, Komminoth P, et al. Von Hippel-Lindau gene alterations in sporadic benign and malignant pheochromocytomas. Int J Cancer. 2003;105(2):190–5.
136. Gimm O, Armanios M, Dziema H, Neumann HP, Eng C. Somatic and occult germ-line mutations in SDHD, a mitochondrial complex II gene, in nonfamilial pheochromocytoma. Cancer Res. 2000;60(24):6822–5.
137. van Nederveen FH, Korpershoek E, Lenders JW, de Krijger RR, Dinjens WN. Somatic SDHB mutation in an extraadrenal pheochromocytoma. N Engl J Med. 2007;357(3):306–8.
138. Burnichon N, Buffet A, Parfait B, Letouze E, Laurendeau I, Loriot C, et al. Somatic NF1 inactivation is a frequent event in sporadic pheochromocytoma. Hum Mol Genet. 2012;21(26):5397–405.
139. Welander J, Larsson C, Backdahl M, Hareni N, Sivler T, Brauckhoff M, et al. Integrative genomics reveals frequent somatic NF1 mutations in sporadic pheochromocytomas. Hum Mol Genet. 2012;21(26):5406–16.
140. Luchetti A, Walsh D, Rodger F, Clark G, Martin T, Irving R, et al. Profiling of somatic mutations in phaeochromocytoma and paraganglioma by targeted next generation sequencing analysis. Int J Endocrinol. 2015;2015:138573.
141. Crona J, Delgado Verdugo A, Maharjan R, Stalberg P, Granberg D, Hellman P, et al. Somatic mutations in H-RAS in sporadic pheochromocytoma and paraganglioma identified by exome sequencing. J Clin Endocrinol Metab. 2013;98(7):E1266–71.
142. Semenza GL. HIF-1, O(2), and the 3 PHDs: how animal cells signal hypoxia to the nucleus. Cell. 2001;107(1):1–3.
143. Gruber M, Simon MC. Hypoxia-inducible factors, hypoxia, and tumour angiogenesis. Curr Opin Hematol. 2006;13(3):169–74.
144. McDonough MA, Li V, Flashman E, Chowdhury R, Mohr C, Lienard BM, et al. Cellular oxygen sensing: crystal structure of hypoxia-inducible factor prolyl hydroxylase (PHD2). Proc Natl Acad Sci U S A. 2006;103(26):9814–9.

145. Min JH, Yang H, Ivan M, Gertler F, Kaelin Jr WG, Pavletich NP. Structure of an HIF-1alpha -pVHL complex: hydroxyproline recognition in signaling. Science. 2002;296(5574):1886–9.
146. Comino-Mendez I, de Cubas AA, Bernal C, Alvarez-Escola C, Sanchez-Malo C, Ramirez-Tortosa CL, et al. Tumoural EPAS1 (HIF2A) mutations explain sporadic pheochromocytoma and paraganglioma in the absence of erythrocytosis. Hum Mol Genet. 2013;22(11):2169–76.
147. Lorenzo FR, Yang C, Ng Tang Fui M, Vankayalapati H, Zhuang Z, Huynh T, et al. A novel EPAS1/HIF2A germline mutation in a congenital polycythemia with paraganglioma. J Mol Med (Berl). 2013;91(4):507–12.
148. Welander J, Andreasson A, Brauckhoff M, Backdahl M, Larsson C, Gimm O, et al. Frequent EPAS1/HIF2alpha exons 9 and 12 mutations in non-familial pheochromocytoma. Endocr Relat Cancer. 2014;21(3):495–504.
149. Cox AD, Der CJ. Ras history: the saga continues. Small GTPases. 2010;1(1):2–27.
150. Karnoub AE, Weinberg RA. Ras oncogenes: split personalities. Nat Rev Mol Cell Biol. 2008;9(7):517–31.
151. Yoshimoto K, Iwahana H, Fukuda A, Sano T, Katsuragi K, Kinoshita M, et al. ras mutations in endocrine tumours: mutation detection by polymerase chain reaction-single strand conformation polymorphism. Jpn J Cancer Res. 1992;83(10):1057–62.
152. Oudijk L, de Krijger RR, Rapa I, Beuschlein F, de Cubas AA, Dei Tos AP, et al. H-RAS mutations are restricted to sporadic pheochromocytomas lacking specific clinical or pathological features: data from a multi-institutional series. J Clin Endocrinol Metab. 2014;99(7):E1376–80.
153. Nikiforov YE, Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. Nat Rev Endocrinol. 2011;7(10):569–80.
154. Agrawal N, Jiao Y, Sausen M, Leary R, Bettegowda C, Roberts NJ, et al. Exomic sequencing of medullary thyroid cancer reveals dominant and mutually exclusive oncogenic mutations in RET and RAS. J Clin Endocrinol Metab. 2013;98(2):E364–9.
155. Ciampi R, Mian C, Fugazzola L, Cosci B, Romei C, Barollo S, et al. Evidence of a low prevalence of RAS mutations in a large medullary thyroid cancer series. Thyroid. 2013;23(1):50–7.
156. Fishbein L, Khare S, Wubbenhorst B, DeSloover D, D'Andrea K, Merrill S, et al. Whole-exome sequencing identifies somatic ATRX mutations in pheochromocytomas and paragangliomas. Nat Commun. 2015;6:6140.
157. Gibbons RJ, Picketts DJ, Villard L, Higgs DR. Mutations in a putative global transcriptional regulator cause X-linked mental retardation with alpha-thalassemia (ATR-X syndrome). Cell. 1995;80(6):837–45.
158. Eid R, Demattei MV, Episkopou H, Auge-Gouillou C, Decottignies A, Grandin N, et al. Genetic inactivation of ATRX leads to a decrease in the amount of telomeric cohesin and level of telomere transcription in human glioma cells. Mol Cell Biol. 2015;35(16):2818–30.
159. Ratnakumar K, Bernstein E. ATRX: the case of a peculiar chromatin remodeler. Epigenetics. 2013;8(1):3–9.
160. Liau JY, Tsai JH, Yang CY, Lee JC, Liang CW, Hsu HH, et al. Alternative lengthening of telomeres phenotype in malignant vascular tumours is highly associated with loss of ATRX expression and is frequently observed in hepatic angiosarcomas. Hum Pathol. 2015;46(9):1360–6.
161. Kurihara S, Hiyama E, Onitake Y, Yamaoka E, Hiyama K. Clinical features of ATRX or DAXX mutated neuroblastoma. J Pediatr Surg. 2014;49(12):1835–8.
162. Marinoni I, Kurrer AS, Vassella E, Dettmer M, Rudolph T, Banz V, et al. Loss of DAXX and ATRX are associated with chromosome instability and reduced survival of patients with pancreatic neuroendocrine tumours. Gastroenterology. 2014;146(2):453–60. e5.
163. Yang M, Pollard PJ. Succinate: a new epigenetic hacker. Cancer Cell. 2013;23(6):709–11.
164. Sciacovelli M, et al. Fumarate is an epigenetic modifier that elicits epithelial-to-mesenchymal transition. "https://www.ncbi.nlm.nih.gov/pubmed/27580029" \o "Nature." Nature. 2016;537(7621):544–7. doi:10.1038/nature19353. [Epub ahead of print].
165. Comino-Méndez I, et al. ATRX driver mutation in a composite malignant pheochromocytoma. https://www.ncbi.nlm.nih.gov/pubmed/?term=cascon+ATRX \o "Cancer genetics." Cancer Genet. 2016;209(6):272–7. doi:10.1016/j.cancergen.2016.04.058.