REVIEW

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Genetics and clinical characteristics of hereditary pheochromocytomas and paragangliomas

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Abstract

Pheochromocytomas (PCCs) and paragangliomas (PGLs) are rare neuroendocrine tumors of the adrenal glands and the sympathetic and parasympathetic paraganglia. They can occur sporadically or as a part of different hereditary tumor syndromes. About 30% of PCCs and PGLs are currently believed to be caused by germline mutations and several novel susceptibility genes have recently been discovered. The clinical presentation, including localization, malignant potential, and age of onset, varies depending on the genetic background of the tumors. By reviewing more than 1700 reported cases of hereditary PCC and PGL, a thorough summary of the genetics and clinical features of these tumors is given, both as part of the classical syndromes such as multiple endocrine neoplasia type 2 (MEN2), von Hippel–Lindau disease, neurofibromatosis type 1, and succinate dehydrogenase-related PCC–PGL and within syndromes associated with a smaller fraction of PCCs/PGLs, such as Carney triad, Carney–Stratakis syndrome, and MEN1. The review also covers the most recently discovered susceptibility genes including *KIF1Bβ, EGLN1/PHD2, SDHAF2, TMEM127, SDHA*, and *MAX*, as well as a comparison with the sporadic form. Further, the latest advances in elucidating the cellular pathways involved in PCC and PGL development are discussed in detail. Finally, an algorithm for genetic testing in patients with PCC and PGL is proposed.

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Introduction

Pheochromocytomas (PCCs) and paragangliomas (PGLs) are neuroendocrine tumors that arise in the adrenal medulla or the extra-adrenal sympathetic and parasympathetic paraganglia (DeLellis *et al*. 2004). Paraganglia are small organs that mainly consist of neuroendocrine cells derived from the embryonic neural crest that have the ability to synthesize and secrete catecholamines (McNichol 2001). As defined by the World Health Organization, a PCC is an intra-adrenal PGL that arises from the chromaffin cells of the adrenal medulla (DeLellis *et al*. 2004). The term PCC means 'dusky-colored tumor' and was historically derived from the color change that occurs when the tumor tissue is immersed in chromate salts. Extra-adrenal PGLs, nowadays often referred to as only PGLs, are classified as sympathetic or parasympathetic depending on the type of paraganglia in which they have their origin. Sympathetic PGLs arise from chromaffin cells of paraganglia along the sympathetic chains and are usually located in the chest, abdomen, or pelvis (Fig. 1). Parasympathetic PGLs arise from the glomera that are distributed along parasympathetic nerves in the head, neck, and upper mediastinum and are therefore also referred to as head and neck PGLs.

PCCs and PGLs are rare tumors. Their prevalence is unknown but has been estimated to lie between 1:6500 and 1:2500 in the United States (Chen *et al*. 2010). Autopsy series have revealed a higher prevalence of about 1:2000, suggesting that many tumors remain undiagnosed (McNeil *et al*. 2000). The annual incidence has been reported to be two to ten cases per million

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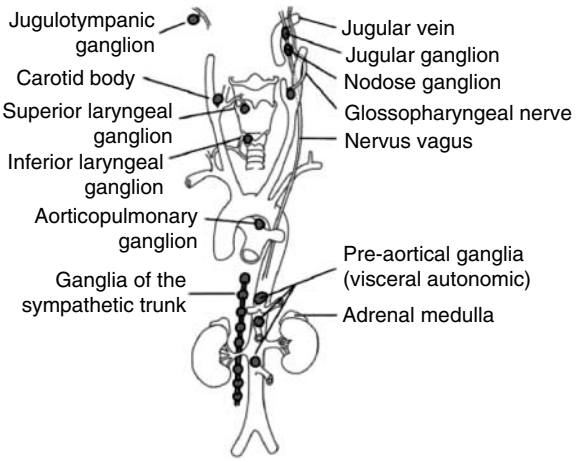

Figure 1 Anatomical distribution of paraganglia. Pheochromocytomas arise in the medulla of the adrenal gland, whereas sympathetic paragangliomas arise along the sympathetic chains in the pelvis, abdomen, and chest. Parasympathetic paraganglioma arise along the parasympathetic nerves in the head, neck, and mediastinum, the most common location being the carotid body. Adapted from Lips *et al.* (2006) with permission.

(Beard *et al*. 1983, Stenstrom & Svardsudd 1986, Ariton *et al*. 2000). The tumors may occur in all ages but have the highest incidence between 40 and 50 years, with an approximately equal sex distribution (O'Riordain *et al*. 1996, Favia *et al*. 1998, Goldstein *et al*. 1999, Erickson *et al*. 2001, Cascon *et al*. 2009*b*, Mannelli *et al*. 2009). In 693 unselected PCC/PGL patients, about 69% of the patients had PCC, 15% had sympathetic PGL, and 22% had parasympathetic PGL (some had a combination of tumors), providing an approximate measure of the relative incidence of the different tumor types (Cascon *et al*. 2009*b*, Mannelli *et al*. 2009).

PCCs and sympathetic PGLs are very similar histologically as well as functionally (DeLellis *et al*. 2004). They generally produce large amounts of catecholamines, mainly adrenaline and noradrenaline, at rates many times higher than normal, resulting in a high concentration of these fight-or-flight response causing hormones in the bloodstream (reviewed by Karagiannis *et al*. (2007)). The tumors usually cause hypertension, which may be either paroxysmal or sustained. Typical symptoms are recurring episodes of headache, sweating, and palpitations. Other symptoms may include anxiety, tremors, nausea, pallor, and abdominal or chest pain. Up to 10% of the patients have only minor or no signs of clinical symptoms and an increasing number of tumors are incidentally found during imaging studies (Kopetschke *et al*. 2009). In other cases, the tumors can cause severe cardiovascular or neurological manifestations such as shock, heart failure, seizures, and stroke, which can become life threatening and also obstruct a correct diagnosis (Spencer *et al*. 1993, Sibal *et al*. 2006).

Parasympathetic PGLs are histologically similar to PCCs and sympathetic PGLs (McNichol 2001), but whereas the latter two tumor forms are almost always clinically functional, parasympathetic PGLs are usually not (DeLellis *et al*. 2004). They typically have no or only a low production of catecholamines (Erickson *et al*. 2001, van Duinen *et al*. 2010) and commonly present as a slow-growing, painless cellular mass (DeLellis *et al*. 2004). Consequently, many patients are non-symptomatic. However, depending on site, the space occupation by the tumors may cause symptoms such as pain, hearing disturbances, hoarseness, and dysphagia.

The majority of PCCs and PGLs are benign. Malignancy is defined as the presence of distant metastases (DeLellis *et al*. 2004) and occurs in ~5–13% of PCCs (Goldstein *et al*. 1999, DeLellis *et al*. 2004, Mannelli *et al*. 2009), 15–23% of sympathetic PGLs (O'Riordain *et al*. 1996, Goldstein *et al*. 1999, Mannelli *et al*. 2009), and 2–20% (depending on site) of parasympathetic PGLs (DeLellis *et al*. 2004, Mannelli *et al*. 2009). The most common sites for metastasis are bone, liver, and lung tissue (Chrisoulidou *et al*. 2007). Currently, malignancy cannot be predicted with certainty, although some histological or gene expression features might be suggestive of malignancy (Strong *et al*. 2008). The prognosis of malignant PCC and PGL is poor, with a 5-year mortality rate >50% (Lee *et al*. 2002, Chrisoulidou *et al*. 2007). There is currently no effective or curative treatment, but surgery, chemotherapy, and radiotherapy are beneficial in some patients.

Genes and syndromes associated with PCC and PGL

Most PCCs and PGLs occur as sporadic tumors, and historically about 10% of the tumors were associated with hereditary syndromes, mainly multiple endocrine neoplasia type 2 (MEN2), von Hippel–Lindau disease (VHL), and neurofibromatosis type 1 (NF1) (Maher & Eng 2002). A small fraction is associated with other syndromes, including Carney triad, Carney–Stratakis syndrome, and, very rarely, MEN1. During the last decade, mutations in the genes encoding different subunits of the succinate dehydrogenase (SDH) complex have been linked to familial PCC–PGL syndrome, and subsequent genetic screenings have revealed that about 30% of PCCs and PGLs are caused by hereditary mutations (Amar *et al*. 2005, Mannelli *et al*. 2009). In addition, several novel susceptibility

genes, such as kinesin family member 1B (*KIF1Bβ*; Schlisio *et al*. 2008), EGL nine homolog 1, also termed *PHD2* (*EGLN1/PHD2*; Ladroue *et al*. 2008), transmembrane protein 127 (*TMEM127*; Qin *et al*. 2010), and MYC-associated factor X (*MAX*; Comino-Mendez *et al*. 2011), have recently been added to the list. The predisposing genes that have been identified seem at a first glance to have entirely different functions but, in spite of this, malfunction of their different gene products can give rise to clinically and histologically undistinguishable tumors. Nevertheless, some clinical features may be quite different, e.g. patients with *SDHB* mutations have considerably higher risk of malignancy than many other PCC/PGL patients (Gimenez-Roqueplo *et al*. 2003). The following section gives an overview of clinical characteristics of PCCs and PGLs with different genetic backgrounds, which is summarized in Table 1.

RET

Gene and protein function

RET is a proto-oncogene of 21 exons, located on chromosome 10q11.21. The gene was discovered in 1985 by transfection of NIH 3T3 cells with human lymphoma DNA (Takahashi *et al*. 1985). As it was activated by a rearrangement during the process, the name 'Rearranged during Transfection' was suggested. The gene product, RET, is a transmembrane receptor tyrosine kinase for members of the glial cell line-derived neurotropic factor (GDNF) family (Durbec *et al*. 1996, Jing *et al*. 1996, Trupp *et al*. 1996). RET is normally activated by the binding of one of its ligands, which induces dimerization (Treanor *et al*. 1996). A subsequent phosphorylation of specific tyrosine residues by RET is then believed to activate multiple intracellular pathways involved in cell growth and differentiation. The RET protein is mainly expressed in urogenital and neural crest precursor cells and is essential for the development of the kidneys as well as the sympathetic, parasympathetic, and enteric nervous system (Ichihara *et al*. 2004). Alternative splicing of the gene results in three isoforms, RET9, RET43, and RET51, which seem to differ slightly in function. Oncogenic activation of RET has been shown to activate both PI3K/AKT- and RAS/RAF/MAPK-dependent cell signaling (Besset *et al*. 2000, Califano *et al*. 2000, Segouffin-Cariou & Billaud 2000).

Gain-of-function mutations of the *RET* gene is the underlying genetic cause of the MEN2 syndrome (Donis-Keller *et al*. 1993, Mulligan *et al*. 1993, Hofstra *et al*. 1994). They are mostly missense and located in exons 10, 11, 13, 14, 15, and 16 (Raue & Frank-Raue 2010). Inherited inactivating mutations can be scattered throughout the gene and do instead predispose for Hirschsprung disease, which is a congenital disorder characterized by lack of ganglion cells in the colon (Edery *et al*. 1994, Romeo *et al*. 1994). Interestingly, some overlap has been reported between MEN2 and Hirschsprung phenotypes (Mulligan *et al*. 1994). A rare sequence variant (rs36119840) in the RET ligand gene *GDNF* has also been detected in the germline of one PCC patient, and it was suggested that *GDNF* variants may influence PCC susceptibility, although no further studies have been performed (Woodward *et al*. 1997).

MEN2 syndrome

MEN2 is an autosomal dominantly inherited tumor syndrome with a prevalence of ∼1/40 000 individuals (reviewed by Raue & Frank-Raue (2010)). Clinically, it can be divided into three types: MEN2A (55% of all cases), MEN2B (5–10%), and familial medullary thyroid carcinoma (FMTC, 35–40%). MEN2A and MEN2B patients have almost 100% risk of developing MTC and ∼50% risk of developing PCC. Patients with MEN2A also possess a risk (15–25%) of developing primary hyperparathyroidism, which is not a feature of MEN2B. MEN2B is the least common but often considered the most aggressive form with higher morbidity and mortality of MTC and an earlier onset, although the difference in aggressiveness has been argued (Leboulleux *et al*. 2002). FMTC is the mildest variant in which patients have familial, often more benign, MTC and by definition no incidence of other endocrine neoplasms.

RET-associated PCCs and PGLs

Activating *RET* mutations predispose to PCCs, which are often recurrent and bilateral, but typically have a low risk of malignancy (Table 1). Four large studies of PCCs in MEN2 (Lairmore *et al*. 1993, Modigliani *et al*. 1995, Quayle *et al*. 2007, Rodriguez *et al*. 2008), including a total of 514 MEN2 patients with PCCs (479 MEN2A and 35 MEN2B), are summarized in this review. In these studies, ∼63% of the patients displayed bilateral PCC and only 3% had malignant disease. The mean age at PCC presentation was 36 years, and PCC was diagnosed before MTC in 12–25% of the cases (Modigliani *et al*. 1995, Rodriguez *et al*. 2008). PGLs are very rare in MEN2 and were not reported in any of the above patients, but a few cases of sympathetic and parasympathetic PGL have been described (Neumann *et al*. 1993, Nilsson *et al*. 1999, Erickson *et al*. 2001, Boedeker *et al*. 2009).

Table 1 Characteristics of pheochromocytomas and paragangliomas associated with hereditary syndromes and/or susceptibility genes

Gene with germline mutation	Syndrome	Proportion of all PCCs/PGLs (%)	Mean age at presentation (years; range)	Penetrance of PCC/PGL (%)	Frequency of PCC (%)	Frequency of PGL (%; sPGL/psPGL)	Frequency of malignancy (%)	Frequency of bilateral PCC (%)	Frequency of multiple PGLs (%)
RET	MEN2	5.3	35.6 (4–73)	50	100	∼0[c,d]	2.9	63.2	0
VHL	VHL	9.0	28.6 (5–67)	10–26	90.3	18.6 (5.9/8.8)[e]	3.4	43.5	0
NF1	NF1	2.9	41.6 (1–74)	0.1–5.7	95.3	6.1 (6.1/0)	9.3	14.1	0
SDHD	PGL1	7.1	35.0 (10–96)	86[b]	23.9	91.5 (22.0/84.4)	3.5	0	56.4
SDHAF2	PGL2	∼0	32.2 (20–59)	∼100[b]	0	100 (0/100)	0	NA	86.7
SDHC	PGL3	0.5	42.7 (13–73)	U	∼0[c]	100 (7.1/92.9)	∼0[c]	U	16.7
SDHB	PGL4	5.5	32.7 (6–77)	77	25.2	77.5 (70.7/24.4)	30.7	0	20.8
SDHA	–	<3	40.0 (27–55)	U	16.7	83.3 (50.0/33.3)	0–14.3	0	0
KIF1Bβ[a]	–	∼0	46 (22–70)	U	100	0	0	100	NA
EGLN1[a]	–	∼0	43 (single patient)	U	0	100 (100/0)	0	NA	100
TMEM127	–	<2	42.8 (21–72)	U	95.7	8.7 (4.3/4.3)	4.3	39.1	4.3
MAX	–	U[f]	32.2 (17–47)	U	100	0	25.0	66.7	NA
Unknown	Carney triad	∼0	27.5 (12–48)	NA	16.2	91.9[d]	10.8	2.7	21.6
SDHB,C,D	Carney–Stratakis	∼0	33 (10–61)	U	9.1	100[d]	0	0	72.7
MEN1	MEN1	∼0	30.5 (29–32)	U	100	0	14.3	0	NA
No mutation	Sporadic disease	∼70	48.3 (5–93)	NA	72.9	29.1 (8.8/20.3)	8.9	6.2	1.2

Frequencies of different characteristics are given in relation to the total number of patients with tumors. Not all characteristics were available in all studies; please refer to the text for details and references. The proportion of all PCCs and PGLs associated with each gene was estimated from genetic screenings (Amar *et al*. 2005, Mannelli *et al*. 2009, Yao *et al*. 2010*a*,*b*, Korpershoek *et al*. 2011). PCC, pheochromocytoma; PGL, paraganglioma; sPGL, sympathetic PGL, psPGL, parasympathetic PGL; NA, not applicable; U, unknown.

[a]Only one to three PCC/PGL patients with mutations have been reported.

[b]Valid only for paternally inherited mutations, penetrance after maternal transmission is ∼0, putatively due to maternal imprinting.

[c]One or a few cases have been reported.

[d]Both sPGL and psPGL have been reported, but frequencies are unknown.

[e]Separate frequencies for sPGL and psPGL were not reported in all studies, here causing the sum of frequencies of sPGL and psPGL to be less than the total frequency of PGL.

[f]Not yet determined, but *MAX* mutations are likely to be found in more cases (see text).

von Hippel–Lindau

Gene and protein function

VHL is a tumor suppressor gene of three exons, mapping to chromosome 3p25.3. The gene was identified by positional cloning in 1993 (Latif *et al*. 1993). There are three *VHL* gene products: a full-length 213 amino acid protein and two shorter isoforms, resulting from an alternative splicing excluding the second exon and an alternative translation initiated from an in-frame ATG codon respectively (Richards *et al*. 1996, Kaelin 2008). VHL is involved in oxygen-dependent regulation of hypoxia-inducible factor (HIF) by constituting a part of the E3 ubiquitin ligase complex that ubiquitinates HIF-1α, thereby targeting it for proteasomal degradation (Maynard & Ohh 2007, Kaelin 2008).

VHL is considered a classical tumor suppressor gene in the sense that, in accordance with Knudsons two-hit model, biallelic inactivation is usually required for tumorigenesis (Knudson 1971, 1996). Loss of heterozygosity (LOH) of the wild-type allele is frequent in VHL-associated tumors, including PCCs (Crossey *et al*. 1994), and hypermethylation of the wild-type allele as an alternative mechanism of gene inactivation has also been reported (Herman *et al*. 1994, Prowse *et al*. 1997), although not in PCCs (Bender *et al*. 2000). Disease-causing mutations in *VHL* can be missense, nonsense, as well as deletions and insertions (indels), with missense mutations being more frequent in families with PCC/PGL (Woodward & Maher 2006).

VHL syndrome

Germline mutations that inactivate the *VHL* gene result in VHL, an autosomal dominantly inherited tumor syndrome occurring in ∼1/36 000 individuals (Woodward & Maher 2006). The disease is characterized by several different tumors such as clear cell renal carcinomas, PCCs, PGLs, pancreatic islet cell tumors, lymphatic sac tumors, and hemangioblastomas of the retina, cerebellum, kidney, and pancreas. About 10–26% of VHL patients develop PCC or PGL, but the risk varies between different families (Richard *et al*. 1994, Walther *et al*. 1999*b*, Baghai *et al*. 2002).

VHL-associated PCCs and PGLs

VHL mutations predispose to unilateral or bilateral PCCs and, much less frequently, to sympathetic or parasympathetic PGLs (Table 1). Six studies of VHL-associated PCCs and PGLs (Neumann *et al*. 1993, Richard *et al*. 1994, Walther *et al*. 1999*b*, Baghai *et al*. 2002, Amar *et al*. 2005, Mannelli *et al*. 2009), including a total number of 236 patients, have been analyzed. Of these patients, 90% had PCC and 19% had PGL. Bilateral PCC was seen in 44% of the patients, and only 3% displayed malignant tumors. The mean age at diagnosis of PCC/PGL was 29 years. PCC or PGL was the first manifestation of VHL disease in 30–55% of the cases (Richard *et al*. 1994, Baghai *et al*. 2002).

Neurofibromatosis type 1

Gene and protein function

NF1 is a large gene of 60 exons, located on chromosome 17q11.2 and encoding the protein neurofibromin (Boyd *et al*. 2009). The gene was discovered in 1990 (Viskochil *et al*. 1990) and has one of the highest spontaneous mutation rates in the human genome (Boyd *et al*. 2009). Alterations of the gene include missense, nonsense, and splice-site mutations as well as indels and chromosomal rearrangements. The gene product is mainly expressed in the nervous system, where it suppresses cell proliferation by promoting the conversion of RAS into its inactive form, thereby inhibiting the oncogenic RAS/RAF/MAPK signaling cascade (Ballester *et al*. 1990, Martin *et al*. 1990). Neurofibromin also inhibits the PI3K/AKT/mTOR pathway via suppression of RAS (Johannessen *et al*. 2005, 2008). NF1-related tumors, including PCCs, often display alterations of both alleles, normally including one germline mutation and one acquired mutation or LOH of the wild-type allele, implying that *NF1* functions as a classical tumor suppressor gene (Bausch *et al*. 2007, Boyd *et al*. 2009).

NF1 syndrome

Mutations in *NF1* result in NF1, also termed von Recklinghausen's disease, which occurs in ∼1 of 3500 persons (reviewed by Boyd *et al*. (2009)). It is inherited as an autosomal dominant disease, but 30–50% of the patients have new, spontaneous mutations that, if postzygotic, can give rise to a mosaic phenotype (Kehrer-Sawatzki & Cooper 2008). NF1 syndrome can be usually diagnosed early in childhood and the diagnostic features include neurofibromas, café au lait patches, skinfold freckling, iris Lisch nodules, optic pathway gliomas, and bone dysplasia (Boyd *et al*. 2009). Patients may also suffer from malignant peripheral nerve sheath tumors, other CNS gliomas, and cognitive impairment. PCCs and PGLs are not among the most common manifestations of NF1 but occur in 0.1–5.7% of the patients (3.3–13.0% at autopsy), representing a considerably higher incidence than in the general population (Walther *et al*. 1999*a*).

NF1-associated PCCs and PGLs

NF1-associated PCCs and PGLs typically have characteristics similar to those of sporadic tumors, with a relatively late mean age of onset and about 10% risk of malignancy (Table 1). A summary of a thorough review (Walther *et al*. 1999*a*) together with more recent studies (Amar *et al*. 2005, Bausch *et al*. 2007, Mannelli *et al*. 2009, Zinnamosca *et al*. 2011), including a total of 216 NF1 patients with PCC or PGL, showed that 95% of the patients had PCC and 6% had PGL, all of which were sympathetic. Fourteen percent of the patients displayed bilateral PCC, 9% developed malignant disease, and the mean age at presentation was 42 years.

SDHx

Genes and protein functions

SDH is a mitochondrial enzyme complex consisting of four subunits: SDHA, SDHB, SDHC, and SDHD, which are all encoded by the nuclear genome (reviewed by Rutter *et al*. (2010)). The enzyme, also known as mitochondrial complex II, is involved both in the tricarboxylic acid cycle, where it catalyzes the oxidation of succinate to fumarate, and in the respiratory electron transfer chain, where it transfers electrons to coenzyme Q. The gene *SDHA* is located on chromosome 5p15.33 and consists of 15 exons. It encodes a protein that functions as a part of the catalytic core and contains the binding site for succinate. The other part of the catalytic domain, which also forms an interface with the membrane anchor, is encoded by *SDHB*, a gene of eight exons located on chromosome 1p36.13. *SDHC* on chromosome 1q23.3 and *SDHD* on chromosome 11q23.1 contain six and four exons, respectively, and encode two hydrophobic proteins that anchor the complex to the mitochondrial inner membrane.

The link between SDH and neuroendocrine tumors was first established in the year 2000, when germline mutations in *SDHD* were discovered in patients with familial PGLs (Baysal *et al*. 2000). *SDHD* mutations were subsequently found also in apparently sporadic PCCs (Gimm *et al*. 2000) and PGLs (Dannenberg *et al*. 2002) as well as in familial PCCs (Astuti *et al*. 2001*a*). Shortly after, germline mutations were also identified in *SDHB* in both PCCs and PGLs (Astuti *et al*. 2001*b*). *SDHC* mutations were reported in PGLs in 2000 (Niemann & Muller 2000) and were also recently found in PCCs (Peczkowska *et al*. 2008). During several years, homozygous and compound heterozygous mutations in the gene encoding the fourth subunit, *SDHA*, were associated with a rare early-onset neurodegenerative disorder called Leigh syndrome (Bourgeron *et al*. 1995, Horvath *et al*. 2006), but neither with PCCs nor with PGLs (Bayley *et al*. 2005). This was intriguing since functional analysis showed that SDHA mutations cause SDH deficiency (Briere *et al*. 2005). However, most recently, a germline mutation in *SDHA* was reported in a patient with PGL (Burnichon *et al*. 2010) and subsequently in additional patients including one with PCC (Korpershoek *et al*. 2011), and thus all of the four SDH subunits have now been revealed to be involved in PCC and/or PGL development. In 2009, two factors involved in the assembly of the SDH complex were discovered, SDHAF1 (Ghezzi *et al*. 2009) and SDHAF2 (Hao *et al*. 2009). Whereas mutations in the *SDHAF1* gene have been associated with infantile leukoencephalopathy, a brain white matter disease (Ghezzi *et al*. 2009), mutations in *SDHAF2*, a gene of four exons on chromosome 11q12.2, have been identified in two families affected by PGLs (Hao *et al*. 2009, Bayley *et al*. 2010), but so far not in any PCC patients (Bayley *et al*. 2010, Yao *et al*. 2010*a*). Missense, nonsense, frameshift, as well as splice site mutations have been described in *SDHB* and *SDHD*, which are the most commonly altered *SDH* genes (Neumann *et al*. 2004).

The *SDHx* genes are believed to function as classical tumor suppressors since tumors generally display LOH of the non-mutated allele (Baysal *et al*. 2000, Gimenez-Roqueplo *et al*. 2003, Lopez-Jimenez *et al*. 2008, Burnichon *et al*. 2010). Mutations in any of the different *SDHx* genes, regardless of whether its gene product has catalytic or anchorage function, have been demonstrated to cause an abolishment of SDH enzyme activity (Gimenez-Roqueplo *et al*. 2001, Douwes Dekker *et al*. 2003) as well as an absence of SDHB protein expression (van Nederveen *et al*. 2009, Gill *et al*. 2010, Korpershoek *et al*. 2011).

PCC–PGL syndrome

Germline mutations in the *SDHx* genes give rise to familial PCC–PGL syndrome, sometimes only referred to as familial PGL. The syndrome can be divided into PGL1, PGL2, PGL3, and PGL4, which are caused by mutations in *SDHD, SDHAF2, SDHC*, and *SDHB* respectively. They are all inherited in an autosomal dominant manner but with varying penetrance. *SDHD* is putatively maternally imprinted and PGL1 is thus only passed on to children by their father (van der Mey *et al*. 1989), although one exception of maternal transmission has been reported (Pigny *et al*. 2008). To date, PGL2 has also only been diagnosed in individuals with an affected father, suggesting a similar

parent-of-origin-specific inheritance for *SDHAF2* (Kunst *et al*. 2011). No specific PCC/PGL syndrome has yet been described for *SDHA* mutations, but they seem to have a low penetrance of PCC/PGL and do not seem to be associated with a familial presentation (Burnichon *et al*. 2010, Korpershoek *et al*. 2011). The prevalence of PCC–PGL syndrome is unknown, but a summary of the cases reviewed here (about 13% of all PCC/PGL cases) gives an estimate of 1:50 000 to 1:20 000, the majority represented by PGL1 and PGL4.

Apart from PCCs and PGLs, *SDHB* mutations have been associated with renal cell carcinoma (Neumann *et al*. 2004, Vanharanta *et al*. 2004, Ricketts *et al*. 2008, 2010). One *SDHD* mutation carrier with a renal cell tumor has also been described (Ricketts *et al*. 2010), as well as a few cases of *SDHB* and *SDHD* patients with thyroid carcinoma (Neumann *et al*. 2004, Ricketts *et al*. 2010). In addition, mutations in *SDHB, SDHC*, and *SDHD* can give rise to the Carney–Stratakis syndrome (Stratakis & Carney 2009), characterized by the dyad of PGLs and gastrointestinal stromal tumors (GISTs), as will be discussed later. Very recently, *SDHA* mutations were also reported in two patients with GISTs but without PGLs (Pantaleo *et al*. 2011).

SDHx-associated PCCs and PGLs

SDHD mutations (PGL1) predispose most frequently to parasympathetic, often multifocal PGLs, but also to sympathetic PGLs and PCCs (Table 1). Several national and multinational studies have gathered information about tumor characteristics in patients with PCC–PGL syndrome (Neumann *et al*. 2004, Benn *et al*. 2006, Burnichon *et al*. 2009, Mannelli *et al*. 2009, Ricketts *et al*. 2010). Summarizing these studies for 289 patients with *SDHD*-related tumors, 24% had developed PCC, none of which were bilateral. As many as 92% had developed PGL (22% of the patients had sympathetic and 84% had parasympathetic PGL), 56% of the patients had multiple PGLs, and 4% had malignant disease. The mean age at presentation was 35 years and the penetrance of PCC/PGL in *SDHD* mutation carriers has been estimated to 86% by the age of 50 years (Neumann *et al*. 2004).

SDHAF2 mutations (PGL2) have so far been detected in one large Dutch kindred (Hao *et al*. 2009, Kunst *et al*. 2011) and in one Spanish family (Bayley *et al*. 2010), both afflicted with early-onset hereditary PGL and carrying the same mutation. Identity-by-state analysis of genome-wide single nucleotide polymorphism (SNP) data implied that the two families are unrelated (Bayley *et al*. 2010). In the Dutch kindred with PGL2, almost 100% penetrance of the disease has been reported by the age of 45 years (van Baars *et al*. 1981). All reported *SDHAF2*-related tumors have been parasympathetic PGLs, and no metastases have been described (Table 1). Summarizing 15 patients from the two families (Bayley *et al*. 2010, Kunst *et al*. 2011), the mean age at presentation was 32 years, and 87% of the patients had multiple PGLs.

SDHC mutations (PGL3) are rare but have been detected in up to 4% of patients with parasympathetic PGL (Schiavi *et al*. 2005). They are mainly associated with parasympathetic PGLs, much less frequently with sympathetic PGLs and very seldom with PCCs (Mannelli *et al*. 2007, Peczkowska *et al*. 2008). The tumors are typically benign, but malignancy has been reported in one case (Niemann *et al*. 2003). In three different studies (Schiavi *et al*. 2005, Burnichon *et al*. 2009, Mannelli *et al*. 2009), including 42 patients with *SDHC*-related tumors, all patients had PGLs (93% parasympathetic and 7% sympathetic). All tumors were reported as benign, and 17% of the patients had multiple tumors (Table 1). No PCCs were detected in these cohorts. The mean age at presentation was 43 years and only 20–25% of the patients revealed a family history of PGL, suggestive of an incomplete penetrance.

SDHB mutations (PGL4) are generally associated with higher morbidity and mortality than mutations in the other *SDHx* genes (Gimenez-Roqueplo *et al*. 2003). They typically predispose to sympathetic PGLs with a high risk of malignancy, and, less frequently, to benign or malignant PCCs and parasympathetic PGLs (Table 1). Meta-analysis of a number of studies (Neumann *et al*. 2004, Benn *et al*. 2006, Srirangalingam *et al*. 2008, Burnichon *et al*. 2009, Mannelli *et al*. 2009, Ricketts *et al*. 2010), totally including 378 patients with *SDHB*-related tumors, showed that 78% of the patients had PGL (71% had sympathetic and 24% had parasympathetic PGL) and 25% had PCC (none of which were bilateral). The mean age at presentation was 33 years, 21% of the patients presented with multiple PGLs, and as many as 31% of the patients displayed malignant tumors. The penetrance of PCC/PGL in *SDHB* mutation carriers has been estimated to 77% by the age of 50 years (Neumann *et al*. 2004).

SDHA mutations have so far been identified in six different patients with PCC or PGL (Burnichon *et al*. 2010, Korpershoek *et al*. 2011). Among these, one patient suffered from PCC and the other five from PGL (three sympathetic and two parasympathetic). The mean age at presentation was 40 years and no patients displayed metastases or multiple tumors (Table 1). A seventh patient who presented with a malignant sympathetic PGL can be suspected to carry an *SDHA*

mutation due to an immunohistochemically SDHA-negative tumor but could not be genetically tested (Korpershoek *et al*. 2011). The six patients reported by Korpershoek *et al*. (2011) were found among 198 apparently sporadic PCCs and PGLs (3%) in an immunohistochemical screening for the absence of SDHA expression. Interestingly, the identified *SDHA* mutations were also seen in low frequencies in a healthy control group, suggesting a low penetrance of PCC/PGL in *SDHA* mutation carriers (Korpershoek *et al*. 2011).

Kinesin family member 1B

Gene and protein function

KIF1B is a large gene of about 50 exons mapping to chromosome 1p36.22, a region that is frequently deleted in neural crest-derived tumors (Schlisio *et al*. 2008). The gene has two splice variants, *KIF1Bα* and *KIF1Bβ*. The encoded protein isoforms are kinesins that share a common region including a motor domain but have distinguished cargo domains transporting mitochondria and synaptic vesicle precursors respectively (Nangaku *et al*. 1994, Zhao *et al*. 2001). KIF1Bβ functions as a tumor suppressor that is necessary for neuronal apoptosis (Schlisio *et al*. 2008). Findings suggesting a proapoptotic role of KIF1Bβ were also put forward in another study (Munirajan *et al*. 2008), and both studies suggest that haploinsufficiency of *KIF1Bβ* may be adequate for tumorigenesis because the wild-type allele was retained in the tumors. Schlisio *et al*. (2008) discovered two different missense *KIF1Bβ* mutations in PCC patients without other predisposing mutations. Germline DNA was available for one patient, in which the mutation was confirmed to be germline. Three other germline mutations were identified in neuroblastoma patients, and one somatic mutation was detected in a patient with medulloblastoma.

Syndrome

No specific syndrome has been attributed yet, but patients with germline *KIF1Bβ* mutations seem to be predisposed to at least PCCs and neuroblastomas. Ganglioneuroma, leiomyosarcoma, and lung adenocarcinoma have also been reported in a family with *KIF1Bβ* mutations (Yeh *et al*. 2008).

KIF1Bβ-associated PCCs and PGLs

One of the PCC patients reported by Schlisio *et al*. (2008) suffered from neuroblastoma in childhood and developed PCC as an adult. Pedigree analysis revealed that the proband's paternal grandfather had also been diagnosed with PCC, while the proband's father did not show any signs of the disease (Yeh *et al*. 2008). Both patients displayed bilateral PCC, with an onset at 22 and 70 years respectively (Table 1). No PCC metastases were reported.

EGL nine homolog 1

Gene and protein function

EGLN1 is a gene of five exons located on chromosome 1q42.1, encoding the EGLN1 protein. EGLN1 is a member of the EGLN prolyl hydroxylase family, consisting of EGLN1, EGLN2, and EGLN3 (also termed PHD2, PHD1, and PHD3). In the presence of oxygen, the EGLN proteins catalyze a proline hydroxylation of HIF-α, allowing it to be recognized and targeted for degradation by the VHL containing E3 ubiquitin ligase complex (Maynard & Ohh 2007). EGLN1 appears to be the main HIF prolyl hydroxylase under conditions of normal oxygen levels (Berra *et al*. 2003).

In 2008, a germline mutation in *EGLN1* was reported in a patient with erythrocytosis and recurrent PGL (Ladroue *et al*. 2008). Germline mutations in *EGLN1* have previously been reported in patients with erythrocytosis, but not in association with tumors (Percy *et al*. 2006). The detected mutation was shown to affect EGLN1 function and stabilized HIF-1α and HIF-2α in HEK-293 cells. LOH was detected in the tumors, suggesting that *EGLN1* may possess a tumor suppressor function.

In a recent study, mutation analysis of *EGLN1, EGLN2*, and *EGLN3* was performed in 82 patients with features of inherited PGL and absence of mutations in known susceptibility genes, but no mutations were detected (Astuti *et al*. 2011). No studies on genetic alterations in *EGLNx* have been reported for PCC patients.

Syndrome

Only one PGL patient, suffering from recurrent PGL and erythrocytosis, has been reported to have a germline mutation in *EGLN1*, but no tumors have been reported in the relatives of the patient and no syndrome has been described yet (Ladroue *et al*. 2008).

EGLN1-associated PCCs and PGLs

The patient with *EGLN1* mutation was 43 years old at presentation with sympathetic PGL (Ladroue *et al*. 2008). A recurrent tumor was diagnosed 3 years later, but no metastases have been reported (Table 1).

Transmembrane protein 127

Gene and protein function

TMEM127 is a gene of four exons located on 2q11.2, a locus identified as a PCC susceptibility locus in 2005

(Dahia *et al*. 2005*a*). The transmembrane protein was recently revealed to function as a tumor suppressor, and germline mutations in *TMEM127* were detected in PCCs (Qin *et al*. 2010). Qin *et al*. also demonstrated that *TMEM127* is a negative regulator of mechanistic target of rapamycin, formerly mammalian target of rapamycin (mTOR), thus linking a critical signaling pathway for cell proliferation and cell death to the initiation and development of PCC. Both missense and nonsense mutations in *TMEM127* have been reported. LOH of the gene was detected in tumors of all tested mutation carriers, suggesting a classical two-hit model of inactivation.

Syndrome

So far, no specific syndrome has been described for *TMEM127*. Other tumors, including MTC, breast cancer, and myelodysplasia, have been identified in carriers of *TMEM127* mutations, but a causal relationship between the tumors and the mutations remains to be established (Jiang & Dahia 2011). A clear family history in only a fourth of the patients suggests an incomplete penetrance, and in a single family, the penetrance of PCC was 64% by the age of 55 years (Yao *et al*. 2010*b*).

TMEM127-associated PCCs and PGLs

Among 990 patients with PCC or PGL, negative for *RET, VHL*, and *SDHB/C/D* mutations, *TMEM127* mutations were identified in 20 (2.0%) of the cases, all of which had PCC (Yao *et al*. 2010*b*). Another study revealed one additional PCC patient with a *TMEM127* mutation (Burnichon *et al*. 2011). No *TMEM127* mutations were detected in 129 sympathetic and 60 parasympathetic PGLs (Yao *et al*. 2010*b*), but in a recent study, germline missense variants were detected in two out of 48 patients with multiple PGLs (Neumann *et al*. 2011), one of which also displayed bilateral PCC. Summarizing the 23 reported patients, all but one (96%) had PCC and 39% had bilateral PCC (Table 1). Two (9%) had PGL, of which one had sympathetic and the other multiple parasympathetic PGLs. The mean age at presentation was 43 years, and one patient (4%) displayed a malignant tumor.

MYC-associated factor X

Gene and protein function

MAX is a gene of five exons, located on chromosome 14q23.3. It encodes a transcription factor, MAX, that belongs to the basic helix–loop–helix leucine zipper (bHLHZip) family and plays an important role in regulation of cell proliferation, differentiation, and death as a part of the MYC/MAX/MXD1 network (Grandori *et al*. 2000). Members of the MYC family are proto-oncoproteins and their expression correlates with growth and proliferation, whereas expression of MXD1 (also known as MAD) is associated with differentiation. Heterodimerization of MAX with MYC family members results in sequence-specific DNA-binding complexes that act as transcriptional activators. In contrast, heterodimers of MAX with MXD1 family member repress transcription of the same target genes by binding to the same consensus sequence and thus antagonize MYC–MAX function.

Interestingly, PC12 cells, derived from a rat PCC, express only a mutant form of MAX incapable of dimerization, and a reintroduction of normal MAX in these cells resulted in a repressed transcription and inhibited growth (Hopewell & Ziff 1995). This suggests that some tumors can grow in the absence of MYC–MAX dimers and may imply that MAX can function as a tumor suppressor. A tumor suppressor role of MAX was most recently confirmed when germline *MAX* mutations were discovered in PCC patients by next-generation exome sequencing (Comino-Mendez *et al*. 2011). The mutations were missense, nonsense, splice site, or altering the start codon, and immunohistochemical analysis confirmed the lack of full-length MAX in the tumors. LOH of 14q, caused either by uniparental disomy or by chromosomal loss, was seen in investigated tumors in agreement with classical tumor suppressor behavior.

Syndrome

MAX mutations segregate with the disease in families with PCC (Comino-Mendez *et al*. 2011), but no specific syndrome has been described yet. A paternal origin of the mutated allele in investigated cases, together with the absence of PCC in persons who inherited a mutated allele from their mother, may suggest a paternal transmission of disease similar to that of PGL1 (*SDHD*) and PGL2 (*SDHAF2*).

MAX-associated PCCs and PGLs

Comino-Mendez *et al*. (2011) reported 12 PCC patients with MAX mutations, of which three were discovered with exome sequencing and four were relatives of those. The remaining five were found in a subsequent screening of 59 PCC patients lacking mutations in other known susceptibility genes but suspected to have hereditary disease (due to bilateral tumors, early age of onset, and/or familial antecedents with the disease). Of the 12 patients, eight (67%) had bilateral PCC and the mean age at presentation was

32 years (Table 1). Notably, 25% of the patients (38% of the probands) showed metastasis at diagnosis, suggesting that *MAX* mutations are associated with a high risk of malignancy. So far, no studies on PGLs have been reported.

Carney triad

Carney triad syndrome

Carney triad is a condition that includes a triad of tumors: PGLs, GISTs, and pulmonary chondromas (Carney 1999). PCCs and other lesions such as esophageal leiomyomas and adrenocortical adenomas have also been described (Stratakis & Carney 2009). The prevalence of Carney triad is not known, but <100 cases have so far been reported worldwide. The syndrome primarily affects young women, with a mean age of 21 years at presentation. About 20% of the patients have all three tumor types; the remaining has two of the three, most commonly GIST and pulmonary chondroma. In a study of 79 patients with Carney triad, 47% presented with PGL and/or PCC (Carney 1999).

Gene and protein function

Carney triad does not appear to run in families and no responsible gene has been discovered so far (Stratakis & Carney 2009). Yet, the coexistence of several rare tumor types and the young age of the affected individuals do implicate an inherited genetic defect, but the lack of familial cases has hampered linkage studies and positional cloning. Patients have been tested for mutations in *SDHA, SDHB, SDHC*, and *SDHD*, which are involved in familial PGLs, and also *KIT* and *PDGFRA*, which are the most frequently mutated genes in GISTs, but no mutations have so far been detected (Matyakhina *et al*. 2007, Stratakis & Carney 2009).

Carney triad-associated PCCs and PGLs

In a study of 37 Carney triad-patients with PCC and/or PGL, 92% presented with PGL, including both sympathetic and parasympathetic tumors, and 16% presented with PCC (Carney 1999). Multiple PGLs were found in 22% of the patients and bilateral PCC in 3%. Metastasis occurred in 11% of the patients and the mean age at presentation was 28 years (Table 1).

Carney–Stratakis syndrome

Carney–Stratakis syndrome

Carney–Stratakis syndrome, also termed Carney dyad, is a condition that includes PGLs and GISTs, but not pulmonary chondromas as in Carney triad (Carney & Stratakis 2002). The condition is inherited in an autosomal dominant manner but with incomplete penetrance. The prevalence is unknown, but so far about 20 kindreds with Carney–Stratakis syndrome have been identified (Stratakis & Carney 2009). The syndrome is, in contrast to Carney triad, equally common in men and women, with an average age of 23 years at presentation. Among 12 patients with Carney–Stratakis syndrome, 33% displayed both tumor forms, 58% showed only PGL/PCC and 8% showed only GIST (Carney & Stratakis 2002).

Genes and protein function

The majority of patients with Carney–Stratakis syndrome have been found to carry germline mutations in *SDHB, SDHC*, or *SDHD* (McWhinney *et al*. 2007, Pasini *et al*. 2008), which encode subunits of the SDH complex (described earlier). This has revealed a novel molecular mechanism behind GISTs, which are usually caused by gain-of-function mutations in *KIT* or *PDGFRA* (Hirota *et al*. 1998, 2003).

Carney–Stratakis syndrome-associated PCCs and PGLs

Among 11 patients with Carney–Stratakis-related PGL/PCC, 100% had PGL and one patient (9%) also presented with unilateral PCC, with a mean age of 33 years at presentation (Carney & Stratakis 2002). Multiple PGLs, which could be both sympathetic and parasympathetic, were seen in 73% of the patients, and none of the tumors were malignant (Table 1).

Multiple endocrine neoplasia type 1

Gene and protein function

MEN1 is a tumor suppressor gene consisting of ten exons on chromosome 11q13, which was identified by positional cloning in 1997 (Chandrasekharappa *et al*. 1997). Missense, nonsense, frameshift, as well as splice-site mutations in *MEN1* have been reported, and tumors frequently have LOH of the *MEN1* gene, consistent with a classical tumor suppressor function (reviewed by Lemos & Thakker (2008)). The gene product, menin, is a nuclear protein that interacts with several proteins involved in transcriptional regulation, genome stability, and cell proliferation. It has been demonstrated to bind JunD and suppress its activity and also to enhance the activity of c-Jun (Agarwal *et al*. 1999, Ikeo *et al*. 2004), but the precise mechanism for menin's role as a tumor suppressor still remains unclear.

MEN1 syndrome

Mutations in *MEN1* is the genetic cause of MEN1, an autosomal dominant disorder occurring in ~1 of 30 000 individuals (Agarwal *et al*. 2009). MEN1 is characterized by a combined occurrence of tumors in the parathyroid glands, pancreatic islet cells, and anterior pituitary gland, and some patients may also develop adrenal cortical tumors, carcinoid tumors, facial angiofibromas, collagenomas, and lipomas. PCC is a very infrequent and rarely described manifestation of the MEN1 syndrome (Schussheim *et al*. 2001).

MEN1-associated PCCs and PGLs

To our knowledge, no cases of PGL and only seven cases of PCC in the MEN1 syndrome have been reported in the literature (Alberts *et al*. 1980, Trump *et al*. 1996, Carty *et al*. 1998, Marx *et al*. 1998, 1999, Dackiw *et al*. 1999), previously summarized by (Schussheim *et al*. 2001). However, the authors know from personal communication that more unpublished cases exist, and the real incidence is thus not known. The reported tumors were unilateral in all cases and malignant in one case (14%). Age information was available for two patients, who were 29 and 32 years at onset respectively (Table 1).

Sporadic PCCs and PGLs

Apparently, sporadic tumors constitute the majority of PCCs and PGLs. The patients are generally somewhat older at onset and have a lower rate of multiple tumors than those with familial disease (Table 1). The rate of inherited mutations in patients with a negative family history has been reported to be 11–24% (Neumann *et al*. 2002, Amar *et al*. 2005, Cascon *et al*. 2009*b*, Mannelli *et al*. 2009), around the lower figure in patients with a single tumor, and without syndromic features. Somatic mutations in any of the identified familial disease genes are rare (Maher & Eng 2002, Korpershoek *et al*. 2007, van Nederveen *et al*. 2007, Waldmann *et al*. 2009).

Among 340 PCC/PGL patients with apparently sporadic PCC or PGL, 73% had PCC and 29% had PGL (9% had sympathetic and 20% had parasympathetic PGL; Mannelli *et al*. 2009). Bilateral PCC was seen in 6% of the patients and multiple PGLs in only 1%. When also including 228 patients with PCC or sympathetic PGL after a similar genetic screening (Amar *et al*. 2005), the average age at presentation was 48 years, and 9% of the patients had malignant disease. The summarized patients were negative for mutations in *RET, VHL, SDHB, SDHC*, and *SDHD* and showed no clinical signs of NF1 syndrome, but mutations in any of the more recently discovered susceptibility genes cannot be ruled out.

Gene expression and cellular pathways

Distinct gene expression profiles revealed by microarray analysis

Microarray studies of genome-wide mRNA expression have revealed that hereditary PCCs and PGLs cluster into two distinct groups based on their transcription profile: tumors with *VHL* mutations resemble those with mutations in any of the *SDHx* genes and display a different transcription profile compared to tumors caused by *RET* or *NF1* mutations (Eisenhofer *et al*. 2004, Dahia *et al*. 2005*b*). By unsupervised hierarchical cluster analysis of sporadic and hereditary PCCs, Dahia *et al*. (2005*b*) could identify two dominant expression clusters, where the first cluster contained all *VHL*- and *SDHx*-mutant tumors whereas the second contained all *RET*- and *NF1*-mutant tumors. Interestingly, the sporadic tumors were represented in both clusters. The VHL/SDH cluster showed a transcription signature associated with angiogenesis, hypoxia, and a reduced oxidative response, suggesting common molecular pathways in the development or preservation of these tumors. In contrast, the RET/NF1 cluster displayed a signature of genes involved in translation initiation, protein synthesis, and kinase signaling. Similar results were obtained in yet other independent studies which, in addition, could further divide the VHL/SDH cluster into SDH and VHL tumors by performing unsupervised clustering using either genes involved in oxidative phosphorylation (Favier *et al*. 2009), or target genes of HIF-1α and HIF-2α (Lopez-Jimenez *et al*. 2010). Subsequent studies have revealed that microarray transcription profiles of tumors with mutations in *KIF1Bβ* (Yeh *et al*. 2008), *TMEM127* (Qin *et al*. 2010, Burnichon *et al*. 2011), and *MAX* (Comino-Mendez *et al*. 2011) all cluster with the RET/NF1 group. As would be expected, both *SDHAF2*-mutant (Hensen *et al*. 2009) and *SDHA*-mutant (Burnichon *et al*. 2010) tumors have shown gene expression profiles similar to those of other *SDHx*-mutant tumors.

HIF-α regulation and pseudohypoxia

VHL and *SDH* mutations are linked by their ability to cause a so-called pseudo-hypoxic response by stabilizing HIFs under normoxic conditions (Fig. 2). HIFs are sequence-specific DNA-binding transcription factors that activate several genes promoting adaptation and survival under conditions of reduced oxygen

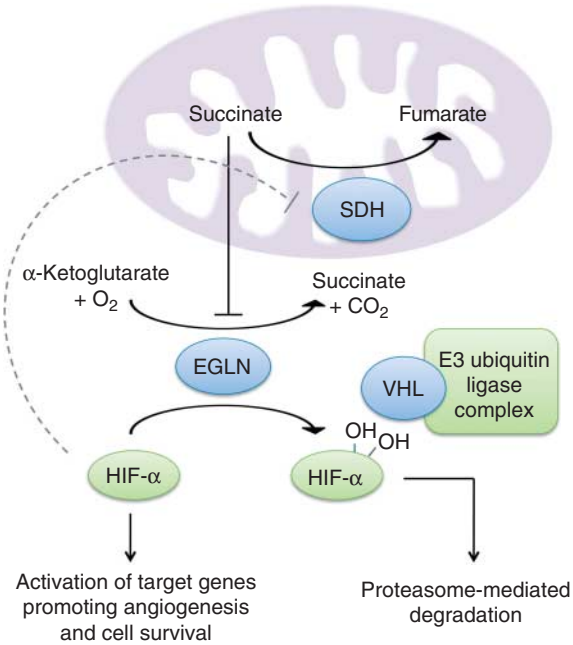

Figure 2 Regulation of HIF-α. Proteins that have been found inactivated by germline mutations in PCCs/PGLs are indicated in blue color. Inactivations of SDH, VHL, or EGLN1 are believed to cause a pseudo-hypoxic response where HIF-1α and/or HIF-2α escape ubiquitination and are allowed to accumulate. Downregulation of SDHB by high HIF-1α levels (dashed line), which would further enhance the pseudo-hypoxic response, has been suggested (Dahia *et al.* 2005*b*).

levels (hypoxia; reviewed by Maynard & Ohh (2007), Tennant *et al*. (2009), and Favier & Gimenez-Roqueplo (2010)). Active HIF is a heterodimer consisting of one α and one β subunit. There are three human HIF-α genes: *HIF-1α, HIF-2α*, and *HIF-3α*. The term HIF-α will here primarily refer to HIF-1α and HIF-2α, which are best characterized and appear to be the most important players in PCC and PGL. The β subunit HIF-1β, also called the aryl hydrocarbon receptor nuclear translocator, is stably expressed, and HIF activity is therefore regulated by the levels of HIF-α.

The VHL protein, pVHL, is part of an E3 ubiquitin ligase complex that ubiquitinates HIF-α and thereby targets it for degradation by the 26S proteasome (Maynard & Ohh 2007). The interaction requires proline hydroxylation of HIF-α in order for it to be recognized by the E3 complex. This hydroxylation is performed by members of the EGLN/PHD family, where EGLN1, which has been found to be mutated in PGL, appears to be the main HIF prolyl hydroxylase under normoxic conditions (Berra *et al*. 2003). The reaction is dependent on molecular oxygen (O_2) and α-ketoglutarate and produces succinate and CO_2 (Tennant *et al*. 2009). In the absence of functional pVHL or under conditions of hypoxia, HIF-α is allowed to accumulate and bind to HIF-1β and induce transcription of several genes involved in angiogenesis (e.g. *VEGF*), energy metabolism, survival, and growth. Thus, pVHL deficiency induces the same cellular response as hypoxia, a process referred to as pseudo-hypoxia.

The SDH complex, which catalyzes oxidation of succinate to fumarate in the tricarboxylic acid cycle, has also been associated with a pseudo-hypoxic response (Favier & Gimenez-Roqueplo 2010). An inactivation of SDH causes accumulation of succinate, which can diffuse out in the cytosol and has been shown to be a competitive inhibitor of EGLN, blocking the binding site of α-ketoglutarate (Briere *et al*. 2005). The succinate accumulation thus inhibits the EGLN enzyme activity, thereby leading to HIF-α stabilization and activation (Selak *et al*. 2005). It has been proposed that high HIF-1α levels may downregulate SDHB, suggesting a positive regulatory loop that further enhances the pseudo-hypoxic response (Dahia *et al*. 2005*b*). This model is supported by findings of suppressed SDHB protein levels in tumors with *VHL* mutation (Dahia *et al*. 2005*b*, Pollard *et al*. 2006) and might explain some of the similarities in transcription profile between *SDH*- and *VHL*-mutant tumors.

HIF-1α and HIF-2α (or sometimes exclusively HIF-2α) as well as several of their target genes have been shown to be overexpressed in *SDH*- and *VHL*-mutated PCCs and PGLs (Pollard *et al*. 2006, Favier *et al*. 2009, Lopez-Jimenez *et al*. 2010). This suggests a critical role for HIF-1α and/or HIF-2α and hypoxia in these tumors, although their precise role in tumor development remains unclear. A link between PCC/PGL and hypoxia is also consistent with the early and intriguing findings that persons exposed to chronic hypoxia, due to dwelling on high altitude, appear to have a higher prevalence of PGL compared with those living at sea level (Saldana *et al*. 1973, Rodriguez-Cuevas *et al*. 1998).

Activation of kinase signaling pathways

The genes of the second gene expression cluster, *RET* and *NF1*, are linked by their association with oncogenic kinase signaling pathways (Fig. 3). Oncogenic activation of RET triggers an activation of the RAS/RAF/MAPK pathway (Besset *et al*. 2000, Califano *et al*. 2000) and has also been associated with activation of the PI3K/AKT signaling pathway (Besset *et al*. 2000, Segouffin-Cariou & Billaud 2000). Both kinase cascades promote cell proliferation, growth, and survival and are frequently dysregulated

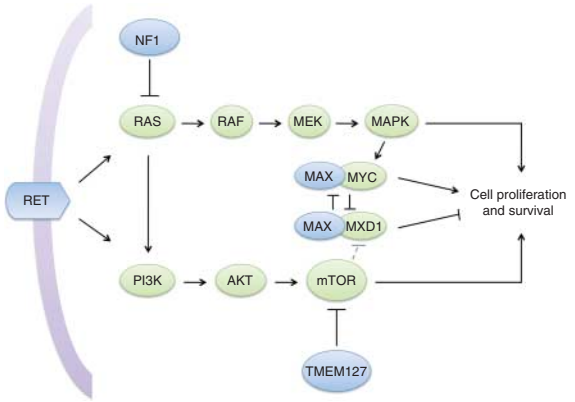

Figure 3 Kinase signaling pathways putatively involved in the development of PCCs/PGLs. Proteins that have been found altered by germline mutations (activating in the case of *RET* and inactivating in the others) in PCCs/PGLs are indicated in blue color. Activation of mTOR may constitute a common mechanism for tumor development caused by mutations in *RET, NF1*, or *TMEM127*. MYC may, at least in PC12 cells, function without forming dimers with MAX (Hopewell & Ziff 1995). In this context, MAX may control cell proliferation by forming dimers with MXD1 that antagonize the transcriptional activity of MYC.

in human cancers (reviewed in Vivanco & Sawyers (2002) and McCubrey *et al*. (2007)).

The *NF1* gene product, neurofibromin, promotes the conversion of RAS into its inactive form, and *NF1* mutations can thus also lead to an activation of the RAS/RAF/MAPK signaling pathway (Ballester *et al*. 1990, Martin *et al*. 1990). In addition, mutations in *NF1* can also activate the PI3K/AKT signaling cascade, an activation that is dependent on enhanced RAS activity (Johannessen *et al*. 2005, 2008).

As the microarray transcription profile of *TMEM127*-mutant tumors clustered with the RET/NF1 group and displayed a similar enriched expression of kinase receptor signals, it is tempting to hypothesize that TMEM127 regulates either RAS/RAF/MAPK or PI3K/AKT signaling (Qin *et al*. 2010). However, Qin *et al*. (2010) showed that this was not the case; instead *TMEM127* mutations enhanced mTOR activity in a RAS/RAF/MAPK- and PI3K/AKT-independent manner. Activation of mTOR, a kinase that is dysregulated in many human cancers, is a downstream signal of both *RET* and *NF1* mutations via the PI3K/AKT pathway, possibly suggesting a common mechanism for mutations in *RET, NF1*, and *TMEM127* (Fig. 3). Microarray expression analysis of *KIF1Bβ*-mutant (Yeh *et al*. 2008) as well as *MAX*-mutant (Comino-Mendez *et al*. 2011) tumors also revealed transcription patterns similar to that of the *RET/NF1*-mutant tumors, but the potential roles of *KIF1Bβ* and *MAX* in this context remain to be elucidated. A link between the MYC/MAX/MXD1 network and the other two pathways has been suggested since activation of the PI3K/AKT/mTOR and RAS/RAF/MAPK signaling cascades may promote the degradation of MXD1, thereby inhibiting it from antagonizing MYC transcription activity (Zhu *et al*. 2008). It is also well established that RAS/RAF/MAPK activation promotes MYC stability (Sears *et al*. 2000).

Developmental apoptosis of neuronal precursor cells

Despite the existence of two distinct groups of PCCs and PGLs, defined by their gene expression profiles, other studies have proposed that the different susceptibility genes converge into a single common pathway (Lee *et al*. 2005, Schlisio *et al*. 2008). According to this model, *RET, VHL, NF1*, and *SDHx* germline mutations all cause a defect in the apoptosis of neuronal progenitor cells, which normally occurs during embryogenesis as nerve growth factor (NGF) becomes limiting (Fig. 4). The neuronal apoptosis is induced by c-Jun, which is activated upon loss of NGF (Estus *et al*. 1994, Palmada *et al*. 2002). The *NF1* gene

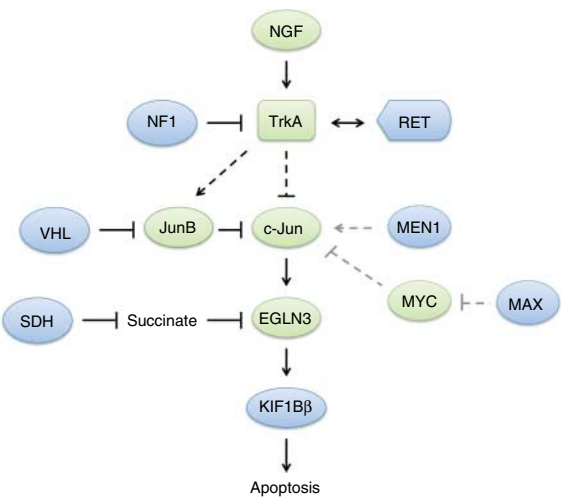

Figure 4 Model linking familial PCC/PGL genes to neuronal apoptosis when NGF becomes limiting. Proteins that have been found altered by germline mutations (activating in the case of *RET* and inactivating in the others) in PCCs/PGLs are indicated in blue color. The model was proposed by (Lee *et al*. 2005, Schlisio *et al*. 2008) and suggests that germline mutations in any of the predisposing genes cause a susceptibility to neural crest-derived tumors by allowing neuronal progenitor cells to escape from c-Jun/EGLN3-dependent apoptosis. Dashed lines suggest possible roles of *MAX* and *MEN1* in this context: the *MEN1* gene product, menin, can enhance c-Jun activity, whereas MYC (which may be antagonized by MAX–MXD1) can block c-Jun upregulation.

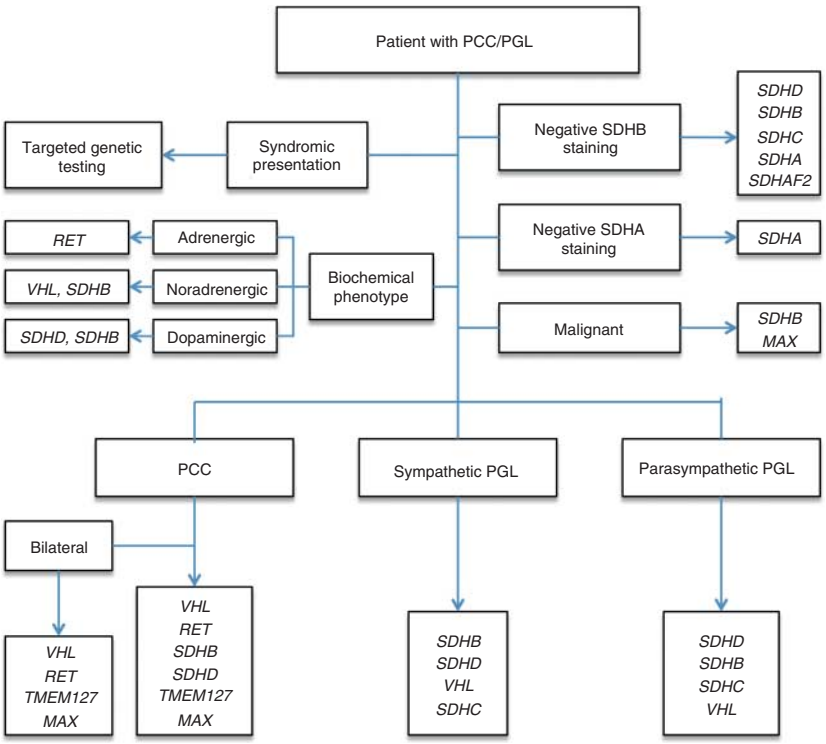

Figure 5 Proposed genetic testing algorithm for patients with PCC or PGL based on the clinical features of the tumor(s). The flow chart was based on the cases reviewed here and previous publications stated in the text. Genes are listed after descending priority from top to bottom/left to right. Biochemical phenotype (please refer to Karasek *et al.* (2010) for definitions) and immunohistochemical staining for SDHB (perhaps in combination with SDHA) can, when available, be used as a complement guide to what genes should be prioritized. Owing to the large size of the *NF1* gene and the usually very typical and early-onset skin lesions and other characteristics in patients with NF1 syndrome, *NF1* mutations are normally deduced on the basis of phenotype. The algorithm can be used as a guide for efficient genetic screening. However, in individual cases, no gene can be ruled out until tested.

product neurofibromin can inhibit the NGF receptor TrkA, and loss of neurofibromin promotes the survival of embryonic sympathetic neurons in the absence of NGF (Vogel *et al*. 1995). It has also been shown that RET and TrkA can cross talk and possibly activate each other (Tsui-Pierchala *et al*. 2002, Peterson & Bogenmann 2004). Lee *et al*. (2005) showed that elevated levels of the transcription factor JunB blocked apoptosis in PC12 cells and suggested inhibition of c-Jun by JunB. Further, loss of pVHL as well as oncogenic activation of RET leads to an induction of JunB, resulting in decreased apoptosis in PC12 cells after NGF withdrawal. Lee *et al*. (2005) also demonstrated that EGLN3, but not the other members of the EGLN family, induces neuronal apoptosis and placed it downstream of c-Jun in the NGF signaling pathway. Accumulation of succinate due to SDH inactivation inhibits EGLN3, and SDH inhibition was shown to reduce apoptosis in PC12 cells. An shRNA screening for preventing EGLN3-induced cell death resulted in the finding of *KIF1Bβ*, which was a target for one of the identified shRNAs (Schlisio *et al*. 2008). Introduction of KIF1Bβ into PC12 cells was sufficient to induce apoptosis, and siRNA knockdown of human EGLN3 (but not EGLN1) in HeLa cells decreased KIF1Bβ levels, suggesting that KIF1Bβ acts downstream of EGLN3.

In summary, Lee *et al*. (2005) and Schlisio *et al*. (2008) proposed a model where germline mutations in *RET, VHL, NF1, SDHx*, or *KIF1Bβ* allow neuronal progenitor cells to escape from c-Jun/EGLN3-dependent apoptosis during early development (Fig. 4) and that these cells are capable of forming PCCs and PGLs later in life. The theory is supported by the fact that somatic mutations of the familial disease genes, as opposed to the case in many other cancers, are rare in sporadic PCCs and PGLs. However, the model does not provide an explanation for the two distinct transcription profiles seen in these tumors. Augmentation of c-Jun activity induced by menin (Agarwal *et al*. 1999, Ikeo *et al*. 2004) and blocking of c-Jun upregulation by MYC (Vaque *et al*. 2008) may suggest potential roles for *MEN1* and *MAX* mutations in this context, although it remains to be investigated.

Whether there are any links between *EGLN1* or *TMEM127* and neuronal apoptosis also still remains to be determined.

Concluding remarks

During the past few years, numerous advances have taken place in the field of PCC and PGL biology, revealing an increasingly versatile genetic background of these intriguing tumors. Several novel susceptibility genes have been discovered, and even though only about 10% of the patients have a positive family history, genetic screenings have revealed that about 30% have germline mutations in any of the identified genes (Mannelli *et al*. 2009). The frequency is likely to increase as additional susceptibility genes probably remain to be discovered, an assumption supported by the existence of families with PCC or PGL without an identified genetic cause and the young age of some apparently sporadic cases (Comino-Mendez *et al*. 2011), the existence of at least one additional potential susceptibility locus (Dahia *et al*. 2005*a*), and the so far unidentified but plausibly genetic cause of Carney triad (Stratakis & Carney 2009).

Genetic testing can be of great importance for patients and their relatives, especially in cases of malignant or multiple tumors or a young age of onset. In light of the cases reviewed herein and further publications (Cascon *et al*. 2009*a*, Erlic *et al*. 2009, Petri *et al*. 2009, Karasek *et al*. 2010, Waguespack *et al*. 2010), we propose a genetic testing algorithm that may constitute a guide for a time- and cost-efficient genetic screening (Fig. 5). Measurements of plasma concentrations of catecholamines and their metabolites (Karasek *et al*. 2010, Eisenhofer *et al*. 2011) and SDHB and SDHA immunohistochemistry (van Nederveen *et al*. 2009, Gill *et al*. 2010) may be valuable tools to further guide the order of genetic testing and thereby reduce the costs. Knowledge of the clinical features linked to different hereditary backgrounds can be crucial for decision making regarding treatment and surveillance. For example, complete unilateral adrenalectomy may be the best alternative in *SDHB* mutation carriers with PCC, considering the high risk of malignancy and the low risk of bilateral PCC, whereas MEN2 and VHL patients with high risk of bilateral tumors and low risk of malignancy might benefit from cortical-sparing surgery.

Studies of genome-wide transcription patterns have shed new light on the molecular characteristics of PCCs and PGLs and revealed cellular pathways that might be potential targets of future therapeutic approaches. *VHL*- and *SDHx*-related tumors share a similar gene expression profile linked to hypoxia and angiogenesis, where a stabilization of HIF-1α and/or HIF-2α under normoxic conditions may play a central role in the pathogenesis. In contrast, the profile displayed by *RET*-, *NF1*-, *KIF1Bβ*-, *TMEM127*-, and *MAX*-related tumors can be linked to an activation of kinase signaling pathways. Interestingly, sporadic tumors can belong to either of the two distinct groups. Apart from these two models, an additional model has been suggested that links the different familial disease genes to a common pathway, where germline mutations would cause tumor susceptibility by allowing neuronal progenitor cells to escape from apoptosis during embryogenesis. It is hoped that our increasing knowledge of the underlying pathogenesis of these tumors will lead to the development of new treatment modalities.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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