Published in final edited form as:

Cancer Genet. 2012 January ; 205(1-2): 1–11. doi:10.1016/j.cancergen.2012.01.009.

Pheochromocytoma and Paraganglioma: Understanding the Complexities of the Genetic Background

Lauren Fishbein^a and Katherine L. Nathanson^{b,c,*}

^aDepartment of Medicine, Division of Endocrinology, Diabetes and Metabolism, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104

^bDepartment of Medicine, Division of Translational Medicine and Human Genetics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104

^cAbramson Cancer Center, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104

Abstract

Pheochromocytomas and paragangliomas (PCC/PGL) are tumors derived from the adrenal medulla or extra adrenal ganglia, respectively. They are rare and often benign tumors which are associated with high morbidity and mortality due to mass effect and high circulating catecholamines. Although most pheochromocytomas and paragangliomas are thought to be sporadic, over one third are associated with ten known susceptibility genes. Mutations in three genes causing well characterized tumor syndromes are associated with an increased risk of developing pheochromocytomas and paragangliomas including *VHL* (von Hippel-Lindau disease), *NF1* (Neurofibromatosis Type 1), and *RET* (Multiple Endocrine Neoplasia Type 2). Mutations in any of the succinate dehydrogenase (SDH) complex subunit genes (*SDHA*, *SDHB*, *SDHC*, *SDHD*) can lead to pheochromocytomas and paragangliomas with variable penetrance, as can mutations in the subunit cofactor, *SDHAF2*. Recently, two additional genes have been identified, *TMEM127* and *MAX*. Although these tumors are rare in the general population, occurring in 2–8 per million people, they are more commonly associated with an inherited mutation than any other cancer type. This review summarizes the known germline and somatic mutations leading to pheochromocytoma and paraganglioma development, as well as biochemical profiling for PCC/PGL and screening of mutation carriers.

Keywords

Pheochromocytoma; paraganglioma; genetics; mutations; screening

INTRODUCTION

Pheochromocytomas and paragangliomas (PCC/PGL) are tumors of the autonomic nervous system which are derived from chromaffin tissue in the adrenal medulla or extra adrenal

© No copyright information found. Please enter manually.

Corresponding Author: Katherine L. Nathanson, Division of Translational Medicine and Human Genetics, Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, 351 BRB II/III, 421 Curie Blvd, Philadelphia, PA 19104, Phone: 215-573-9840, Fax: 215-573-7945, knathans@exchange.upenn.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ganglia, respectively. The tumors are rare, occurring in 2–8 per million people with a peak incidence in the third to fourth decade of life (1). PCC/PGL are often benign, but associated with high morbidity and mortality secondary to mass effect and high circulating catecholamines. This hormonal hypersecretion can lead to hypertension, stroke and even death. Classically, episodic headaches, palpitations, diaphoresis, and anxiety are thought to be hallmarks of a secreting PCC/PGL. Nevertheless, these symptoms do not occur in all patients despite excessive catecholamines in the circulation (2), which can sometimes hinder diagnosis. PGLs can be divided into sympathetic and parasympathetic tumors; PCCs fall into the former category. Sympathetic PGLs can be located anywhere along the sympathetic ganglia in the thorax, abdomen and pelvis and, like adrenal PCC, they tend to hypersecrete catecholamines. In contrast, parasympathetic PGL are predominantly found in the head and neck region; the vast majority are non-secretory.

Approximately a quarter of PCC/PGL are malignant, defined as the presence of chromaffin tissue in sites where it is not normally found, most commonly in bone, liver, lymph nodes and lung (1). Metastases can occur at initial diagnosis, or even 20 years later; unfortunately, there currently are no good predictors of malignancy. The histological PASS (Pheochromocytoma of the Adrenal gland Scaled Score) system is a graded score developed to help predict potential malignancy of adrenal based PCC, taking into account various histologic findings such as number of mitoses, amount of necrosis, and vascular density (3). Although the PASS is widely recorded for adrenal PCC, it has high intra- and inter-observer variability, making it unreliable (4). Some studies suggest malignancy is associated with larger primary tumors (over 4–5 cm in diameter), increased vascular density or expression of molecular markers of vascularity such as CD32 or VEGF (5–8). However, currently the only reliable predictor of malignancy is a germline mutation in *Succinate dehydrogenase subunit B* (*SDHB*), one of the known susceptibility genes (discussed further below).

Population-based studies have found approximately one-third of patients with apparently sporadic PCC/PGL have a germline mutation in a known susceptibility gene (9–11). This number can be as high as 79% in patients with a positive family history of non-syndromic PCC/PGL (12) and 54% in patients with head and neck PGL (13). Ten known susceptibility genes for PCC/PGL have been identified to date: three genes for three well known cancer susceptibility syndromes, *VHL* for von Hippel-Lindau disease (vHL), *NF1* for Neurofibromatosis Type 1 (NF1) and *RET* for Multiple Endocrine Neoplasia Type 2 (MEN 2); the *Succinate Dehydrogenase* (*SDH*) complex subunit genes (*SDHA*, *SDHB*, *SDHC*, *SDHD*); one of the SDH complex cofactors, *SDHAF2*; and two recently recognized susceptibility genes, *TMEM127* and *MAX* (Table 1). Therefore, although PCC/PGLs are infrequent, they are more commonly associated with an inherited mutation than any other cancer type. This article reviews the current literature regarding genetics in PCC/PGL.

CANCER SUSCEPTIBILITY SYNDROMES

von Hippel-Lindau Disease

von Hippel-Lindau disease (vHL) is an autosomal dominant cancer susceptibility syndrome with an incidence of approximately one in 36,000 births per year (14, 15). It is characterized by a variety of benign and malignant tumors including hemangioblastomas of the central nervous system (in the brain, spinal cord and retina), renal cysts and clear cell renal cell carcinoma (RCC), pancreatic cysts and pancreatic neuroendocrine tumors, endolymphatic sac tumors and epididymal cystadenomas (reviewed in detail elsewhere (16)).

Pheochromocytomas occur in about 10–20% of patients with vHL and may be one of the earliest manifestations of the disease. In fact, the mean age of PCC presentation in patients with vHL is 30 years old (17). The rate of malignant PCC in vHL disease is about 5% (16). vHL associated PCCs tend to be located in the adrenal gland and are often bilateral;

nevertheless, occasional patients do develop extra adrenal and head and neck PGLs (18, 19). Screening with annual plasma free metanephrines for PCC in vHL patients should begin at 5 years of age for families with high risk of PCC (20).

The *VHL* gene is located on chromosome 3p25–26 and consists of 639 nucleotides which code for two VHL proteins, one full length 213 amino acids and a smaller protein that lacks the first 53 amino acids (OMIM *608537). The VHL protein forms a complex with elongin B, elongin C, RBx 1 and Cul2 which has ubiquitin ligase E3 activity (21). The normal function of the VHL protein is to regulate transcription of hypoxia inducible genes. Under normoxic conditions VHL binds to the hydroxyproline residue on Hypoxia Inducible Factors (HIFs) α targeting it for ubiquitination and proteosomal degradation (22). Under hypoxic conditions, or if there is a mutation in *VHL*, this interaction cannot take place, resulting in the loss of ubiquitination of HIF α , and thus allowing it to bind to the HIF β subunit and target transcription of hypoxia inducible genes (21).

VHL is a tumor suppressor gene and most vHL-associated PCCs show loss of heterozygosity (LOH) of the wild type allele as the second hit according to Knudson's two hit hypothesis. The risk of PCC in vHL patients varies according to the clinical subtype and underlying *VHL* mutation (23–28). Patients with Type 1 vHL disease have truncating mutations or exon deletions with a lower penetrance for PCC and a higher penetrance for RCC. Patients with Type 2 vHL disease have missense mutations in the *VHL* gene, which are associated with development of RCC or PCC depending on the location of the missense mutation. Specifically, mutations which disrupt the VHL-HIF protein interaction tend to lead to RCC development, whereas mutations in other parts of the VHL protein tend to lead to PCC development (27, 28). This genotype-phenotype correlation suggests that PCC formation is independent of HIF regulation. Furthermore, missense mutations which affect the surface of the folded protein have a higher risk of PCC development than missense mutations which affect the deeper protein core (29).

Neurofibromatosis Type 1

Neurofibromatosis Type 1 (NF1), also called von Recklinghausen's disease, is an autosomal dominant genetic disorder caused by inactivating mutations in the tumor suppressor gene, *NF1*. The *NF1* gene is a large gene of 360kb and over 60 exons located on chromosome 17q11.2 (OMIM *162200). The protein, neurofibromin, is 2818 amino acids and the most well characterized function is as a GTPase to inactivate Ras and inhibit the MAPK signaling pathway. When *NF1* is mutated, there is constitutive activation of Ras and hence, the downstream MAPK, PI3K and mTOR pathways, leading to uncontrolled cellular growth and differentiation (30–32). Up to 50% of NF1 patients have a *de novo* mutation, and there is variable penetrance and expressivity of the disease even in patients with the same mutation (33).

The diagnosis of NF1 is made based on clinical diagnostic criteria rather than genetic testing since the gene is large and there is no hot spot for mutations. Patients must have at least two of the following features: six or more café-au-lait macules of specific size depending on age; two or more cutaneous neurofibromas or a single plexiform neurofibroma; inguinal or axillary freckling; two or more Lisch nodules (benign iris hamartomas); optic nerve glioma; dysplasia of the long bones; and a first degree relative with NF1 (34). Several other cancers have been associated with NF1 at a higher frequency than the general population, including malignant peripheral nerve sheath tumors and chronic myeloid leukemia (33).

The estimated rate of PCC in NF1 is 5–7%; however, an autopsy series found a PCC in 13% of NF1 patients (35). The mean age of presentation is 42 years, similar to the general population. Screening with plasma metanephrines is suggested only for hypertensive NF1

patients (36). Most NF1 associated PCC are unilateral adrenal tumors and malignant PCC rates are about 12% (37).

Multiple Endocrine Neoplasia Type 2

Multiple Endocrine Neoplasia Type 2 (MEN 2) is an autosomal dominant syndrome caused by activating mutations in the *RET* proto-oncogene. The gene is located on chromosome 10q11.2 and encodes an 860 amino acid transmembrane tyrosine kinase protein (OMIM *171400). When ligand binds to the RET receptor, or there is an activating mutation, a cell signaling cascade is triggered through the PI3 kinase pathway to regulate cell proliferation and apoptosis (38).

There are three sub-types of MEN 2 and strong genotype-phenotype correlations (38–41). Ninety percent of MEN 2 patients are in sub-type 2A, characterized by medullary thyroid carcinoma, PCC and primary hyperparathyroidism in 95%, 50% and 15–30% of patients, respectively. The majority of MEN 2A patients have a mutation in codon 634 in exon 11 of *RET* (39). MEN 2B is characterized by medullary thyroid carcinoma in 100% of patients, PCC in 50% of patients, marfanoid habitus and multiple mucosal ganglioneuromas. Almost all cases of MEN 2B are caused by a single missense mutation in codon 918 in exon 16 of *RET* (p.Met918Thr) (38). The third subgroup is called familial medullary thyroid carcinoma (FMTC) in which patients have only medullary thyroid cancer. The diagnosis of FMTC is based on the absence of PCC and hyperparathyroidism in two or more generations within a family or by providing evidence of a *RET* mutation in a codon associated with susceptibility to only medullary thyroid cancer, including codons 768, 790, 804 and 891 (38).

MEN 2 associated PCC are usually adrenal and are often bilateral (greater than 50% of the time) (42, 43). The risk of PCC in MEN 2 is associated with specific *RET* mutations, with the highest risk associated with mutations codons 634, 883, 918, and double mutations in codons 804 plus 805 or 806 (41). Very rarely parasympathetic PGL have been described in the head and neck (18). The frequency of malignant disease is less than 5% (44). MEN 2 associated PCC usually present between 30 and 40 years of age. The age to begin screening with annual biochemistries is suggested based on risk of PCC by mutation as discussed above. Screening begins as early as age 8 for all MEN 2B patients and for MEN 2A patients with mutations in codons 630 or 634, and screening begins at age 20 for all other MEN 2A associated mutations (41).

PARAGANGLIOMA SYNDROMES

Mutations in any one of the Succinate dehydrogenase (SDH) complex subunits can lead to PCC/PGL formation. The SDH complex, also known as complex II of the mitochondrial respiratory chain, is a highly conserved heterotetrameric protein and is the only complex in the electron transport chain also involved in the Krebs' cycle. The catalytic subunits SDHA and SDHB are in the mitochondrial matrix and anchored to the inner membrane by subunits SDHC and SDHD. The SDH enzyme catalyzes the oxidation of succinate to fumarate in the Krebs' cycle and couples that with electron transfer to the terminal acceptor ubiquinone in the electron transport chain.

PGL1 (*SDHD*)

Mutations in *SDHD* lead to PGL1 (45–47). *SDHD*, located on chromosome 11q23, has four exons spanning 19kb and encodes a 103 amino acid protein (48, 49). *SDHD* (OMIM *602690) encodes the small anchoring subunit with an ubiquinone binding site to which the electrons are transferred from the iron sulfur clusters within the SDHB subunit. Mutations in *SDHD* are inherited in an autosomal dominant fashion with a parent of origin effect. Disease susceptibility occurs when the mutation is inherited from the father, suggesting this gene is

maternally imprinted (13, 50). However, proving this gene is maternally imprinted has been difficult as the data has been mixed. One study showed only paternal mutant expression of *SDHD* in tumor samples (51), while other studies have demonstrated bi-allelic expression in normal tissues including adrenal gland and in some PCC tissue (46, 52, 53). Interestingly, *Sdh* knockout mice do not develop PCC/PGL at any age (54). This finding suggests that other modifier genes or additional mutated loci nearby are needed to contribute to tumorigenesis. Complicating the issue further, there have been a few reported cases of a maternally transmitted *SDHD* mutant allele (55, 56). In 2008, Pigny et al. described a case report of a male with a head and neck PGL who shared a *SDHD* germline mutation with his mother. However, this finding should be interpreted cautiously as the tumor for this patient was not removed surgically, and therefore, not investigated on a molecular level (55). In 2011, Yeap et al. described a three generation kindred with PCC/PGL who share a maternally transmitted *SDHD* mutation found in both germline and tumor DNA analysis (56). In addition, the molecular analysis showed that two independent recombination events occurred in the tumor. The authors suggest that the predominant factor underlying the parent of origin effect is the need to lose or inactivate one or more genes that would normally be transcribed on the maternal 11p15 allele (56). Thus far, maternal transmission appears to be a rare event; however, it should be a consideration during genetic counseling.

The penetrance for PCC/PGL in *SDHD* mutation carriers reaches 90% or higher by age 70 (50, 57). Most patients develop multiple head and neck PGL, but other extra adrenal tumors have been seen as well. In fact, the risk by age 60 of developing a head and neck PGL is 71% with a mean age of 40 years, and the risk of developing an extra adrenal PGL is 29% with a mean age of 21 years (50). *SDHD* mutation carriers can develop adrenal based PCC, but usually this is one of multiple PCC/PGL tumors throughout the body (58). The associated risk of malignancy is less than 5% for *SDHD* mutation carriers (50).

PGL2 (*SDHAF2*)

The gene for PGL2 syndrome been identified as *SDHAF2*, also known as *SDH5* (59). This gene maps to chromosome 11q12.2 and encodes for the 167 amino acid protein necessary for flavanation of the SDHA protein in complex II, which is essential for the enzyme activity (OMIM *601650). One common mutation, c.232G>A; p.Gly78Arg, was identified in two distinct families with head and neck PGL (one family with Dutch decent and one with Spanish decent) (59–61). Inheritance is autosomal dominant with a parent of origin effect similar to *SDHD*, with cancer susceptibility associated only with paternal transmission. Recently, the clinical characteristics of the largest PGL2 pedigree known to date were described (61). Out of 57 family members, 24 had an *SDHAF2* mutation; and 91% of affected individuals had more than one head and neck PGL. In this family, the average age of onset of PGL was 33 years with the range from 22–47 years old; and furthermore, there was 100% penetrance by age 50. None of the tumors were malignant. These data are based on small numbers of patients but suggest that individuals with multiple head and neck PGL who are negative for other SDH gene mutations, should be tested for *SDHAF2* mutations. However, even in this subset, mutations in *SDHAF2* are quite rare as one study found no mutations in 201 patients with head and neck PGL who had already been screened, and were negative, for mutations in *SDHD*, *SDHC*, or *SDHB* (60).

PGL3 (*SDHC*)

Mutations in *SDHC* are responsible for PGL3 syndrome, which is inherited in an autosomal dominant manner with no parent of origin effect. The gene is located on chromosome 1q23.3 and encodes the large subunit with the cytochrome b in the mitochondrial complex II (OMIM *602413). The gene contains six exons spanning 35kb (62) and encodes a 140 amino acid protein (49). Mutations in *SDHC* are less common compared to those in *SDHD*

and *SDHB*, and occur in only 0–6.6% of PCC/PGL patients (13, 63–66). The mean age of initial diagnosis is 38 years old, with a range of 17–70 years old (13). Patients with *SDHC* mutations tend to have solitary head and neck PGL, but rare extra adrenal PGLs and even PCC have been described (13, 67). Furthermore, there is very low risk of malignant transformation associated with this gene mutation.

PGL4 (*SDHB*)

Mutations in *SDHB* are responsible for PGL4 syndrome, conferring tumor susceptibility with an autosomal dominant inheritance. The *SDHB* gene is located on chromosome 1p36.1–p35 with 8 exons spanning 40kb and encodes the iron sulfur subunit of the succinate dehydrogenase complex (OMIM *185470). *SDHB* acts as a tumor suppressor gene, with LOH seen in the tumors as the second hit (68, 69). Although the precise mechanism for tumorigenesis is unknown, mutations in *SDHB* are associated with dysregulation of the hypoxia pathway including over-expression of HIF α and hypoxia inducible gene products such as VEGF (69–72). Mutations range from point mutations and small insertions and deletions to large deletions or duplications of the *SDHB* gene loci (reviewed in (73)). A genotype-phenotype correlation has not been delineated.

Most *SDHB* associated tumors are extra adrenal, occurring in the abdomen and pelvis although they can be found in any location including the adrenal gland and head and neck. The mean age at initial diagnosis ranges from 28.7 to 36.7 years, depending on the study, with a range from 6–77 years old (10, 12, 13). The penetrance of disease ranges from 80–100% by age 70 (13, 50, 74, 75). The largest study to date, including 295 patients with *SDHB* mutations, found that the lifetime risk by age 60 of a *SDHB* mutation carrier developing a non-head and neck PGL was 52% with a mean age of diagnosis of 27 years old; the risk of developing a head and neck PGL was 29% with a mean age of diagnosis of 42 years old (50).

SDHB mutation associated tumors carry a substantial risk of malignancy which has been estimated to range from 31–71% (50, 57, 75–78). The absolute risk of malignancy is difficult to define given that metastases can occur even up to 20 years after primary tumor diagnosis, so many studies do not have the length of follow up required to capture the true risk of malignancy. In addition, the definition of malignant PCC/PGL can vary depending on the study, with some including local extension and invasion, and others only distant metastatic disease, despite WHO guidelines defining malignant PCC/PGL as metastasis at sites where chromaffin tissue is not normally present (1). Thus, the wide range of malignancy risk is due to the potential long latency time to development of metastases and differing criteria for malignant disease.

Interestingly, mutations in *SDHB* may confer susceptibility to other cancers, as carriers of *SDHB* mutations have an increased incidence of gastrointestinal stromal tumors (GIST), papillary thyroid cancer, neuroblastoma and various types of renal cell carcinoma, including clear cell and papillary RCC (50, 57, 79–82). A similar phenomenon was noted with *BRCA1* and *BRCA2* mutations, which were initially identified as associated with breast and ovarian cancers, but are now known to confer increased risks for multiple cancer types (83–85). The risk of associated RCC is reported as high as 14% in one large study of 295 *SDHB* mutation carriers (50) compared to the 1.49% lifetime risk in the general population (86). Of note, mutations in *SDHB* (and also in *SDHD* and *SDHC*) have been associated with Carney-Stratakis syndrome (GIST and PGL), but have not yet been found in Carney triad (extra adrenal PGL, GIST and pulmonary chondroma), suggesting the triad syndrome may be the result of a yet unidentified genetic cause (87–90). More research is needed to define the risk of other cancers associated with *SDHB* mutations.

SDHA

The *SDHA* gene is located on chromosome 5p15 and encodes a 621 amino acid protein which is the flavoprotein and one of the catalytic subunits in the succinate dehydrogenase complex (OMIM *600857). Biallelic mutations in *SDHA* lead to Leigh's syndrome, characterized by an early onset neurodegenerative disorder, and mutations can also cause a form of cardiomyopathy (91–93). Mutations in this subunit of complex II were not identified in patients with PCC/PGL until recently. Burnichon et al. identified the first heterozygous germline missense mutation associated with an abdominal PGL (94). Subsequently, the same group published a series demonstrating that *SDHA* mutations represented approximately three percent of germline mutations in apparently sporadic PCC/PGL (95). Given the small number of mutations found thus far (six in total), true prevalence of mutations and rates of malignancy are yet to be determined. Genetic testing for *SDHA* mutations is complicated by the existence of two known pseudogenes on chromosomes 3 and 5; and therefore, it currently is not offered by clinical genetic testing laboratories (94).

NEWLY IDENTIFIED SUSCEPTIBILITY GENES

TMEM127

TMEM127 was recently identified as a PCC/PGL susceptibility gene. *TMEM127* is located on chromosome 2q11.2 (OMIM *613403). *TMEM127* contains four exons and encodes a 238 amino acid protein which has three transmembrane domains (96). The role it plays in cell signaling is still being determined, but evidence to date shows it is involved in the mTORC1 signaling pathway and co-localizes with the early endosome in the cell (96). The penetrance of mutations in *TMEM127* is still not well defined. Mutations confer disease susceptibility in an autosomal dominant fashion with LOH being a common second hit in tumors. Initial studies suggested that all mutations led to adrenal based PCC, typically unilateral and in patients with no prior family history (96, 97). However, more recently, bilateral adrenal PCC, extra adrenal and head and neck PGL have been described in patients with germline mutations in *TMEM127*, so more data are needed to define the associated clinical syndrome (98, 99). The average age of onset for tumors appears to be 45 years and the risk of malignancy is very low (96, 97). Clinical genetic testing for mutations in this gene is available.

MAX

Most recently inherited mutations in *MAX* were identified as associated with PCC/PGL susceptibility. Comino-Mendez et al. identified three patients with familial PCC/PGL but no identified mutation in a known susceptibility gene. Tumors from these patients clustered on expression array analysis with tumors having known mutations in *RET*, *NF1* and *TMEM127* (100). Using whole exome sequencing on germline DNA from these patients, they identified *MAX* as a PCC/PGL susceptibility gene (100). *MAX*, *myc-associated factor X*, is located on chromosome 14q23 and was cloned in 1991 (101). The protein encodes a basic helix-loop-helix leucine zipper protein which heterodimerizes with MYC to act as a transcription factor for genes involved in cellular proliferation, differentiation and apoptosis (OMIM *154950). Comino-Mendez et al. found bilateral adrenal PCC in eight of twelve cases studied with *MAX* mutations; moreover, three of eight probands had malignant PCC (100). Future research is needed to define the penetrance of disease associated with this mutation and the true risk of malignancy.

BIOCHEMICAL TESTING FOR PCC/PGL

Initial screening for PCC/PGL relies heavily on biochemical testing for plasma free metanephrines and/or urinary fractionated metanephrines and catecholamines. Measuring

plasma free metanephrines has at least equal or higher sensitivity and specificity (98% and 92%, respectively) as urinary fractionated metanephrines and has the advantage of being more easily obtained than a 24 hour urine collection (102, 103). The pattern of metanephrine and catecholamine expression, as well as the location of the tumors, can guide the order of genetic testing to help contain the costs associated with the analysis (104, 105).

The biochemical profile of PCC/PGLs in vHL patients differs from other PCC/PGLs because of low expression of phenylethanolamine-N-methyltransferase (the enzyme that converts norepinephrine to epinephrine), and these patients often have excessive production of the precursors, norepinephrine and normetanephrine, but not their metabolites, epinephrine and metanephrine (105, 106). MEN 2 associated PCCs often over-express phenylethanolamine N-methyltransferase, so the biochemical profile is unique by showing predominant elevations of epinephrine (105, 106). NF1 associated PCC show elevation of both norepinephrines and normetanephrines and their metabolites (105). Biochemical profile for *SDHB* mutated tumors is similar to that of vHL patients with a normetanephrine predominance, but they can also have high methoxytyramine excretion (the o-methylated metabolite of dopamine) in addition to, or as the only elevated biochemical marker (105). Tumors associated with *SDHC*, *SDHD*, and *SDHAF2* mutations, often located in the head and neck, are derived from parasympathetic ganglia which usually do not secrete catecholamines. If biochemical testing results show high levels of catecholamine secretion in patients with known head and neck PGL, imaging studies should be done to identify another possible primary tumor, most commonly located in the abdomen or pelvis. Finally, the biochemical profiles of tumors associated with mutations in *TMEM127*, *SDHA*, and *MAX* have not been well established.

SCREENING FOR MUTATION CARRIERS

Screening for PCC/PGL in vHL and MEN 2 patients is based on standard screening recommendations for these well known cancer susceptibility syndromes as discussed above (20, 38, 41). NF1 patients who have hypertension at any age should be screened for PCC (36); NF1 patients who have had one PCC should be screened annually with plasma free metanephrines for the possible development of a contralateral PCC.

As no formal guidelines exist for screening carriers of mutations in the other known susceptibility genes, screening is based on expert opinion. Screening for both unaffected and affected mutation carriers should include annual 24 hour urine and/or plasma metanephrines and catecholamines (given the possibility of methoxytyramine only secretion). Even for those syndromes associated with a high risk of head and neck PGL, which are thought to be mostly parasympathetic and non-secreting tumors, catecholamines should be evaluated, as these tumors may only have elevated methoxytyramine (or its precursor dopamine). It is important to note that catecholamine measurements, compared to metanephrines, are more likely to be falsely positive, in particular due to medication interactions (107). As described in the previous section, if biochemical testing results show high levels of secretion in patients with known head and neck PGL, imaging studies should be done of the torso to exclude another possible primary tumor.

In patients with metastatic PCC/PGL, biomarkers are used for screening for disease recurrence and for therapeutic monitoring. In addition to testing standard plasma metanephrines, chromogranin A is commonly measured as these levels correlate well with tumor size and malignancy (108). Most recently, Eisenhofer et al. showed that methoxytyramine also is highly correlated with the presence of metastases in patients with or without *SDHB* mutations, and therefore, has high utility in monitoring disease (109).

Mutation carriers also should undergo regular imaging studies as part of the screening process as not all PCC/PGLs will be secreting (regardless of location) and may be missed through biochemical studies alone. For unaffected mutation carriers, the type of imaging study will be guided by the specific gene mutation. Unaffected carriers of *SDHB*, *SDHC*, *SDHD*, and *SDHAF2* mutations who have normal plasma metanephrines and catecholamine results, should have screening imaging with MRI (or CT scan) of the thorax, abdomen and pelvis and head and neck every two years. If available, rapid full body MRI should be considered as a screening modality to reduce cost, time of study and radiation exposure. Clearly, the frequency of imaging studies for any previously unaffected mutation carrier should increase in the presence of elevated metanephrines or catecholamines, and then also may include functional imaging studies such as I-123 MIBG scans.

PCC/PGLs, even malignant and multi-focal tumors, have been reported in children as young as five to eight years old in association with *SDHB* and *SDHD* mutations (9, 13, 110); therefore, screening for unaffected mutation carriers should begin between ages five and ten. Biochemical profiling in the pediatric population should be done annually. However, it remains unclear whether imaging studies should be done in childhood with the same frequency as in adults.

Recommendations for surveillance of affected mutation carriers will vary depending on location of the tumor, presence of a single or multiple tumors, genetic mutation and evidence of metastases. As tumors associated with mutations in *SDHB* have a higher chance of malignant progression, more frequent follow-up after diagnosis of an initial tumor may be warranted. In particular, for patients with metastatic PCC/PGL, functional imaging may be useful for disease localization including I-123 MIBG scans and PET imaging. The appropriate frequency of screening for *TMEM127*, *SDHA*, and *MAX* mutation carriers is not yet clear given the rarity of these cases.

SOMATIC GENETICS

Several studies have screened specific candidate genes for somatic mutations in PCC/PGL, but overall, few somatic mutations have been identified. Somatic point mutations in the *SDHx* genes are rare occurring approximately one percent of the time (47, 73, 111, 112). Ten to fourteen percent of sporadic PCC have somatic mutations in *RET* or *VHL* (112–118). Other studies have examined candidate genes frequently mutated in other cancers including *K-*, *N-* and *H-RAS*, *GNAS*, *p53*, *BRAF*, and *CDKN2A*; and the results across these very small studies have been inconsistent with no mutational hotspot identified (115, 119–126). Although no *PTEN* mutations have been found in human PCC/PGL, LOH at the *PTEN* loci was found in about 16% of tumors examined (five of 31) (127, 128). Interestingly, mice with heterozygous *Pten* mutations, or conditional knockouts, develop malignant PCC/PGL (129). Animal models for PGL have been difficult to create as *Sdh* knockout mice do not develop PCC/PGL, despite it being a known human susceptibility gene (54).

Copy number profiling has been done in a limited number of PCC/PGL. Array based comparative genomic hybridization (aCGH) studies have found that most PCC/PGL have losses of chromosomes 1p and 3q (113, 130, 131), whereas *VHL* associated tumors have selective loss of chromosome 11 (132). Loss of chromosome 22q also seems to be common in PCC/PGL (133, 134). Finally, studies have examined expression profiling for PCC/PGL, but they have not provided novel insights into the disease. The data have shown expected results of increased expression of hypoxia related genes in tumors with germline mutations in *VHL* or *SDH* genes (135–137). One of the largest studies by Burnichon et al. examined 202 PCC/PGL for expression array analysis and the results were consistent with previous data clustering together the *RET/NFI/TMEM127* mutated tumors separately from *VHL*/

SDHx mutated tumors based on upregulation of MAPK signaling and neuroendocrine differentiation gene expression vs. upregulation of genes involved in angiogenesis and hypoxia, respectively. A novel finding in this study is that the expression profiling data can further subdivide *VHL* from *SDH* tumors based on upregulation of genes involved in cell adhesion in *SDHx* tumors vs. glycolysis in *vHL* related tumors (112). While interesting, this work will need to be verified with an independent sample set.

CONCLUSIONS AND FUTURE DIRECTIONS

Understanding whether the genetic predisposition to PCC/PGL is present provides important clinical information regarding the patient's risk for developing multifocal lesions, malignant PCC/PGL and other associated malignancies. We suggest that all patients with multiple PGLs, or even one extra adrenal or head and neck tumor, should have genetic testing. This suggestion is based on data showing that at least a third of patients with apparently sporadic PCC/PGL and over half of patients with head and neck PGL, have an identified germline mutation in one of the known susceptibility genes (9, 10, 12, 13). These numbers may be under-estimates, as not all of the currently identified genes have been included in many studies. In addition, patients with only adrenal PCC have up to a 21% mutation detection rate (10, 12), and the rate can be as high as 59% if diagnosed by 18 years of age (9). These data suggest that even patients with only adrenal PCC should have clinical genetic testing, especially when diagnosed at a young age. Ten known susceptibility genes have been identified to date, making PCC/PGL more commonly associated with an inherited mutation than any other cancer type. In order to guide treatment for both unaffected carriers of germline mutations in known PCC/PGL susceptibility genes and patients with PCC/PGLs, we need to have an increased understanding of the underlying somatic genetic and genomic changes, as well as pathway activation in these tumors. Significant strides have been made in this area over the last decade, however, there is still much more to learn about the germline and somatic genetics in PCC/PGL to provide insights into the biology of the tumors, and hopefully provide new targets for novel therapeutics.

Acknowledgments

Salary support for LF is funded by NIH training grant 2-T32-DK007314-31. Support to LF and KLN is provided by the Pheo-Para Alliance.

References

1. DeLellis, RA.; Lloyd, RV.; Heitz, PU., et al. World Health Organization Classification of Tumours. In: DeLellis, RVL.; Ronald, A.; Heitz, Philipp U.; Eng, Charis, editors. Pathology and Genetics of Tumours of Endocrine Organs. Lyon, France: IARC Press; 2004.
2. Cohen DL, Fraker D, Townsend RR. Lack of symptoms in patients with histologic evidence of pheochromocytoma: a diagnostic challenge. *Ann N Y Acad Sci.* 2006; 1073:47–51. [PubMed: 17102071]
3. Thompson LD. Pheochromocytoma of the Adrenal gland Scaled Score (PASS) to separate benign from malignant neoplasms: a clinicopathologic and immunophenotypic study of 100 cases. *Am J Surg Pathol.* 2002; 26(5):551–66. [PubMed: 11979086]
4. Wu D, Tischler AS, Lloyd RV, et al. Observer variation in the application of the Pheochromocytoma of the Adrenal Gland Scaled Score. *Am J Surg Pathol.* 2009; 33(4):599–608. [PubMed: 19145205]
5. Ayala-Ramirez M, Feng L, Johnson MM, et al. Clinical risk factors for malignancy and overall survival in patients with pheochromocytomas and sympathetic paragangliomas: primary tumor size and primary tumor location as prognostic indicators. *J Clin Endocrinol Metab.* 2011; 96(3):717–25. [PubMed: 21190975]

6. Feng F, Zhu Y, Wang X, et al. Predictive factors for malignant pheochromocytoma: analysis of 136 patients. *J Urol*. 2011; 185(5):1583–90. [PubMed: 21419457]
7. Huang KH, Chung SD, Chen SC, et al. Clinical and pathological data of 10 malignant pheochromocytomas: long-term follow up in a single institute. *Int J Urol*. 2007; 14(3):181–5. [PubMed: 17430251]
8. Szalat A, Fraenkel M, Doviner V, et al. Malignant pheochromocytoma: predictive factors of malignancy and clinical course in 16 patients at a single tertiary medical center. *Endocrine*. 2011; 39(2):160–6. [PubMed: 21069578]
9. Neumann HP, Bausch B, McWhinney SR, et al. Germ-line mutations in nonsyndromic pheochromocytoma. *N Engl J Med*. 2002; 346(19):1459–66. [PubMed: 12000816]
10. Mannelli M, Castellano M, Schiavi F, 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. [PubMed: 19223516]
11. Neumann HP, Erlic Z, Boedeker CC, et al. Clinical predictors for germline mutations in head and neck paraganglioma patients: cost reduction strategy in genetic diagnostic process as fall-out. *Cancer Res*. 2009; 69(8):3650–6. [PubMed: 19351833]
12. Cascon A, Pita G, Burnichon N, et al. Genetics of pheochromocytoma and paraganglioma in Spanish patients. *J Clin Endocrinol Metab*. 2009; 94(5):1701–5. [PubMed: 19258401]
13. Burnichon N, Rohmer V, Amar L, 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. [PubMed: 19454582]
14. Maher ER, Iselius L, Yates JR, et al. Von Hippel-Lindau disease: a genetic study. *J Med Genet*. 1991; 28(7):443–7. [PubMed: 1895313]
15. Neumann HP, Wiestler OD. Clustering of features and genetics of von Hippel-Lindau syndrome. *Lancet*. 1991; 338(8761):258. [PubMed: 1676812]
16. Maher ER, Neumann HP, Richard S. von Hippel-Lindau disease: a clinical and scientific review. *Eur J Hum Genet*. 2011; 19(6):617–23. [PubMed: 21386872]
17. Delman KA, Shapiro SE, Jonasch EW, et al. Abdominal visceral lesions in von Hippel-Lindau disease: incidence and clinical behavior of pancreatic and adrenal lesions at a single center. *World J Surg*. 2006; 30(5):665–9. [PubMed: 16617420]
18. Boedeker CC, Erlic Z, Richard S, et al. Head and neck paragangliomas in von Hippel-Lindau disease and multiple endocrine neoplasia type 2. *J Clin Endocrinol Metab*. 2009; 94(6):1938–44. [PubMed: 19336503]
19. Gaal J, van Nderveen FH, Erlic Z, et al. Parasympathetic paragangliomas are part of the Von Hippel-Lindau syndrome. *J Clin Endocrinol Metab*. 2009; 94(11):4367–71. [PubMed: 19808854]
20. Schimke, RN.; Collins, DL.; Stolle, CA. Von Hippel-Lindau Syndrome. In: Pagon, RA.; Bird, TD.; Dolan, CR.; Stephens, K., editors. *GeneReviews*. Seattle (WA): 1993.
21. Kaelin WG Jr. Molecular basis of the VHL hereditary cancer syndrome. *Nat Rev Cancer*. 2002; 2(9):673–82. [PubMed: 12209156]
22. Min JH, Yang H, Ivan M, et al. Structure of an HIF-1 α -pVHL complex: hydroxyproline recognition in signaling. *Science*. 2002; 296(5574):1886–9. [PubMed: 12004076]
23. Chen F, Slife L, Kishida T, et al. Genotype-phenotype correlation in von Hippel-Lindau disease: identification of a mutation associated with VHL type 2A. *J Med Genet*. 1996; 33(8):716–7. [PubMed: 8863170]
24. Glavac D, Neumann HP, Wittke C, et al. Mutations in the VHL tumor suppressor gene and associated lesions in families with von Hippel-Lindau disease from central Europe. *Hum Genet*. 1996; 98(3):271–80. [PubMed: 8707293]
25. Maher ER, Webster AR, Richards FM, et al. Phenotypic expression in von Hippel-Lindau disease: correlations with germline VHL gene mutations. *J Med Genet*. 1996; 33(4):328–32. [PubMed: 8730290]
26. Zbar B, Kishida T, Chen F, et al. Germline mutations in the Von Hippel-Lindau disease (VHL) gene in families from North America, Europe, and Japan. *Hum Mutat*. 1996; 8(4):348–57. [PubMed: 8956040]

27. Rechsteiner MP, von Teichman A, Nowicka A, et al. VHL Gene Mutations and Their Effects on Hypoxia Inducible Factor HIF{alpha}: Identification of Potential Driver and Passenger Mutations. *Cancer Res.* 2011; 71(16):5500–11. [PubMed: 21715564]
28. Forman JR, Worth CL, Bickerton GR, et al. Structural bioinformatics mutation analysis reveals genotype-phenotype correlations in von Hippel-Lindau disease and suggests molecular mechanisms of tumorigenesis. *Proteins.* 2009; 77(1):84–96. [PubMed: 19408298]
29. Ong KR, Woodward ER, Killick P, et al. Genotype-phenotype correlations in von Hippel-Lindau disease. *Hum Mutat.* 2007; 28(2):143–9. [PubMed: 17024664]
30. Le LQ, Parada LF. Tumor microenvironment and neurofibromatosis type I: connecting the GAPs. *Oncogene.* 2007; 26(32):4609–16. [PubMed: 17297459]
31. Johannessen CM, Johnson BW, Williams SM, et al. TORC1 is essential for NF1-associated malignancies. *Curr Biol.* 2008; 18(1):56–62. [PubMed: 18164202]
32. Johannessen CM, Reczek EE, James MF, et al. The NF1 tumor suppressor critically regulates TSC2 and mTOR. *Proc Natl Acad Sci U S A.* 2005; 102(24):8573–8. [PubMed: 15937108]
33. Ferner RE, Huson SM, Thomas N, et al. Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. *J Med Genet.* 2007; 44(2):81–8. [PubMed: 17105749]
34. Neurofibromatosis. Conference statement. *Arch Neurol*; National Institutes of Health Consensus Development Conference; 1988. p. 575-8.
35. Walther MM, Reiter R, Keiser HR, et al. Clinical and genetic characterization of pheochromocytoma in von Hippel-Lindau families: comparison with sporadic pheochromocytoma gives insight into natural history of pheochromocytoma. *J Urol.* 1999; 162(3 Pt 1):659–64. [PubMed: 10458336]
36. Williams VC, Lucas J, Babcock MA, et al. Neurofibromatosis type 1 revisited. *Pediatrics.* 2009; 123(1):124–33. [PubMed: 19117870]
37. Bausch B, Borozdin W, Neumann HP. Clinical and genetic characteristics of patients with neurofibromatosis type 1 and pheochromocytoma. *N Engl J Med.* 2006; 354(25):2729–31. [PubMed: 16790714]
38. Wohllk N, Schweizer H, Erlic Z, et al. Multiple endocrine neoplasia type 2. *Best Pract Res Clin Endocrinol Metab.* 2010; 24(3):371–87. [PubMed: 20833330]
39. Eng C, Clayton D, Schuffenecker I, 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. [PubMed: 8918855]
40. Brandi ML, Gagel RF, Angeli A, et al. Guidelines for diagnosis and therapy of MEN type 1 and type 2. *J Clin Endocrinol Metab.* 2001; 86(12):5658–71. [PubMed: 11739416]
41. Kloos RT, Eng C, Evans DB, et al. Medullary thyroid cancer: management guidelines of the American Thyroid Association. *Thyroid.* 2009; 19(6):565–612. [PubMed: 19469690]
42. Pomares FJ, Canas R, Rodriguez JM, et al. Differences between sporadic and multiple endocrine neoplasia type 2A pheochromocytoma. *Clin Endocrinol (Oxf).* 1998; 48(2):195–200. [PubMed: 9579232]
43. Pacak K, Ilias I, Adams KT, et al. Biochemical diagnosis, localization and management of pheochromocytoma: focus on multiple endocrine neoplasia type 2 in relation to other hereditary syndromes and sporadic forms of the tumour. *J Intern Med.* 2005; 257(1):60–8. [PubMed: 15606377]
44. Modigliani E, Vasen HM, Raue K, et al. Pheochromocytoma in multiple endocrine neoplasia type 2: European study. The Euromen Study Group. *J Intern Med.* 1995; 238(4):363–7. [PubMed: 7595173]
45. Astuti D, Douglas F, Lennard TW, et al. Germline SDHD mutation in familial pheochromocytoma. *Lancet.* 2001; 357(9263):1181–2. [PubMed: 11323050]
46. Baysal BE, Ferrell RE, Willett-Brozick JE, et al. Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science.* 2000; 287(5454):848–51. [PubMed: 10657297]
47. Gimm O, Armanios M, Dziema H, et al. Somatic and occult germ-line mutations in SDHD, a mitochondrial complex II gene, in nonfamilial pheochromocytoma. *Cancer Res.* 2000; 60(24):6822–5. [PubMed: 11156372]

48. Hirawake H, Taniwaki M, Tamura A, et al. Characterization of the human SDHD gene encoding the small subunit of cytochrome b (cybS) in mitochondrial succinate-ubiquinone oxidoreductase. *Biochim Biophys Acta*. 1999; 1412(3):295–300. [PubMed: 10482792]
49. Hirawake H, Taniwaki M, Tamura A, et al. Cytochrome b in human complex II (succinate-ubiquinone oxidoreductase): cDNA cloning of the components in liver mitochondria and chromosome assignment of the genes for the large (SDHC) and small (SDHD) subunits to 1q21 and 11q23. *Cytogenet Cell Genet*. 1997; 79(1–2):132–8. [PubMed: 9533030]
50. Ricketts CJ, Forman JR, Rattenberry E, et al. Tumor risks and genotype-phenotype-proteotype analysis in 358 patients with germline mutations in SDHB and SDHD. *Hum Mutat*. 2010; 31(1):41–51. [PubMed: 19802898]
51. Badenhop RF, Cherian S, Lord RS, et al. Novel mutations in the SDHD gene in pedigrees with familial carotid body paraganglioma and sensorineural hearing loss. *Genes Chromosomes Cancer*. 2001; 31(3):255–63. [PubMed: 11391796]
52. Cascon A, Ruiz-Llorente S, Fraga MF, et al. Genetic and epigenetic profile of sporadic pheochromocytomas. *J Med Genet*. 2004; 41(3):e30. [PubMed: 14985401]
53. Yamashita R, Usui T, Hashimoto S, et al. Predominant expression of mutated allele of the succinate dehydrogenase D (SDHD) gene in the SDHD-related paragangliomas. *Endocr J*. 2009; 56(9):1129–35. [PubMed: 19550080]
54. Bayley JP, van Minderhout I, Hogendoorn PC, et al. Sdhb and SDHD/H19 knockout mice do not develop paraganglioma or pheochromocytoma. *PLoS One*. 2009; 4(11):e7987. [PubMed: 19956719]
55. Pigny P, Vincent A, Cardot Bauders C, et al. Paraganglioma after maternal transmission of a succinate dehydrogenase gene mutation. *J Clin Endocrinol Metab*. 2008; 93(5):1609–15. [PubMed: 18211978]
56. Yeap PM, Tobias ES, Mavraki E, et al. Molecular Analysis of Pheochromocytoma after Maternal Transmission of SDHD Mutation Elucidates Mechanism of Parent-of-Origin Effect. *J Clin Endocrinol Metab*. 2011; 96(12):E2009–13. [PubMed: 21937622]
57. Neumann HP, Pawlu C, Peczkowska M, et al. Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. *JAMA*. 2004; 292(8):943–51. [PubMed: 15328326]
58. Dannenberg H, van Nederveen FH, Abbou M, et al. Clinical characteristics of pheochromocytoma patients with germline mutations in SDHD. *J Clin Oncol*. 2005; 23(9):1894–901. [PubMed: 15774781]
59. Hao HX, Khalimonchuk O, Schraders M, et al. SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. *Science*. 2009; 325(5944):1139–42. [PubMed: 19628817]
60. Bayley JP, Kunst HP, Cascon A, et al. SDHAF2 mutations in familial and sporadic paraganglioma and pheochromocytoma. *Lancet Oncol*. 2010; 11(4):366–72. [PubMed: 20071235]
61. Kunst HP, Rutten MH, de Monnik JP, et al. SDHAF2 (PGL2-SDH5) and hereditary head and neck paraganglioma. *Clin Cancer Res*. 2011; 17(2):247–54. [PubMed: 21224366]
62. Elbehti-Green A, Au HC, Mascarello JT, et al. Characterization of the human SDHC gene encoding of the integral membrane proteins of succinate-quinone oxidoreductase in mitochondria. *Gene*. 1998; 213(1–2):133–40. [PubMed: 9714607]
63. Badenhop RF, Jansen JC, Fagan PA, et al. The prevalence of SDHB, SDHC, and SDHD mutations in patients with head and neck paraganglioma and association of mutations with clinical features. *J Med Genet*. 2004; 41(7):e99. [PubMed: 15235042]
64. Baysal BE, Willett-Brozick JE, Lawrence EC, 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. [PubMed: 11897817]
65. Muller U, Troidl C, Niemann S. SDHC mutations in hereditary paraganglioma/pheochromocytoma. *Fam Cancer*. 2005; 4(1):9–12. [PubMed: 15883704]
66. Schiavi F, Boedeker CC, Bausch B, et al. Predictors and prevalence of paraganglioma syndrome associated with mutations of the SDHC gene. *JAMA*. 2005; 294(16):2057–63. [PubMed: 16249420]

67. Peczkowska M, Cascon A, Prejbisz A, et al. Extra-adrenal and adrenal pheochromocytomas associated with a germline SDHC mutation. *Nat Clin Pract Endocrinol Metab.* 2008; 4(2):111–5. [PubMed: 18212813]
68. Astuti D, Latif F, Dallol A, et al. Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. *Am J Hum Genet.* 2001; 69(1):49–54. [PubMed: 11404820]
69. Gimenez-Roqueplo AP, Favier J, Rustin P, et al. Functional consequences of a SDHB gene mutation in an apparently sporadic pheochromocytoma. *J Clin Endocrinol Metab.* 2002; 87(10):4771–4. [PubMed: 12364472]
70. Pollard PJ, Briere JJ, Alam NA, 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. [PubMed: 15987702]
71. Pollard PJ, El-Bahrawy M, Poulsom R, et al. Expression of HIF-1alpha, HIF-2alpha (EPAS1), and their target genes in paraganglioma and pheochromocytoma with VHL and SDH mutations. *J Clin Endocrinol Metab.* 2006; 91(11):4593–8. [PubMed: 16954163]
72. Guzy RD, Sharma B, Bell E, et al. Loss of the SdhB, but Not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis. *Mol Cell Biol.* 2008; 28(2):718–31. [PubMed: 17967865]
73. Pasini B, Stratakis CA. SDH mutations in tumorigenesis and inherited endocrine tumours: lesson from the pheochromocytoma-paraganglioma syndromes. *J Intern Med.* 2009; 266(1):19–42. [PubMed: 19522823]
74. Benn DE, Gimenez-Roqueplo AP, Reilly JR, et al. Clinical presentation and penetrance of pheochromocytoma/paraganglioma syndromes. *J Clin Endocrinol Metab.* 2006; 91(3):827–36. [PubMed: 16317055]
75. Srirangalingam U, Walker L, Khoo B, et al. Clinical manifestations of familial paraganglioma and pheochromocytomas in succinate dehydrogenase B (SDH-B) gene mutation carriers. *Clin Endocrinol (Oxf).* 2008; 69(4):587–96. [PubMed: 18419787]
76. Amar L, Bertherat J, Baudin E, et al. Genetic testing in pheochromocytoma or functional paraganglioma. *J Clin Oncol.* 2005; 23(34):8812–8. [PubMed: 16314641]
77. Boedeker CC, Neumann HP, Maier W, et al. Malignant head and neck paragangliomas in SDHB mutation carriers. *Otolaryngol Head Neck Surg.* 2007; 137(1):126–9. [PubMed: 17599579]
78. Brouwers FM, Eisenhofer G, Tao JJ, et al. High frequency of SDHB germline mutations in patients with malignant catecholamine-producing paragangliomas: implications for genetic testing. *J Clin Endocrinol Metab.* 2006; 91(11):4505–9. [PubMed: 16912137]
79. Ricketts C, Woodward ER, Killick P, et al. Germline SDHB mutations and familial renal cell carcinoma. *J Natl Cancer Inst.* 2008; 100(17):1260–2. [PubMed: 18728283]
80. Gaal J, Stratakis CA, Carney JA, et al. SDHB immunohistochemistry: a useful tool in the diagnosis of Carney-Stratakis and Carney triad gastrointestinal stromal tumors. *Mod Pathol.* 2011; 24(1):147–51. [PubMed: 20890271]
81. Gill AJ, Chou A, Vilain R, et al. Immunohistochemistry for SDHB divides gastrointestinal stromal tumors (GISTs) into 2 distinct types. *Am J Surg Pathol.* 2010; 34(5):636–44. [PubMed: 20305538]
82. Vanharanta S, Buchta M, McWhinney SR, 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. [PubMed: 14685938]
83. Brose MS, Rebbeck TR, Calzone KA, et al. Cancer risk estimates for BRCA1 mutation carriers identified in a risk evaluation program. *J Natl Cancer Inst.* 2002; 94(18):1365–72. [PubMed: 12237282]
84. Thompson D, Easton DF. Cancer Incidence in BRCA1 mutation carriers. *J Natl Cancer Inst.* 2002; 94(18):1358–65. [PubMed: 12237281]
85. van Asperen CJ, Brohet RM, Meijers-Heijboer EJ, et al. Cancer risks in BRCA2 families: estimates for sites other than breast and ovary. *J Med Genet.* 2005; 42(9):711–9. [PubMed: 16141007]
86. Howlader N, Noone AM, Krapcho M, et al. SEER Cancer Statistics Review, 1975–2008. National Cancer Institute; Bethesda, MD:

87. McWhinney SR, Pasini B, Stratakis CA. Familial gastrointestinal stromal tumors and germ-line mutations. *N Engl J Med*. 2007; 357(10):1054–6. [PubMed: 17804857]
88. Pasini B, McWhinney SR, Bei T, et al. Clinical and molecular genetics of patients with the Carney-Stratakis syndrome and germline mutations of the genes coding for the succinate dehydrogenase subunits SDHB, SDHC, and SDHD. *Eur J Hum Genet*. 2008; 16(1):79–88. [PubMed: 17667967]
89. Bolland M, Benn D, Croxson M, et al. Gastrointestinal stromal tumour in succinate dehydrogenase subunit B mutation-associated familial pheochromocytoma/paraganglioma. *ANZ J Surg*. 2006; 76(8):763–4. [PubMed: 16916404]
90. 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. [PubMed: 19522824]
91. Horvath R, Abicht A, Holinski-Feder E, et al. Leigh syndrome caused by mutations in the flavoprotein (Fp) subunit of succinate dehydrogenase (SDHA). *J Neurol Neurosurg Psychiatry*. 2006; 77(1):74–6. [PubMed: 16361598]
92. Levitas A, Muhammad E, Harel G, et al. Familial neonatal isolated cardiomyopathy caused by a mutation in the flavoprotein subunit of succinate dehydrogenase. *Eur J Hum Genet*. 2010; 18(10):1160–5. [PubMed: 20551992]
93. Parfait B, Chretien D, Rotig A, et al. Compound heterozygous mutations in the flavoprotein gene of the respiratory chain complex II in a patient with Leigh syndrome. *Hum Genet*. 2000; 106(2):236–43. [PubMed: 10746566]
94. Burnichon N, Briere JJ, Libe R, et al. SDHA is a tumor suppressor gene causing paraganglioma. *Hum Mol Genet*. 2010; 19(15):3011–20. [PubMed: 20484225]
95. Korpershoek E, Favier J, Gaal J, et al. SDHA Immunohistochemistry Detects Germline SDHA Gene Mutations in Apparently Sporadic Paragangliomas and Pheochromocytomas. *J Clin Endocrinol Metab*. 2011
96. Qin Y, Yao L, King EE, et al. Germline mutations in TMEM127 confer susceptibility to pheochromocytoma. *Nat Genet*. 2010; 42(3):229–33. [PubMed: 20154675]
97. Yao L, Schiavi F, Cascon A, et al. Spectrum and prevalence of FP/TMEM127 gene mutations in pheochromocytomas and paragangliomas. *JAMA*. 2010; 304(23):2611–9. [PubMed: 21156949]
98. Burnichon N, Lepoutre-Lussey C, Laffaire J, et al. A novel TMEM127 mutation in a patient with familial bilateral pheochromocytoma. *Eur J Endocrinol*. 2011; 164(1):141–5. [PubMed: 20923864]
99. Neumann HP, Sullivan M, Winter A, 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
100. Comino-Mendez I, Gracia-Aznarez FJ, Schiavi F, et al. Exome sequencing identifies MAX mutations as a cause of hereditary pheochromocytoma. *Nat Genet*. 2011; 43(7):663–7. [PubMed: 21685915]
101. Blackwood EM, Eisenman RN. Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. *Science*. 1991; 251(4998):1211–7. [PubMed: 2006410]
102. Chen H, Sippel RS, O'Dorisio MS, et al. The North American Neuroendocrine Tumor Society consensus guideline for the diagnosis and management of neuroendocrine tumors: pheochromocytoma, paraganglioma, and medullary thyroid cancer. *Pancreas*. 2010; 39(6):775–83. [PubMed: 20664475]
103. Eisenhofer G, Siegert G, Kotzerke J, et al. Current progress and future challenges in the biochemical diagnosis and treatment of pheochromocytomas and paragangliomas. *Horm Metab Res*. 2008; 40(5):329–37. [PubMed: 18491252]
104. Karasek D, Frysak Z, Pacak K. Genetic testing for pheochromocytoma. *Curr Hypertens Rep*. 2010; 12(6):456–64. [PubMed: 20938758]
105. Eisenhofer G, Lenders JW, Timmers H, et al. Measurements of plasma methoxytyramine, normetanephrine, and metanephrine as discriminators of different hereditary forms of pheochromocytoma. *Clin Chem*. 2011; 57(3):411–20. [PubMed: 21262951]

106. Eisenhofer G, Walther MM, Huynh TT, et al. Pheochromocytomas in von Hippel-Lindau syndrome and multiple endocrine neoplasia type 2 display distinct biochemical and clinical phenotypes. *J Clin Endocrinol Metab.* 2001; 86(5):1999–2008. [PubMed: 11344198]
107. Eisenhofer G, Goldstein DS, Walther MM, et al. Biochemical diagnosis of pheochromocytoma: how to distinguish true- from false-positive test results. *J Clin Endocrinol Metab.* 2003; 88(6): 2656–66. [PubMed: 12788870]
108. Rao F, Keiser HR, O'Connor DT. Malignant pheochromocytoma. Chromaffin granule transmitters and response to treatment. *Hypertension.* 2000; 36(6):1045–52. [PubMed: 11116123]
109. Eisenhofer G, Lenders JW, Siegert G, et al. Plasma methoxytyramine: A novel biomarker of metastatic pheochromocytoma and paraganglioma in relation to established risk factors of tumour size, location and SDHB mutation status. *Eur J Cancer.* 2011
110. Prodanov T, Havekes B, Nathanson KL, et al. Malignant paraganglioma associated with succinate dehydrogenase subunit B in an 8-year-old child: the age of first screening? *Pediatr Nephrol.* 2009; 24(6):1239–42. [PubMed: 19189136]
111. van Nederveen FH, Korpershoek E, Lenders JW, et al. Somatic SDHB mutation in an extraadrenal pheochromocytoma. *N Engl J Med.* 2007; 357(3):306–8. [PubMed: 17634472]
112. Burnichon N, Vescovo L, Amar L, et al. Integrative Genomic Analysis Reveals Somatic Mutations in Pheochromocytoma and Paraganglioma. *Hum Mol Genet.* 2011
113. Dannenberg H, De Krijger RR, van der Harst E, et al. Von Hippel-Lindau gene alterations in sporadic benign and malignant pheochromocytomas. *Int J Cancer.* 2003; 105(2):190–5. [PubMed: 12673678]
114. Cho NH, Lee HW, Lim SY, et al. Genetic aberrance of sporadic MEN 2A component tumours: analysis of RET. *Pathology.* 2005; 37(1):10–3. [PubMed: 15875728]
115. Hrascan R, Pecina-Slaus N, Martic TN, et al. Analysis of selected genes in neuroendocrine tumours: insulinomas and phaeochromocytomas. *J Neuroendocrinol.* 2008; 20(8):1015–22. [PubMed: 18510707]
116. Kimura T, Yoshimoto K, Yokogoshi Y, et al. Mutations in the cysteine-rich region of the RET proto-oncogene in patients diagnosed as having sporadic medullary thyroid carcinoma. *Endocr J.* 1995; 42(4):517–25. [PubMed: 8556059]
117. Komminoth P, Kunz E, Hiort O, et al. Detection of RET proto-oncogene point mutations in paraffin-embedded pheochromocytoma specimens by nonradioactive single-strand conformation polymorphism analysis and direct sequencing. *Am J Pathol.* 1994; 145(4):922–9. [PubMed: 7943181]
118. van der Harst E, de Krijger RR, Bruining HA, et al. Prognostic value of RET proto-oncogene point mutations in malignant and benign, sporadic phaeochromocytomas. *Int J Cancer.* 1998; 79(5):537–40. [PubMed: 9761126]
119. Moley JF, Brother MB, Wells SA, et al. Low-Frequency of Ras Gene-Mutations in Neuroblastomas, Pheochromocytomas, and Medullary-Thyroid Cancers. *Cancer Res.* 1991; 51(6):1596–9. [PubMed: 1998949]
120. Yoshimoto K, Iwahana H, Fukuda A, et al. ras mutations in endocrine tumors: mutation detection by polymerase chain reaction-single strand conformation polymorphism. *Jpn J Cancer Res.* 1992; 83(10):1057–62. [PubMed: 1452458]
121. Yoshimoto K, Iwahana H, Fukuda A, et al. Rare mutations of the Gs alpha subunit gene in human endocrine tumors. Mutation detection by polymerase chain reaction-primer-introduced restriction analysis. *Cancer.* 1993; 72(4):1386–93. [PubMed: 8339229]
122. Williamson EA, Johnson SJ, Foster S, et al. G protein gene mutations in patients with multiple endocrinopathies. *J Clin Endocrinol Metab.* 1995; 80(5):1702–5. [PubMed: 7745022]
123. Lin SR, Lee YJ, Tsai JH. Mutations of the p53 gene in human functional adrenal neoplasms. *J Clin Endocrinol Metab.* 1994; 78(2):483–91. [PubMed: 8106638]
124. Muscarella P, Bloomston M, Brewer AR, et al. Expression of the p16INK4A/Cdkn2a gene is prevalently downregulated in human pheochromocytoma tumor specimens. *Gene Expr.* 2008; 14(4):207–16. [PubMed: 19110720]

125. Kiss NB, Geli J, Lundberg F, et al. Methylation of the p16INK4A promoter is associated with malignant behavior in abdominal extra-adrenal paragangliomas but not pheochromocytomas. *Endocr Relat Cancer*. 2008; 15(2):609–21. [PubMed: 18509008]
126. Geli J, Kiss N, Karimi M, et al. Global and regional CpG methylation in pheochromocytomas and abdominal paragangliomas: association to malignant behavior. *Clin Cancer Res*. 2008; 14(9): 2551–9. [PubMed: 18451216]
127. Puc J, Placha G, Wocial B, et al. Analysis of PTEN mutation in non-familial pheochromocytoma. *Ann N Y Acad Sci*. 2006; 1073:317–31. [PubMed: 17102102]
128. van Nederveen FH, Perren A, Dannenberg H, et al. PTEN gene loss, but not mutation, in benign and malignant pheochromocytomas. *J Pathol*. 2006; 209(2):274–80. [PubMed: 16538614]
129. Korpershoek E, Loonen AJ, Corvers S, et al. Conditional Pten knock-out mice: a model for metastatic pheochromocytoma. *J Pathol*. 2009; 217(4):597–604. [PubMed: 19142977]
130. Cascon A, Ruiz-Llorente S, Rodriguez-Perales S, et al. A novel candidate region linked to development of both pheochromocytoma and head/neck paraganglioma. *Genes Chromosomes Cancer*. 2005; 42(3):260–8. [PubMed: 15609347]
131. Edstrom E, Mahlamaki E, Nord B, et al. Comparative genomic hybridization reveals frequent losses of chromosomes 1p and 3q in pheochromocytomas and abdominal paragangliomas, suggesting a common genetic etiology. *Am J Pathol*. 2000; 156(2):651–9. [PubMed: 10666394]
132. Lui WO, Chen J, Glasker S, et al. Selective loss of chromosome 11 in pheochromocytomas associated with the VHL syndrome. *Oncogene*. 2002; 21(7):1117–22. [PubMed: 11850829]
133. Jarbo C, Buckley PG, Piotrowski A, et al. Detailed assessment of chromosome 22 aberrations in sporadic pheochromocytoma using array-CGH. *Int J Cancer*. 2006; 118(5):1159–64. [PubMed: 16161042]
134. van Nederveen FH, Korpershoek E, deLeeuw RJ, et al. Array-comparative genomic hybridization in sporadic benign pheochromocytomas. *Endocr Relat Cancer*. 2009; 16(2):505–13. [PubMed: 19153209]
135. Dahia PL, Hao K, Rogus J, et al. Novel pheochromocytoma susceptibility loci identified by integrative genomics. *Cancer Res*. 2005; 65(21):9651–8. [PubMed: 16266984]
136. Thouennon E, Elkahoul AG, Guillemot J, et al. Identification of potential gene markers and insights into the pathophysiology of pheochromocytoma malignancy. *J Clin Endocrinol Metab*. 2007; 92(12):4865–72. [PubMed: 17878247]
137. Lopez-Jimenez E, Gomez-Lopez G, Leandro-Garcia LJ, et al. Research resource: Transcriptional profiling reveals different pseudohypoxic signatures in SDHB and VHL-related pheochromocytomas. *Mol Endocrinol*. 2010; 24(12):2382–91. [PubMed: 20980436]

Figure 1.
Cell signaling pathways for the ten known pheochromocytoma and paraganglioma susceptibility genes. S Succinate; F Fumarate; OH-P hydroxyproline residue

Table 1

Ten Susceptibility Genes for Pheochromocytoma and Paraganglioma

Gene	Locus	Protein function	Inheritance	Primary Location	Malignancy Rate	Biochemistry
<i>NFI</i>	17q11.2	GTPase	AD	Adrenal	12%	MN, MNM
<i>RET</i>	10q11.2	Transmembrane tyrosine kinase	AD	Adrenal (Bilateral)	<5%	E, MN
<i>VHL</i>	3p25-26	Ubiquitin ligase 3E activity	AD	Adrenal	5%	NMN, NE
<i>SDHA</i>	5q15	Complex II catalytic subunit	AD	Any location	?	?
<i>SDHB</i>	1p36.1	Complex II catalytic subunit	AD	Extra adrenal	31-71%	DA or MT, MN, NMN
<i>SDHC</i>	1q23.3	Complex II anchoring subunit	AD	HNPGL	Low	NMN, MN, DA or MT, none
<i>SDHD</i>	11q23.1	Complex II anchoring subunit	AD; Paternal Inheritance	HNPGL (Multifocal)	<5%	NMN, MN, DA or MT, none
<i>SDHAF2 (SHD5)</i>	11q12.2	Cofactor for complex II	AD; Paternal Inheritance	HNPGL (Multifocal)	Low	?
<i>TMEM127</i>	2q11.2	Transmembrane protein	AD	Any location	Low	?
<i>MAX</i>	14q23	BHLHLZ transcription factor	AD	Adrenal (Bilateral)	?	?

NFI Neurofibromatosis Type 1; *RET* rearranged during transfection proto-oncogene; *VHL* von Hippel Lindau; *SDH* succinate dehydrogenase; *SDHA* subunit A; *SDHB* subunit B; *SDHC* subunit C; *SDHD* subunit D; *SDHAF2* cofactor AF2; *MAX* myc-associated factor X; BHLHLZ basic helix loop helix leucine zipper protein; AD autosomal dominant; HNPGL head and neck paraganglioma; MN metanephrine; NMN normetanephrine; E epinephrine; NE norepinephrine; DA dopamine; MT methoxytyramine; ? unknown