Pheochromocytomas and paragangliomas are tumors of the autonomic nervous system; pheochromocytomas are tumors of the adrenal medulla, and paragangliomas are extra-adrenal tumors arising from either the sympathetic nervous system or parasympathetic ganglia. It has previously been estimated that approximately 10%–15% of pheochromocytomas are due to hereditary causes. However, our increased understanding of the three hereditary syndromes (neurofibromatosis 1, multiple endocrine neoplasia type 2, and von Hippel–Lindau syndrome) in which pheochromocytoma is found and the recent discovery that mutations in genes in the succinate dehydrogenase family (SDHB, SDHC, and SDHD) predispose to pheochromocytoma have necessitated a re-evaluation of the genetic basis of pheochromocytoma. These studies indicate that the frequency of germline mutations associated with isolated pheochromocytoma is higher than previously estimated, with both hospital-based series and a large population-based series indicating that the frequency of germline mutations in RET, VHL, SDHB, and SDHD taken together approaches 20%. In all patients with pheochromocytoma, including those with known hereditary syndrome or a positive family history, the frequency of germline mutations in these four genes together approaches 30%. Given the frequency of germline mutations, consideration should be given to genetic counseling for all patients with pheochromocytoma and is particularly important for individuals with a positive family history, multifocal disease, or a diagnosis before age 50. Identification of patients with hereditary pheochromocytoma is important because it can guide medical management in mutation-positive patients and their families. This review provides an overview of the known genetic syndromes that are commonly associated with pheochromocytoma, examines recent data on the association of germline mutations in the succinate dehydrogenase gene family with pheochromocytoma, and suggests guidelines for the genetic evaluation of pheochromocytoma patients. [J Natl Cancer Inst 2003;95:1196–1204]

In general, tumors that arise from the sympathetic nervous system secrete catecholamines, whereas those that arise from the parasympathetic chain do not. The secretion of catecholamines, as measured by fractionated metanephrines in the urine and total plasma metanephrines, is used to diagnose pheochromocytoma. Pheochromocytomas are estimated to account for up to 0.1% of new cases of hypertension per year (2). The most common symptoms of pheochromocytoma are headaches, palpitations, and sweating, although not all patients have all three symptoms. The average age at pheochromocytoma diagnosis is 42 years (3). Approximately 10% of pheochromocytomas are malignant at diagnosis, and approximately 10% of patients have bilateral pheochromocytomas (4).

Familial clustering of pheochromocytoma (MIM 171350) and paraganglioma (MIM 16800) has been described repeatedly in the literature (5–7). Approximately 10%–15% of cases of pheochromocytoma have been thought to be due to hereditary causes; however, in some series up to 50% of cases are due to an underlying susceptibility allele (8,9). Several genetic syndromes, all of which are transmitted in an autosomal dominant fashion, are known to be associated with an increased risk for pheochromocytoma, including von Hippel–Lindau (VHL) syndrome, multiple endocrine neoplasia type 2 (MEN 2), which is associated with mutations in the RET proto-oncogene, and neurofibromatosis type 1 (NF1) (2,9). The genetic etiology of these syndromes has been well defined; however, they account for only approximately half of all familial pheochromocytoma cases (10). Pheochromocytoma and paraganglioma also occur in families without other associated clinical features, i.e., as isolated pheochromocytoma and paraganglioma, with susceptibility transmitted in an autosomal dominant fashion. Thus, an additional gene or genes is probably involved in isolated familial pheochromocytoma and paraganglioma (11). Indeed, germline mutations in members of the succinate dehydrogenase (SDH) gene family—SDHB, SDHC, and SDHD—have recently been identified in families with isolated paraganglioma and/or pheochromocytoma (12–14). Germline mutations in these genes have also been identified in patients with isolated pheochromocytoma.

Pheochromocytomas are rare tumors of the adrenal gland that arise from chromaffin cells in the adrenal medulla. Paragangliomas arise from extra-adrenal chromaffin cells and can originate in either the sympathetic nervous system or parasympathetic ganglia. Paragangliomas that arise from the sympathetic nervous system occur most frequently in the retroperitoneum and are sometimes termed extra-adrenal pheochromocytomas. Paragangliomas that originate in parasympathetic ganglia can be found adjacent to the aortic arch, neck, and skull base and are named according to their location, e.g., carotid body paraganglioma (1).
and a negative family history. Because the prevalence of germ-line mutations in cancer susceptibility genes in pheochromocytoma patients now exceeds 10%, the question has been raised whether genetic evaluation, genetic counseling, and perhaps genetic testing should be considered for all patients with pheochromocytoma or paraganglioma (15,16).

In this review, we provide an overview of the known genetic syndromes that are commonly associated with pheochromocytoma (Table 1). We then review recent data examining the association of germline mutations in the SDH gene family with pheochromocytoma. We end by suggesting guidelines for the genetic evaluation of pheochromocytoma patients. For this review, studies of pheochromocytoma in association with germline mutations in NF1, VHL, RET, SDHB, SDHC, and SDHD published before December 2002 were selected from peer-reviewed journals using MEDLINE; the bibliographies of those studies were also used as sources for articles.

**GENETIC SYNDROMES ASSOCIATED WITH PHEOCHROMOCYTOMA**

**Neurofibromatosis Type 1**

NF1, also known as von Recklinghausen disease, is a common autosomal dominant genetic disorder occurring in about one per 3000–4000 individuals (17). NF1 is characterized by multiple café-au-lait macules, neurofibromas, iris hamartomas (Lisch nodules), and skinfold freckling. The diagnosis of NF1 is based on criteria developed by a National Institutes of Health Consensus Conference in 1987 and is typically diagnosed in childhood (18). In childhood, patients with NF1 can present with optic gliomas; in adolescence, they can present with plexiform neurofibromas. Patients with NF1 also have an increased incidence of other tumor types, including malignant peripheral nerve sheath tumors, pheochromocytomas, and leukemia, particularly, juvenile chronic myelogenous leukemia (19). Pheochromocytomas are not associated with neurofibromatosis type 2 (NF2), which is characterized by acoustic neuromas and multiple meningiomas and schwannomas (20).

The NF1 gene consists of 51 exons that span 350 kilobases (kb) of genomic DNA (21). The open reading frame encodes a 2818-amino-acid protein called neurofibrin, the central region of which demonstrates sequence similarity to the family of GTPase activating proteins that are involved in the inhibition of Ras activity (22). The function of neurofibrin is not completely understood, but it appears that its loss increases activation of Ras, allowing enhanced cellular proliferation (23). Although NF1 acts as a tumor suppressor gene and both alleles are lost in a variety of NF1-associated malignancies, haploinsufficiency of NF1 alone may be sufficient to confer an increased tumor risk (19).

Molecular genetic testing for mutations in NF1 is available, but the diagnosis is typically made on a clinical basis. Because many different types of mutations occur throughout the large NF1 gene, identification of specific mutations is difficult (24—26). The clinical manifestations of NF1 and severity of the disease can vary, even among family members who carry the same mutation. Currently, there is no known relationship between severity of NF1 and age of diagnosis, birth order, parental age, or environmental factors (27). Recent literature (28) suggests that the manifestations of NF1 in a child may more closely resemble those of their parents than previously thought.

**NF1 and Pheochromocytoma**

Pheochromocytoma occurs in 0.1%—5.7% of patients with NF1 and in 20%—50% of NF1 patients with hypertension, as compared with 0.1% of all hypertensive individuals (29,30). Thus, patients with NF1 who have hypertension are more likely to have pheochromocytoma than other hypertensive individuals, and efforts to ensure screening for the tumor and follow-up are more important in these patients. When NF1 patients are examined at autopsy, the prevalence of pheochromocytoma is slightly higher (3.3%—13.0%) (24). The course and presentation of pheochromocytomas in NF1 patients are similar to those in patients with sporadic pheochromocytomas, unlike the pheochromocytomas associated with the other genetic susceptibility syndromes discussed below.

In a review of the literature, Walther et al. (30) characterized the clinical findings of NF1-associated pheochromocytoma in 148 individuals. The mean patient age at pheochromocytoma diagnosis was 42 years, the same as in the general population (3). Eighty-four percent of the patients had unilateral tumors, 10% had bilateral adrenal disease, and 6% had extra-adrenal pheochromocytomas. Of the 125 patients with unilateral tumors, 27 (22%) had no symptoms related to pheochromocytoma or hypertension; the methods of tumor detection were not reported. Malignant pheochromocytomas were identified in 17 of the 148 patients (11%), similar to the frequency of malignancy in the general population. Patients with NF1 also can have adrenal ganglioneuromas, which can be mistaken on radiological examinations for pheochromocytomas that do not secrete catecholamines (30). On occasion, NF1 can be diagnosed concurrently with pheochromocytoma; however, the skin lesions typical of NF1 usually lead to the diagnosis of NF1 in childhood, whereas pheochromocytoma is usually diagnosed in adulthood (18). Screening for pheochromocytoma on a yearly basis is recommended for any NF1 patient with hypertension or if any other suggestive symptoms, such as sweating episodes, are reported (18).

**Multiple Endocrine Neoplasia Type 2**

MEN 2 has two subtypes, MEN 2A and MEN 2B; 90% of MEN 2 cases are of the MEN 2A subtype. MEN 2A is characterized by medullary thyroid carcinoma in 95% of cases, pheochromocytoma in 50% of cases, and hyperplasia of the parathy-
roid glands in 15%–30% of cases (31). MEN 2B patients have medullary thyroid carcinoma in 100% of cases, pheochromocytoma in 50% of cases, and other features, including marfanoid habitus (tall, thin stature) and multiple mucosal neuromas (32). Parathyroid hyperplasia is not associated with MEN 2B. Patients with MEN 2B have a worse prognosis than those with MEN 2A, mainly because cancer develops in the first or second decade of life in MEN 2B patients, as opposed to the second or third decade of life in MEN 2A patients (33). The diagnosis of MEN 2A, MEN 2B, and familial medullary thyroid cancer (FMTMC), in which only medullary thyroid cancer is found, relies on a combination of clinical findings, family history, and molecular genetic testing of the RET proto-oncogene.

Germline mutations in the RET proto-oncogene, which encodes a transmembrane receptor tyrosine kinase and is expressed in cells derived from the neural crest, are responsible for MEN 2 (34). Approximately 95% of the mutations found in MEN 2A occur in exons 10 and 11 of the RET gene, which encode the cysteine-rich extracellular domain of the receptor (35). These are all missense mutations that affect one of five codons (609, 611, 618, and 620 in exon 10 and 634 in exon 11) and cause ligand-independent RET dimerization, leading to constitutive activation of the tyrosine kinase (31). Nearly all cases of MEN 2B are due to a single missense mutation (at codon 918 in exon 16) in the intracellular tyrosine kinase domain (36). RET activates multiple downstream pathways involved in cell growth, survival, and differentiation. A single activating mutation in one allele of the RET proto-oncogene predisposes to neoplastic transformation (37).

**MEN 2A and Pheochromocytoma**

Pheochromocytomas associated with MEN 2A are most commonly diagnosed between the ages of 30 and 40 years. They are diagnosed concurrently with medullary thyroid cancer in 35%–73% of cases (8,38–40) and as the first manifestation of MEN 2A in 9%–27% of cases (8,9,38–41). MEN 2A patients are statistically significantly younger at age of pheochromocytoma diagnosis than patients with sporadic pheochromocytoma (mean ages of 38 and 47 years, respectively; \(P \lt 0.05\)), although results for MEN 2A patients may be biased because diagnosis of pheochromocytomas occurs mainly through routine screening rather than being based on symptoms (8). In the one prospective series (42) of pheochromocytoma in MEN 2A patients, the age of diagnosis was even lower than in the retrospective series (mean of 23.2 years); however, a smaller proportion of patients were diagnosed with pheochromocytomas than in other series, and only a small number of patients were followed up beyond age 30.

Whether pheochromocytoma is diagnosed concurrently with or after medullary thyroid cancer in patients with MEN 2A depends mainly on the patient’s age at initial diagnosis of MEN 2A. Most MEN 2A patients develop bilateral pheochromocytomas, which can be diagnosed either synchronously or asynchronously, in some cases many years apart (39). However, extra-adrenal disease is extremely uncommon (43). Several studies have documented that pheochromocytomas undergo malignant transformation less frequently in MEN 2A patients than in patients with sporadic pheochromocytomas; the frequency of transformation is 4% or less (39,40).

A relationship between RET mutation location and pheochromocytoma has been demonstrated. Eng et al. (44) found that 160 of 186 MEN 2 families with medullary thyroid carcinoma and pheochromocytoma had a mutation at codon 634, whereas only 18 of 43 families without pheochromocytoma had a mutation at that site (\(P \lt 0.001\)). In a population-based study (45) of individuals presenting with sporadic pheochromocytoma, 12 of the 13 RET mutations identified were at codon 634. Thus, the presence of a mutation in codon 634 of RET is strongly associated with the presence of pheochromocytoma in MEN 2A families, and physicians should be aware of the greater likelihood for the development of pheochromocytoma in such patients.

In summary, pheochromocytomas in patients with MEN 2A are diagnosed at an earlier age, more likely to be bilateral, and less likely to undergo malignant transformation than sporadic pheochromocytomas. Annual screening for pheochromocytoma is recommended for all patients with mutations in the RET proto-oncogene associated with MEN 2 (46).

**von Hippel–Lindau Syndrome**

VHL syndrome is an autosomal dominant cancer susceptibility syndrome with an incidence of approximately one in 36,000 births per year. It is characterized by the presence of both benign and malignant tumors, including hemangioblastomas of the brain, spinal cord, and retina; renal cysts and renal cell carcinoma; pheochromocytomas; pancreatic islet cell tumors; and endolymphatic sac tumors. In a series of 934 patients from 23 families, 60% were symptomatic by age 30; however, the phenotypic expression of VHL is highly variable, with marked genotype–phenotype correlation (47,48).

The VHL gene (49) encodes a protein (pVHL) that is expressed in most tissues and has been implicated in a variety of functions, in particular, the regulation of hypoxia-inducible genes, angiogenesis, and fibronectin matrix assembly (50–53). pVHL, which has an elongin C binding region (\(\alpha\)-domain) and a binding site for hypoxia-inducible factor \(\alpha\) (HIF-\(\alpha\)) subunits with a hydrophobic core (\(\beta\)-domain), is part of a large complex that inhibits the accumulation of hypoxia-induced proteins through ubiquitin-mediated degradation of HIF-\(\alpha\) subunits (HIF-\(1\alpha\) and HIF-\(2\alpha\)) under normoxic conditions (54). VHL has been shown to act as a tumor suppressor gene in several studies (55,56); loss of the normal allele appears to be an early event in tumorigenesis because it is seen in renal cysts prior to malignant transformation (57).

Many different types of mutations have been identified in VHL, with partial or complete gene deletions accounting for up to 40% of the mutations identified (58–60). The type of mutation in the VHL gene is related to the clinical phenotype, as shown in Table 2. More than 95% of patients with truncating or null mutations have VHL type 1 (without pheochromocytoma). Patients with VHL type 2 (with pheochromocytoma) have primarily missense mutations. VHL type 2C is defined as pheochromocytoma only, whereas VHL types 2A and 2B have multiple types of VHL-associated tumors. Mutations associated with VHL type 1 disrupt the hydrophobic core of pVHL and are predicted to cause unraveling or complete absence of the protein (61). In contrast, the missense mutations associated with VHL type 2 are within the elongin C binding region (\(\alpha\)-domain) or in the HIF-\(\alpha\) subunit binding site (\(\beta\)-domain). Even those type 2-associated missense mutations that disrupt structural amino acids are predicted to cause only local defects and not to lead to loss of overall structural integrity (61). Recent data have demonstrated that the missense mutations associated with VHL type 2C, in which patients have isolated pheochromocytoma, result in a protein that retains the ability to ubiquinate HIF-\(1\alpha\) but is
defective in the promotion of fibronectin matrix assembly (52, 62,63).

VHL and Pheochromocytoma

As is characteristic of the pheochromocytomas associated with other cancer susceptibility syndromes, the pheochromocytomas associated with VHL are more likely to be bilateral and to be diagnosed at a younger age than sporadic tumors. The largest series of VHL patients with pheochromocytomas, with 64 patients in 38 families, was described by Walther et al. (64); the mean age at diagnosis was 29.9 years (range = 6–54 years), and individuals with missense mutations at nucleotides 595 and 695 presented with pheochromocytoma at a younger age than those with other mutations (P<0.025) (64). The mean age at diagnosis also was statistically significantly younger than the mean age at diagnosis in a control group of patients with sporadic pheochromocytoma (39.7 years); however, there may be bias due to routine screening in the VHL group. The mean age of diagnosis of pheochromocytoma tends to be younger in VHL patients than in MEN 2 patients (means of 29 and 36 years, respectively) (65).

Eighty-eight percent of tumors were adrenal and 12% were extra-adrenal, similar to the distribution in the general population (64). Extra-adrenal pheochromocytomas were found only in families with missense mutations in VHL, most frequently with a mutation at nucleotide 505 (64). Fifty-eight percent of the patients diagnosed with pheochromocytoma had multiple tumors. Interestingly, only 74% of VHL families that have members with pheochromocytomas were found to have missense mutations in VHL, suggesting that missense mutations, rather than being absolutely associated with pheochromocytoma, are associated with a higher penetrance of pheochromocytoma. Other, smaller studies (9,41) are consistent with the findings of Walther et al. (64) and also highlight early age of diagnosis and multiple pheochromocytomas, including extra-adrenal disease, as hallmarks of VHL.

Because mutations in VHL can lead to isolated pheochromocytoma, it is difficult to define how frequently pheochromocytoma is the first sign of VHL. However, depending on the mode of ascertainment, VHL mutations have been identified in 2%–50% of patients with sporadic pheochromocytoma (9,41,66–68). Like MEN 2–associated pheochromocytomas, VHL-associated pheochromocytomas appear to undergo malignant transformation less frequently than sporadic pheochromocytomas; however, further studies are needed to characterize this (41,59,64). In summary, pheochromocytomas associated with VHL are early-onset, multifocal, may be extra-adrenal, and are associated with a relatively lower frequency of malignancy. Screening for pheochromocytoma is recommended in all VHL patients starting at age 5 years (69).

Biochemical Findings in Pheochromocytoma: MEN 2 Versus VHL

A comparison of the patterns of catecholamine excretion from pheochromocytomas associated with MEN 2 and VHL shows that pheochromocytomas associated with MEN 2 are adrenergic, whereas those associated with VHL are noradrenergic because of the lower expression of phenylethanolamine-N-methyltransferase (PNMT), which converts norepinephrine to epinephrine (65). As a result, MEN 2 patients with pheochromocytoma have increases either in both plasma metanephrine and normetanephrine or in plasma metanephrine alone; in contrast, VHL patients with pheochromocytoma usually have increases in plasma normetanephrine only. Thus, patients with MEN 2–associated pheochromocytomas tend to be more symptomatic than those with VHL-associated pheochromocytomas. Lenders et al. (70) have demonstrated that plasma-free metanephrines are the single best test for detecting pheochromocytoma. In hereditary cases of pheochromocytoma, not differentiating among VHL, MEN 2, and NF1, urinary fractionated metanephrines provide sensitivity similar to that of plasma-free metanephrines (96% versus 97%) but lower specificity (82% versus 96%). However, measurement of plasma metanephrines is not universally available, and many physicians continue to rely on 24-hour measurements of total and fractionated urinary metanephrines, catecholamines, and vanillylmandelic acid. The pattern of metanephrine and normetanephrine elevations in the urine associated with MEN 2 and VHL reflects that in the plasma. Because pheochromocytomas associated with VHL are more likely to have elevated levels of normetanephrine and those associated with MEN 2 are more likely to have elevated levels of metanephrine, the pattern of metanephrine secretion in a pheochromocytoma patient can help determine which gene to prioritize for testing.

Succinate Dehydrogenase Gene Family

Germline mutations of SDHD were identified recently in familial paraganglioma through linkage mapping and mutation screening in candidate genes (8). The genes of the succinate dehydrogenase gene family (SDHA, SDHB, SDHC, and SDHD) encode the four subunits of complex II (succinate:ubiquinone oxidoreductase) of the mitochondrial electron transport chain (71). Complex II catalyzes the oxidation of succinate to fumarate and transfers its reducing equivalent to ubiquinone (coenzyme Q), so it is an important enzyme complex in both the tricarboxylic acid cycle and the aerobic respiratory chains of eukaryotic cell mitochondria and prokaryotic cells. The identification of disease-associated SDHD mutations in familial paragangliomas prompted the search for germline mutations in the genes encoding SDHD, SDHB, and SDHC in familial and sporadic cases of

<table>
<thead>
<tr>
<th>VHL disease type</th>
<th>HB</th>
<th>RCC</th>
<th>PHEO</th>
<th>Germline VHL mutation types</th>
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<tbody>
<tr>
<td>1</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>Full gene deletions, partial gene deletions, nonsense mutations, and splice acceptor mutations</td>
</tr>
<tr>
<td>2A</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>Missense mutations</td>
</tr>
<tr>
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<td>High</td>
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</tr>
<tr>
<td>2C</td>
<td>No</td>
<td>No</td>
<td>High</td>
<td>Missense mutations</td>
</tr>
</tbody>
</table>

*HB = hemangioblastoma; RCC = clear-cell renal cell cancer; PHEO = pheochromocytoma.
isolated pheochromocytoma. Germline mutations in SDHA were previously known to be associated with Leigh syndrome (primary lactic acidosis) (72).

**Succinate Dehydrogenase Gene Family and Pheochromocytoma**

Germline mutations in SDHD, SDHB, and SDHC have been identified in patients with paraganglioma, whereas germline mutations in only SDHD and SDHB have been identified in patients with pheochromocytoma. In both paraganglioma and pheochromocytoma, far more germline mutations have been identified in SDHD than in the other succinate dehydrogenase genes. Ninety-seven percent of the germline mutations identified in paraganglioma families are in SDHD (73–75). Only one family has been found to have a mutation in SDHC, and two families with both paragangliomas and pheochromocytomas have been found to have mutations in SDHB (13,14). Frameshift, missense, and nonsense mutations have all been identified in SDHD, with the missense mutation Pro81Leu identified most frequently (12,73,76,77). Interestingly, SDHD appears to be imprinted, with the susceptibility to paraganglioma or pheochromocytoma expressed only after paternal transmission; there is no evidence of imprinting associated with either SDHB or SDHC (78). SDHD has been demonstrated to act as a tumor suppressor gene, with loss of the maternal allele demonstrated in several studies (7,11,73,74,79).

Several series of sporadic paragangliomas have been studied, from the United States, Spain, and the Netherlands. The Dutch population contains two founder mutations in SDHD—Asp92Tyr and Leu95Pro (74,80–82). In the two Dutch series (74,82), a substantial proportion (40% and 56%, respectively) of the paraganglioma patients had mutations in SDHD, predominantly the two founder mutations. Germline mutations in SDHD were associated with multifocal paragangliomas. In the American series (80), ascertained from two otolaryngology clinics, mutations in SDHD and SDHB were identified in five of 10 familial cases, respectively, and in two and one of 37 isolated cases, respectively. Both of the isolated cases with SDHD mutations had multifocal tumors. No mutations were identified in SDHC. Even before the isolation of SDHD, a higher proportion of paragangliomas in the Dutch population than in other populations was thought to be due to hereditary causes (78,83). These data suggest that consideration should be given to referring all paraganglioma patients to a cancer geneticist, with genetic testing focusing on those who have a family history of paraganglioma or pheochromocytoma, who have multifocal disease, or who are of Dutch ancestry.

Germline mutations in the SDH gene family also were good candidates to account for families with multiple cases of isolated pheochromocytoma. Of five such families that were negative for mutations in VHL and RET, one had a mutation in SDHD (77,84). Isolated pheochromocytoma in families is not associated with NF1. The mutation identified in SDHD was identical to that found in an unrelated paraganglioma family (84). Of five nonoverlapping families with multiple cases of isolated pheochromocytoma that were negative for mutations in VHL, RET, and SDH and were tested for mutations in SDHB and SDHC, two had germline mutations in SDHB (77). Although the screening protocol for those with mutations in SDHB and SDHC is still being developed, follow-up including yearly plasma metanephrine tests for elevated normetanephrines and metanephrines and magnetic resonance imaging of the adrenal glands, head and neck region, or both is consistent with the screening recommendations for cancer susceptibility syndromes in general.

**Series of Sporadic Pheochromocytomas**

Many studies have been done of hospital-based series of isolated pheochromocytomas to determine the frequency of germline mutations in VHL and RET. For this review, only studies that have patients’ lymphocytic DNA available are included because studies that estimate frequencies from mutation studies in tumor DNA may not be accurate. In the first such study (41), 4% of 82 patients had germline mutations in RET and 19% had germline mutations in VHL. However, this study has been criticized because the participating hospitals were in the Freiburg area of Germany, which contains a founder mutation in VHL. In addition, the study did not exclude patients with a personal or family history suggestive of MEN 2 or VHL, as subsequent studies have done. In one subsequent study (85), 7% of 77 unselected patients with pheochromocytoma had mutations in RET. However, in other hospital-based studies (66,67,86–88) that excluded patients with a suggestive or personal history of MEN 2 and for which patients’ lymphocytic DNA was available, only one of 137 patients was found to have a germline mutation in RET, with screening limited mainly to exons 10, 11, and 16. After the mutation was identified, the family was found to have a history consistent with MEN 2A. Overall, missense mutations in VHL have been found more frequently than missense mutations in RET in sporadic pheochromocytoma cases, with VHL mutation frequency ranging from 3% to 9% in hospital-based studies (66–68,86), that excluded patients with a personal or family history suggestive of VHL. In all of the studies, single-strand conformational polymorphism analysis (SSCP) was used for mutation detection. This technique has an estimated sensitivity of 72%, so the frequency of mutations may be higher than reported (89). The frequency of mutations in each of the studies was too low to allow any conclusions to be drawn about differences in tumor characteristics based on mutation status.

Following the discovery of germline mutations in SDHD in hereditary paraganglioma, several groups have sought to clarify the role of germline mutations in members of the SDH family in sporadic pheochromocytoma. In two hospital-based case series (76,84), 37 sporadic pheochromocytoma patients were tested for germline mutations in SDHD. Eight percent of the patients had a mutation, but only half the patients had been tested for mutations in VHL and RET, so other hereditary causes of pheochromocytoma had not been fully ruled out. Gimm et al. (76) described a possible association of mutations in SDHD with extra-adrenal disease, but their series was too small to draw a firm conclusion. In a hospital-based series of sporadic pheochromocytoma patients who were negative for mutations in VHL, RET, and SDHD and who were subsequently tested for germline mutations in SDHB and SDHC, only one of 24 (4%) tested individuals had a mutation in SDHB (14), and none had a mutation in SDHC. Although these studies were preliminary, they made it clear that germline mutations in SDHD and SDHB are seen in patients with sporadic pheochromocytoma.

In the most comprehensive study of apparently sporadic pheochromocytoma and paraganglioma to date, Neumann et al. (45) examined a population-based series of 271 patients—241 with pheochromocytoma only, eight with both pheochromocytoma and paraganglioma, and 22 with paraganglioma only. Pa-
Patients with a known genetic syndrome or with a family history of syndrome-related tumors were excluded; these included 11 patients with NF1 (4%), nine with MEN 2 (3%), and five with VHL (2%). All eight exons of SDHB, all four exons of SDHD, all three exons of VHL, and exons 10, 11, and 13–16 of RET were scanned using SSCP analysis and direct sequencing. Overall, 66 patients (24%) who had a germline mutation were identified: 30 in VHL, 13 in RET, 11 in SDHD, and 12 in SDHB. The mean age at presentation of pheochromocytomas associated with a germline mutation ranged from 18.3 years (VHL) to 36.4 years (RET). The age range for patients presenting with tumors associated with a germline mutation in a known susceptibility gene was 5–59 years, whereas that for patients with tumors without a germline mutation in a known susceptibility gene was 4–81 years (P < 0.001). Neumann et al. found that 84% of patients with multifocal tumors carried a germline mutation in one of the four genes. In patients with extra-adrenal tumors, no mutations in RET were identified, consistent with previous studies. In contrast, SDHB mutations were associated solely with extra-adrenal disease. Importantly, for targeting genetic testing, only one of the 83 patients who were older than 50 years at diagnosis was found to carry a germline mutation in one of the four genes analyzed. In summary, the results of this population-based series of pheochromocytomas support those of hospital-based studies and suggest that genetic counseling and potentially genetic testing should be offered to all patients diagnosed with pheochromocytoma before age 50.

**SUMMARY**

As discussed in this review, genetic syndromes associated with a susceptibility to pheochromocytoma include NF1, VHL, and MEN 2. Pheochromocytoma has rarely been reported in association with other autosomal dominant cancer susceptibility syndromes, including Carney syndrome and multiple endocrine neoplasia type 1 (3,90). Familial isolated pheochromocytoma has been associated with mutations in VHL, SDHB, and SDHD. Strikingly, the protein products of all three genes are involved in the response to cellular hypoxia, suggesting a possible common etiology for hereditary susceptibility to pheochromocytoma.

The literature we have reviewed suggests that the proportion of pheochromocytomas due to susceptibility alleles is higher than the 10%–15% often cited. When the hospital-based studies of germline mutations in VHL, RET, SDHD, and SDHB are considered in aggregate, more than 20% of cases of sporadic pheochromocytomas (those in patients with no known family history of a syndrome that is linked to pheochromocytoma) are associated with mutations in one of the four genes—VHL (6%), SDHD (8%), SDHB (4%), or RET (<1%), as shown in Table 3.

<table>
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<td>9</td>
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</tbody>
</table>

The recent population-based series (45) is generally consistent with the hospital-based literature in that germline mutations in one of the four genes were found in 24% of patients with apparently sporadic pheochromocytoma (11% of patients had a VHL mutation, 5% an RET mutation, 4% an SDHD mutation, and 4% an SDHB mutation), with the exception of the relatively high mutation frequency in the RET proto-oncogene.

One of the reasons that the percentage of pheochromocytoma cases that are hereditary may previously have been underestimated is that the SDHD gene undergoes imprinting; consequently, individuals with genetic susceptibility due to mutations in SDHD may not have a positive family history of pheochromocytoma or paraganglioma. On the basis of both the hospital- and population-based series of patients, the total percentage of pheochromocytoma that is due to germline mutations in a susceptibility allele, if those with a suggestive personal or family history are included, is at least 30% (41,85). Comparing this percentage with the percentage of other tumors due to hereditary causes serves to emphasize how striking it is. For example, medullary thyroid cancer is the hallmark of MEN 2, and up to 25% of medullary thyroid cancers are due to mutations in the RET proto-oncogene (91–93). Genetic testing for mutations in RET is considered the standard of care for all patients with medullary thyroid cancer, so that patients with MEN 2 can be identified. A 10% prior probability of identifying a mutation in a cancer susceptibility gene is considered the standard threshold for genetic testing (94). The study by Neumann et al. (45), in agreement with the previous literature, demonstrates that the *a priori* risk of finding a germline mutation in patients with sporadic pheochromocytoma exceeds this criterion in patients up to age 50 years.

Given the prevalence of germline mutations in pheochromocytoma, it is not surprising that several experts have advocated genetic counseling for everyone who has a pheochromocytoma (15). Genetic testing should be considered when the results will affect medical management. The benefits of determining which patients have germline mutations in susceptibility alleles include identifying patients with a genetic syndrome that may have additional implications in terms of risks for other cancers, increasing vigilance in screening for additional adrenal and extra-adrenal pheochromocytomas, and identifying other family members at risk. However, because the practical implications of counseling and testing for mutations in four genes can be daunting, we recommend that all patients with pheochromocytoma first be referred for a cancer genetics risk evaluation. An evaluation of the patient should include 1) an assessment of the likelihood of a genetic syndrome associated with pheochromocytoma, with a review of the personal and family history for any features suggestive of VHL, MEN 2, NF1, MEN 1, or Carney syndromes; 2) consideration of genetic testing; and 3) if testing is positive, recommendations for management of patients with a germline mutation. In addition, the biochemical properties of the pheochromocytoma can help guide the physician in determining which of the four genes should be prioritized for genetic testing (65). All patients with early-onset pheochromocytoma (before age 50), multifocal disease, or any family history of pheochromocytoma or paraganglioma should have synchronized genetic testing, starting with VHL and SDHD. We include a table to assist physicians in prioritizing genes for genetic testing (Fig. 1). Commercial genetic testing is generally available for mutations...
in the RET proto-oncogene and is available in specialized laboratories for VHL, SDHB, and SDHD\(^1\). Current evidence does not support a contribution of mutations in SDHC to inherited pheochromocytoma susceptibility, and unless information to the contrary becomes available, there is no reason to test pheochromocytoma patients for mutations in SDHC. If patients are found to have a mutation in one of the four genes, they should have carefully monitored screening, and at-risk family members should be offered genetic counseling. Patients with pheochromocytoma who do not meet the criteria for genetic testing should be counseled on a case-by-case basis and be made aware of the availability of research studies on the genetic underpinnings of pheochromocytoma.

**REFERENCES**


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**Fig. 1.** Guidelines for approaching genetic testing in pheochromocytoma. Thorough medical and family histories and physical examination will help pinpoint features of a genetic syndrome. The table at the bottom is intended to help guide the physician in prioritizing genetic testing for patients younger than 50 years with sporadic pheochromocytoma. VHL = von Hippel–Lindau syndrome; MEN 2 = multiple endocrine neoplasia type 2; SDHB = pheochromocytoma/paraganglioma syndrome due to mutations in the SDHB gene; SDHD = pheochromocytoma/paraganglioma syndrome due to mutations in the SDHD gene; MTC = medullary thyroid cancer; HPT = hyperparathyroidism.


Point mutation within the tyrosine kinase domain of the RET proto-oncogene is a frequent event in neurofibromatosis type 1. Hum Genet 1998;102:457–62.


Notes

*Testing for mutations in the VHL gene is available from the Boston University School of Medicine Center for Human Genetics (Boston, MA), the Johns Hopkins Hospital DNA Diagnostic Laboratory (Baltimore, MD), and the Children’s Hospital of Philadelphia Molecular Genetics Laboratory (Philadelphia, PA). Testing for mutations in the SDHD gene is available from the Boston University School of Medicine Center for Human Genetics; the University of Pittsburgh Medical Center, Division of Molecular Diagnostics (Pittsburgh, PA); and the Children’s Hospital of Philadelphia Molecular Genetics Laboratory. Testing for mutations in the SDHB gene is available from the University of Pittsburgh Medical Center, Division of Molecular Diagnostics. GeneTests-Geneclinics (http://www.genetests.org/servlet/access?) has an updated list of testing locations and also lists international laboratories where testing can be obtained.

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