The pressure rises: update on the genetics of phaeochromocytoma

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Phaeochromocytomas are neoplasias of neural crest origin arising from the adrenal medulla. Extra-adrenal phaeochromocytomas occur and may be referred to as paragangliomas, although this term is also used to describe vascular head and neck tumours, which most commonly develop at the carotid bifurcation. Historically, genetic factors have been implicated in up to 10% of phaeochromocytoma cases, but recent data suggest that germline mutations may be detected in ~25% of unselected cases. The most frequent causes of phaeochromocytoma susceptibility are von Hippel–Lindau disease (VHL), multiple endocrine neoplasia type 2 (MEN 2), the newly delineated phaeochromocytoma–paraganglioma syndrome and, less commonly, neurofibromatosis type 1. Germline mutations in three of the succinate dehydrogenase (SDH, mitochondrial complex II) subunits (SDHD, SDHB and SDHC) cause susceptibility to head and neck paragangliomas, and may be found in ~20% of unselected patients. In addition, germline SDHD and SDHB mutations may cause phaeochromocytoma susceptibility with or without associated head and neck paragangliomas. Recent studies suggest that germline SDHD and SDHB mutations are an important cause of familial and isolated phaeochromocytoma. The mechanism by which SDH subunit mutations predispose to phaeochromocytomas has not been defined in detail, but dysregulation of hypoxia-responsive genes and impairment of mitochondria-mediated apoptosis have both been suggested.

Phaeochromocytomas are catecholamine-secreting tumours that can present with hypertension and are benign in ~90% of cases. Phaeochromocytomas usually arise within the adrenal medulla, but develop in extra-adrenal sympathetic ganglia ~10% of the time. Extra-adrenal phaeochromocytoma are sometimes referred to as paragangliomas. Vascular head and neck tumours, which usually arise from the carotid body (see later), will be referred to as head and neck paragangliomas or glomus tumours. Inherited cancer syndromes with phaeochromocytomas as a component feature include von Hippel–Lindau syndrome (VHL; MIM 193300) and multiple endocrine neoplasia type 2 (MEN 2; MIM 171400) and, to a much lesser extent, type 1 neurofibromatosis. Germline mutations in the VHL tumour suppressor gene cause VHL and germline mutations in the RET proto-oncogene cause MEN 2 (1–3). A subset of phaeochromocytoma-only families also were found to segregate germline VHL mutations but not RET mutations (4–6). For at least 6 years, molecular genetic investigation, whether for research or for clinical purposes, of phaeochromocytoma was limited to VHL and RET. Recently, both fundamental knowledge about the genesis of these tumours and the practice of clinical cancer genetics have benefited from the discovery that germline mutations in SDHD, encoding a subunit of mitochondrial complex II, predispose to a subset of families with head and neck paragangliomas, typified by carotid body glomus tumours (7). The spectrum of SDHD-defined disease was quickly expanded to include phaeochromocytomas (8,9). Subsequently, germline mutations in related subunits of mitochondrial complex II, SDHB and SDHC, were also implicated in the genesis of hereditary paragangliomas (10,11).

VHL TUMOUR SUPPRESSOR GENE AND HEREDITARY PHAEOCHROMOCYTOMA

VHL disease is a dominantly inherited familial cancer syndrome with variable expression resulting from mutations in the VHL tumour suppressor gene (1,12,13). VHL disease has an approximate incidence of 1 in 36 000 live births (14). The three major features of VHL disease are retinal and central nervous system (CNS) haemangioblastomas and clear cell renal cell
carcinoma (RCC), and the lifetime risk for each of these tumours has been estimated as >70% (14). However, tumour-specific risks are influenced by allelic heterogeneity, and four phenotypic subclasses of VHL disease have been distinguished. Interfamilial differences in phenotype are most marked for phaeochromocytoma. Thus, many kindreds demonstrate a type 1 phenotype in which phaeochromocytoma is absent and the most frequent manifestations are retinal and cerebellar haemangioblastomas and RCC. Type 2 VHL kindreds contain an individual with phaeochromocytoma, but these are further subdivided into 2A, 2B and 2C phenotypes. Excluding Germany, European type 2 VHL families usually have a 2B phenotype characterized by the development of phaeochromocytoma, RCC, and retinal and cerebellar haemangioblastomas. However, in south-west Germany and in North American families of German origin, type 2A kindreds are more common (15). Type 2A kindreds are at risk for phaeochromocytoma and retinal and CNS haemangioblastomas, but RCC is rare and the mortality rate appears to be similar to the general population (whereas median life expectancy is ~60 years in type 1 and 2B families) (16). Finally, type 2C VHL disease is defined by the detection of a germline VHL gene mutation in kindreds with a phaeochromocytoma-only phenotype (~40% of patients with non-syndromic familial or bilateral phaeochromocytoma may have a germline VHL mutation) (5) (E.R. Maher et al., unpublished observations).

Phaeochromocytoma susceptibility and genotype–phenotype correlations in VHL disease

Although the overall frequency of phaeochromocytoma in VHL disease is ~10–15%, phaeochromocytoma is often the most frequent manifestation in type 2A and 2B kindreds and, by definition, is absent in type 1 kindreds and the sole feature in type 2C families. An association between phaeochromocytoma susceptibility and the development of pancreatic islet cell tumours (which occurs in 5–10% of patients) has been suggested (17).

The VHL gene specifies two translation products: a full-length 213-amino-acid protein (pVHL103) and a shorter protein (pVHL106), which is translated from an internal translation initiation site at codon 54 and produces a 160-amino-acid protein. Germline VHL gene mutations have been identified in >500 kindreds (18–22) (http://www.umd.necker.fr), but no mutations have been reported in the first 53 amino acids included in pVHL103. Convenietly for genotype–phenotype correlations, germline VHL mutations fall into three major groups. Germline deletions and protein truncating mutations account for ~70% of germline mutations (~40% and ~30% respectively) and a greater proportion of mutations in type 1 families, whereas all type 2A and 2C, and the vast majority of type 2B kindreds, have missense mutations (5, 6, 18, 21–23). Maher et al. (22) estimated that whereas the phaeochromocytoma risk in patients with VHL gene deletions or truncating mutations is up to 5% at 50 years, this is increased 10-fold in patients with missense mutations (although these are heterogeneous, and, in some cases, risks are even higher). Recurrent VHL gene mutations (e.g. 694C > T, 712C > T, 713G > A and 505T > C) mostly represent multiple de novo mutations at hypermutable sequences, but the 505T > C (Y98H) (‘Black Forest’) mutation common in south-west Germany and in North American kindreds of German origin is a founder mutation (15). Phenotypic variability in VHL disease may reflect allelic heterogeneity, genetic modifiers or stochastic events (23). Relatively few VHL mutations have occurred in multiple and/or extensive pedigrees that would enable precise genotype–phenotype correlations. However, in addition to the Y98H prototypic type 2A mutation, the recurrent R167Q mutation is associated with a type 2B phenotype and L188V is the best defined type 2C-associated mutation (6, 22).

VHL tumour suppressor gene function and relationship to genotype-phenotype correlations

The complex genotype–phenotype correlations in VHL disease suggest that the VHL gene product has multiple and/or tissue-specific functions. Consistent with this hypothesis, pVHL has been implicated in several processes, including the regulation of hypoxia-inducible gene expression via the heterodimeric transcription factors HIF-1 and HIF-2, cell cycle control, mRNA stability, and control of extracellular fibronectin matrix assembly (24–26).

The most well-defined function of pVHL is the targeting of the HIF-1α and HIF-2α subunits for ubiquitylation and proteolytic degradation (27–29). This aspect of pVHL function and the role of pVHL in oxygen sensing are reviewed in detail elsewhere (30). The elucidation of the fundamental role of pVHL in oxygen sensing was preceded by the finding that pVHL formed a multimeric complex with elongins B and C, Cul-2 and Rbx1 (31–33). Structural and sequence motif homologies between VHL, elongins C and B and Cul-2 and the yeast SCF (Skp1-Cdc53/Cul1-F-box) E3 ligase complex suggested that pVHL might have an ‘F-box-like’ function and target specific proteins for ubiquitylation and proteosomal degradation (32). In addition, Rbx1 was demonstrated to associate with the pVHL-containing complex and to be an essential general component of SCF complexes (33). Elucidation of the crystal structure of the pVHL–elongin C–elongin B Complex (VCBC) demonstrated that pVHL has two principal domains, an ~100 residue N-terminal domain rich in β sheet (the β domain) and a smaller C-terminal α-helical domain (the α domain). A large portion of the α-domain surface interacts with elongin C, and many disease-causing VHL mutations disrupt the α domain and elongin C binding. Other VHL mutations map to the surface protein binding site in the α domain (34). The α domain site binds the HIF-α subunits (and potentially other proteins), while the other components of the general ubiquitylation complex are bound via the α-domain interaction with elongin C. Subsequent studies have demonstrated that wild-type pVHL, but not mutant, promotes HIF-α subunit ubiquitylation in vitro (28, 29, 35). Thus, VHL inactivation results in loss of HIF-α subunit ubiquitylation, abnormal normoxic expression of HIF-1 and HIF-2, and upregulation of a wide range of hypoxia-inducible genes [including angiogenic growth factors such as vascular endothelial growth factor (VEGF)] that promote angiogenesis and regulate glucose metabolism, apoptosis and matrix metabolism. While HIF dysregulation associated with VHL inactivation provides a plausible explanation for the vascular nature of VHL tumours, the relevance to
growth suppression is less clear. However, recent reports suggest that HIF-2 overexpression may be oncogenic per se (36, 37).

Although the pVHL–ubiquitin ligase complex might also target additional protein substrates for pVHL-dependent ubiquitylation, and pVHL-null cells demonstrate impaired extracellular fibronectin matrix assembly, disordered cell cycle control and increased expression of cyclin D1 and a GLUT-1 RNA-binding protein (hnRNP A2) (24–26, 38), the precise relevance of these (and other putative) functions to pVHL tumour suppressor activity is uncertain. Definition of the relationships between pVHL mutations associated with different phenotypes and loss of specific pVHL functions might provide critical insights into the relevance of pVHL functions to tumour suppression. Structural analysis of the VCBC (34) demonstrated that missense mutations associated with a type 1 phenotype often occur at codons within the hydrophobic core mutations and would be predicted to cause complete disruption to the pVHL structure. Furthermore, such mutations usually lead to loss of elongin C or HIF-1α binding and are unable to promote HIF-1α ubiquitylation (29, 35). Type 2 (phaeochromocytoma-associated) mutations show a trend against hydrophobic core mutations (Y98H maps to the β-domain surface-binding site and R167Q to the α-domain elongin-binding site), consistent with a strong bias against total loss-of-function mutations in type 2 kindreds (34). Furthermore, in vitro analysis of pVHL function demonstrates partial retention of pVHL binding to elongin C or HIF-1α with phaeochromocytoma associated missense mutations (29). These findings would suggest that complete loss of pVHL function (generally) does not predispose to, or is incompatible with, phaeochromocytoma development. Analysis of HIF-1 regulation by overexpression of mutant VHL proteins demonstrated complete or partial dysregulation with types 1, 2A and 2B-associated mutations, but type 2C mutants retained the ability to regulate HIF-1 (although fibronectin binding was impaired) (29, 35). These findings would apparently suggest that HIF-1 dysregulation is not necessary for phaeochromocytoma development in VHL disease, but abnormal fibronectin matrix assembly may be implicated. The effect of specific mutations associated with type 2 phenotypes on other putative pVHL functions has not been studied in detail, although it is reported that the L188V type 2C substitution retains the ability to downregulate cyclin D1 expression (38).

**RET PROTEO-ONCOGENE AND HEREDITARY PHAEOMOCYTOMA**

Multiple endocrine neoplasia type 2 (MEN 2)

MEN 2 is an autosomal dominant inherited cancer syndrome occurring in 1 in 300,000 live births and comprises three clinical subtypes—MEN 2A, MEN 2B and familial medullary thyroid carcinoma (FMTC)—depending on the clinical phenotype (reviewed in 39). Thus, depending on clinical features, MEN 2A, the most common clinical subtype, is characterized by the classic triad of medullary thyroid carcinoma (MTC), phaeochromocytoma and hyperparathyroidism (HPT). MEN 2B is much less common, and is characterized by MTC and phaeochromocytoma, but the age of onset of these tumours could be a median 10 years earlier than that for MEN 2A. FMTC is characterized by the occurrence of MTC only in the family. Approximately 50% of MEN 2 patients develop phaeochromocytoma.

Germline mutations in the RET proto-oncogene cause MEN 2, without genetic heterogeneity (34–43). Approximately 95% of MEN 2 cases are accounted for by germline RET mutations (3). Germline RET mutations have been found in ~98% of MEN 2A cases, 97% of MEN 2B cases and perhaps 85% of FMTC cases (3, 44, 45).

Germline RET genotype dictates phaeochromocytoma phenotype

Genotype–phenotype association analyses on a referral series of MEN 2 suggested that germline RET mutations at codon 634 were associated with the development of phaeochromocytoma (2). This association has been confirmed by other single-institution and referral series (46–48). Thus, the International RET Mutation Consortium was founded in the summer of 1994 to analyse genotype–phenotype associations (49). This comprised >20 centres of excellence around the world, which provided almost 300 unrelated MEN 2 families (3). In the Consortium analysis, germline mutations at RET codon 634 were significantly associated with phaeochromocytoma development (3).

Conversely, RET genotypes associated with FMTC should be inversely associated with the development of phaeochromocytoma (3). In general, this genotype–phenotype association is robust, although there have been anecdotal reports of phaeochromocytoma development in a single family with germline RET V804L mutations, previously associated with FMTC only (50). Whether the unilateral phaeochromocytomas at later ages is component of the MEN 2 spectrum, in particular associated with this mutation, is as yet unknown.

**PHAEOMOCYTOMA—PARAGANGLIOMA SYNDROMES CHARACTERIZED BY GERMLINE SDHx MUTATIONS**

Phaeochromocytomas and paragangliomas

Paragangliomas are tumours derived from the paraganglia, which can be of sympathetic origin, and localized mainly in the retroperitoneum, although they can also occur in the thorax as catecholamine-secreting, ‘functioning’ extra-adrenal phaeochromocytomas. Paragangliomas can also be of parasympathetic origin, occurring adjacent to the aortic arch, neck and skull base as local ‘non-functioning’ masses, commonly referred to as head and neck paragangliomas, glomus tumours or chemodectomas. The carotid body is the most common site of head and neck paragangliomas. Like most parasympathetic paraganglia, the carotid body does not secrete but serves a chemosensory role, specifically linked to oxygen sensing. Carotid bodies sense hypoxia and send signals to increase respiratory and heart rate by stimulating central ventilatory centres. The carotid body can be exposed to chronic (relative) hypoxia in individuals living at high altitude, and this chronic
hypoxia has been shown to be associated with neoplastic enlargement of the carotid body (51). It is this observation and the knowledge that mitochondrial complex II is intimately involved in hypoxia and oxidation–reduction that led investigators to hypothesize that defects in this complex could be aetologic for predisposition to carotid glomus tumours (reviewed in (52)).

**Germline SDHD mutations predispose to paraganglioma and phaeochromocytoma**

Familial clustering of glomus tumours was first recognized and reported in 1964 (53). After studying such families, Dutch investigators noted that glomus tumours were inherited from the paternal line (54,55). Based on clinical observations, the gene causing familial glomus tumours was believed to be maternally imprinted (paternally expressed). Working on this premise, these investigators mapped a gene for familial glomus tumours to 11q23 and showed by haplotype analysis that this predisposition was most likely due to a founder effect in the Dutch population (55,56). This locus was labelled *PGL1* (MIM 168000). Subsequently, using standard positional cloning and a candidate gene approach, Baysal and colleagues (7) demonstrated that SDHD was the *PGL1* locus and that germline mutations in this gene were associated with a subset of families segregating head and neck glomus tumours. They also demonstrated that two founder mutations, R92Y and L139P, account for almost all cases of familial paraganglioma in the Netherlands (57). Approximately 5% of Dutch head and neck paraganglioma patients also had phaeochromocytomas. Interestingly, monoallelic expression in tumours from these germline mutation-positive families could not be conclusively demonstrated (7).

The clinical spectrum of SDHD-associated disease was expanded when germline mutations in *SDHD* were found in familial phaeochromocytoma-only families, who were known to be mutation-negative in *VHL* and *RET* (Fig. 1) (5,9). Further, in a pilot series of 17 non-familial, non-syndromic phaeochromocytomas, up to 18% were found to harbour occult germline *SDHD* mutations (8).

**Germline SDHC and SDHB mutations in familial paraganglioma and phaeochromocytoma**

Apart from SDHD, mitochondrial complex II (succinate dehydrogenase, succinate:ubiquinone oxidoreductase) is also composed of three other subunits (SDHA, SDHB and SDHC), which participate in aerobic electron transport and the Krebs tricarboxylic acid cycle. SDHA and SDHB are the enzymatic subunits, and SDHC and SDHD anchor the heterotetrameric complex to the mitochondrial membrane. Homozygous germline mutations in *SDHA* cause an inherited neurological disorder (Leigh syndrome), as well as a syndrome of optic atrophy, ataxia and myopathy (58,59).

To date, germline mutations in *SDHC*, the *PGL3* locus (MIM 605373) on 1q21, have only been described in a single two-generation German family with paragangliomas (11). It is unclear from the description of this family whether phaeochromocytomas are also a feature of *PGL3*. Subsequently, germline mutations were described in *SDHB*, the *PGL4* locus (MIM 185470) on 1p36, in two of five families with phaeochromocytoma only, two of three families with both phaeochromocytoma and paraganglioma, and interestingly, 1 of 24 individuals with non-familial phaeochromocytoma (10). While the *SDHB* R90X was described at least three times in probands of European origin (Fig. 1), this mutation could not be shown to be due to a founder effect. The putative *PGL2* locus, on 11q13, was shown to be linked to paragangliomas in a single large Dutch family (60), but no susceptibility gene has been identified to date.

**FREQUENCY OF GERMLINE MUTATIONS IN PREDISPOSING GENES IN PARAGANGLIOMA AND PHAEOCHROMOCYTOMA**

The frequency of germline mutations within predisposing genes lends clues to the contribution of each gene to the pathogenesis of a certain condition. This frequency is important in practice, since it would dictate clinical management. In the practice of clinical cancer genetics, a patient with some likelihood of harbouring a germline mutation is usually offered clinically based mutation analysis (61). The most accurate estimate of mutation frequency is ideally obtained from a population-based study. Unfortunately, only one such study exists, while few other studies, based on clinic patients (i.e. referral-based), have been reported.

The frequencies of germline mutations in *SDHB*, *SDHC* and *SDHD* were examined in a referral-based clinic series of 47 unrelated head and neck paraganglioma probands (62). This series originated from two otolaryngology clinics—one on the east coast and one on the west coast of the USA. Amongst these 47 referred probands, belonging to 10 familial and 37 apparently sporadic cases 9 (20%) were found to have mutations in *SDHD* or *SDHB* (Fig. 1). No mutations were found in *SDHC*. Interestingly, half of the 10 families were found to harbour germline *SDHD* mutations, while germline mutations were found in 2 of the 37 (5%) of the apparently sporadic cases. However, these 2 mutation-positive apparently sporadic cases did present with multifocal disease. In contrast, germline mutations in *SDHB* were found in 2 of the 10 (20%) families with paragangliomas and in 1 of the 33 (3%) with apparently sporadic cases. Thus, if this series could be extrapolated to the general population, *SDHD* and *SDHB* could account for 70% of head and neck paragangliomas in the familial setting, and perhaps 8% of apparently sporadic head and neck paragangliomas.

In this referral-based setting, two recurrent germline mutations in *SDHD* were confirmed: P81L and R38X (62). Haplotype analyses revealed that these mutations were most likely not due to a founder effect but instead represented recurrent mutations at CpG dinucleotides. In this small series and a previous report on *SDHB* mutations, no founder mutations were found (10,62).

In a single population-based study of clinical presentations of phaeochromocytomas using the registries of Germany and central Poland, 271 non-syndromic phaeochromocytomas without family history were ascertained (63). Of these unrelated registrants, 66 (24%) were found to have a germline mutation in one of *VHL* (30), *RET* (13), *SDHB* (12) or *SDHD* (11). Of note, all individuals carrying the Black Forest
founder mutation in VHL were counted as one individual only. Interestingly, of the 66 with germline mutations, only 21 presented with multifocal disease. While young age of onset was associated with finding a germline mutation, it should be noted that 35% of those found to have germline mutations presented after the age of 30 years and 8% after the age of 40 years. Extra-adrenal tumours were found to be associated with heredity and, in particular, with germline mutations in SDHD.

The relatively high frequency of apparently sporadic disease in SDHD mutation carriers might be attributed to maternal imprinting. No evidence of genomic imprinting is evident for SDHB in this and other studies to date (10, 62, 63). The findings from the population-based study are important for clinical practice as well. These data suggest that all presentations of pheochromocytoma and paraganglioma, irrespective of family history, syndromic manifestations or age at diagnosis, should be subjected to clinical genetic testing for these four genes. The relative contribution of each of these four genes might suggest beginning clinical mutation analysis with VHL, although the presence of extra-adrenal disease might prompt a clinical cancer geneticist to commence with SDHD mutation analysis. Another important clinical point from these two studies is that it is probably not necessary to examine SDHC for mutations when faced with either individuals or families with pheochromocytoma or paraganglioma.

Given the available data, it would appear that individuals or families with pheochromocytoma, or at least presenting with pheochromocytoma, are over-represented by germline mutations in the 5′ portion of SDHD (Fig. 1). In contrast, head and neck paragangliomas appear to have a mutational spectrum favouring the 3′ portion of the gene. While the number of unrelated probands with head and neck paragangliomas found to have SDHB mutations is relatively small, it would appear that those with pheochromocytomas have a mutation spectrum throughout exons 2–7, while those with head and neck tumours seem to favour the 5′ portion (exons 2 and 3) of SDHB (Fig. 1).

**PUTATIVE FUNCTION OF MITOCHONDRIAL COMPLEX II COMPONENTS AND PHEOCHROMOCYTOMA GENESIS**

Few functional studies specifically examining complex II component dysfunction and carcinogenesis have been performed given the relatively recent link between the two. Complex II is crucial for both the tricarboxylic acid cycle and the aerobic respiratory chains of mitochondria. As noted above, since the carotid body contains oxygen chemoreceptors, it has been suggested that chronic hypoxic stimulation could account for the high frequency of sporadic occurrence of glomus...
tumours in individuals who live at high altitude, and hence for the involvement of the SDHX proteins in the pathogenesis of hereditary paragangliomas and (by extrapolation) phaeochromocytomas (7,51,52).

It is believed that mitochondria can act as oxygen sensors by generating reactive oxygen species required for HIF-1 DNA-binding activity with subsequent increased transcription of VEGF and glycylcotic enzymes (64). Based on these data, Gimenez-Roqueplo and colleagues (65) examined the genetic, expression and functional status of SDHD in a phaeochromocytoma originating from a family with germline SDHD R22X mutation compared with eight sporadic phaeochromocytomas, the latter presumably without somatic or occult germine SDHD mutations. The phaeochromocytoma from the hereditary case exhibited loss of the remaining wild-type allele. Further, this phaeochromocytoma was shown to have complete yet selective abrogation of complex II electron transfer activity, while the sporadic phaeochromocytomas retained full activity. Levels of HIF-1α, HIF-2α/EPAS1, VEGF and its receptor VEGF-R1 were shown to be increased in the SDHD-related phaeochromocytoma.

Despite these data and the link between oxygen sensing and regulation of HIF-1α, it is not clear that this link can explain how dysfunction of SDHB and SDHC can lead to phaeochromocytoma and paraganglioma genesis. In this context, it should be noted that the germline mutations in VHL that predispose families to phaeochromocytoma in particular do not impair HIF-1α ubiquitylation (29,35). Therefore, another plausible explanation for the association of SDHX mutation and neoplasia is the role of mitochondria in apoptosis. In other words, failure of apoptosis in neuroendocrine progenitor cells could result in the development of phaeochromocytoma and paraganglioma (reviewed in 66). This hypothesis, however, has not yet been directly tested in the context of paraganglioma.

**SOMATIC GENETICS OF PHAEOCHROMOCYTOMA**

Human cancer genetics provides many examples of how the identification of a rare inherited cancer gene has provided insights into the pathogenesis of sporadic cases. Thus, somatic inactivation of the VHL tumour suppressor gene occurs in most sporadic clear cell RCC, and somatic RET mutations are common in sporadic medullary thyroid cancer (42,67–71). In VHL-associated RCC, haemangioiblastomas and phaeochromocytoma, the remaining wild-type VHL allele is inactivated by somatic mutation, methylation or deletion (72). Therefore, VHL conforms to the classical tumour suppressor gene model with two somatic hits in sporadic tumours. However, somatic intragenic VHL mutations are infrequent (<5%) in sporadic phaeochromocytoma, and promoter methylation has not been detected (70,73; E.R. Maher et al., unpublished observations). Similarly, somatic RET mutations are found in only ~10% of sporadic phaeochromocytomas, suggesting that VHL and RET are relatively minor players in the pathogenesis of sporadic phaeochromocytoma (70,73). Furthermore, mutation analysis of SDHD in a total of 91 sporadic phaeochromocytomas and of SDHB in 24 sporadic phaeochromocytoma revealed only a single somatic SDHD mutation (8–10,74,75). Although epigenetic inactivation has not been excluded, it would appear that intragenic somatic SDHB and SDHD mutations do not play a major role in the pathogenesis of sporadic phaeochromocytoma. Why RET, VHL, SDHB and SDHD mutations should be a frequent cause of hereditary phaeochromocytoma, while somatic mutations are rare in sporadic tumours, is unclear, but possibly mutations in these genes only promote tumorigenesis if they are present at a specific stage of cell development.

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