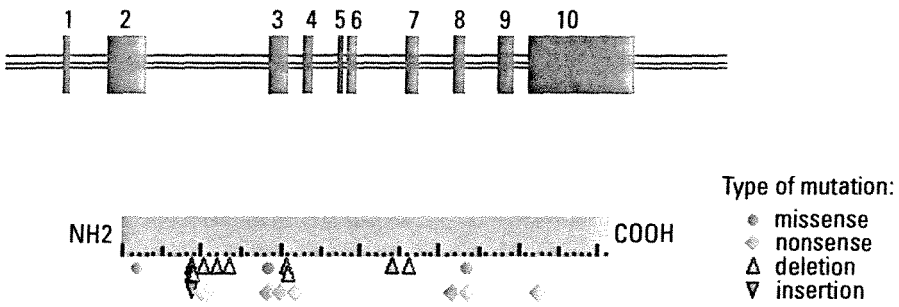

Fig. 5.17 MEN 1. Structure of the *MEN1* gene and examples of germline mutations spread over the coding sequence. From I. Lemmens et al. {1272}.

total or total parathyroidectomy should reduce the risks of thymic carcinoid. Bronchial carcinoids are less common in MEN 1 and occur predominantly in women {1560}. Most bronchial tumours in MEN 1 are typical carcinoids and treatment requires curative resection with a risk of locoregional failure.

Gastric ECLomas

ECLomas are presumed to originate from proliferation of enterochromaffin-like (ECL) cells in gastric mucosa and such tumours are mainly recognized during gastric endoscopy for Zollinger-Ellison syndrome (ZES) in MEN 1 {228}. These tumours are often small and multiple and they may be observed in about 10% of MEN 1 patients. ECLoma may be found in antro-pyloric and fundus mucosa and may be induced both by hypergastrinaemic conditions and genetic predisposition to MEN 1 {227}. Prognosis of ECLomas in patients with ZES-MEN 1 is good. Metastases are rare and tumour-related deaths are exceptional. ECLomas measuring less than 1 cm should be treated by endoscopic polypectomy and survey {274}. Considering the good prognosis of these tumours, aggressive surgery could be limited to selected patients.

Central nervous system tumours

Spinal ependymomas have been observed in rare MEN 1 patients and localize mostly in intratentorial cervical or lumbar regions {723,1049}. They are rapidly symptomatic and need surgery. One epidemiological study of a large series of MEN 1 patients has assessed that uncommon forms of meningioma and astrocytoma may occur in the context of genetic predisposition to MEN 1 {277}.

Soft tissue tumours

Oesophageal leiomyoma and renal angiomyolipoma have been described in rare MEN 1 cases {1466,2333}. Malignant gastrointestinal stromal tumour (GIST) represents an atypical presentation of MEN 1 syndrome but has been considered as non-fortuitous {1680}.

Genetics of MEN1

Chromosomal location

The *MEN1* gene (GenBank acc.no. U93237) is localized on chromosome 11q13 {1234}. This has mainly been shown by deletion mapping in tumours from MEN 1 patients {272}. Most tumours in MEN 1 affected patients, including less common lesions such as thoracic and gastric carcinoids, ECLomas and cutaneous tumours, show somatic loss of the wild-type allele (loss of heterozygosity (LOH) at 11q13 {558}. This observation is consistent with the fact that *MEN1* is a tumour suppressor gene with most pathogenic mutations corresponding to a loss of function.

Gene structure

The *MEN1* gene consists of 10 exons, spanning ~9 kb of genomic sequence, and encoding a protein of 610 aminoacids, menin {351,1272}. The first exon is noncoding and constitutes most of the 111 nt 5'-UTR. The sequence around the start codon (gccATGg) of the 610-amino acid open reading frame (ORF) is identical to the "Kozak consensus". The 797 nt 3' UTR has an unusual polyA signal (AATACA) located at -13. The exon sizes range from 41 bp to 1296 bp, and the introns range in size from 79nt to 1,563nt. Menin does not reveal homologies to any other known proteins. The only motifs which have been recognised in the menin sequence are two leucine zippers, and two nuclear localization sequences (NLS) in the carboxyterminal part of the protein {791}. Menin has orthologs not only in mouse and rat, but also in zebrafish and drosophila (98%, 97%, 75%, and 47% homology, respectively), but there is no homologue known in the yeast Saccharomyces cerevisae {792,1072, 2130}.

Gene expression

The *MEN1* transcript is 2.9 kb (GenBank acc.no. U93236) in all tissues, with an additional 4.2 kb transcript also being present in the pancreas and thymus {1272}. Western blot analysis showed strong expression of menin as a 68 kDa protein in all types of human cell lines and tissues tested, and mostly in brain cortex, kidney, pituitary, testis, thymus, adrenal glands with lower or undetectable levels in pancreas, liver, lung and skin {2361}. Menin is a nuclear protein whose expression is cell cycle regulated {1020}. In all cell lines tested, menin is found both in the nucleus and the cytoplasm, but its localization depends on the phase of the cell-cycle: during interphase, menin localizes in the nucleus; during and immediately after cell division, it migrates in the cytoplasm {933}. Various transcripts of *MEN1* vary in the content of their 5'-untranslated region. All transcript variants display upstream exons correctly spliced to *MEN1* exon 2. Further identification of 5' promoting regions will be relevant to identify tissue-specific promotion and the promoter(s) of the menin minor 4,2 Kb transcript in pancreas and thymus {1071}. Mouse models of MEN 1 have produced via inactivation of the mouse *Men1* gene through homologous recombination (knock-out mice) {425}. Homozygous inactivation of the *Men1* gene is lethal early during embryogenesis. *Men1+/-* heterozygotes develop mostly hyperplastic pancreatic islets and small tumours from 9 months of age. Other tumours were also observed in these mice, e.g. parathyroid hyperplasia and adenoma, pituitary adenoma, and adrenocortical adenoma/carcinoma.

Gene function

Menin is supposed to play a role in control pathways of cell growth and differen-

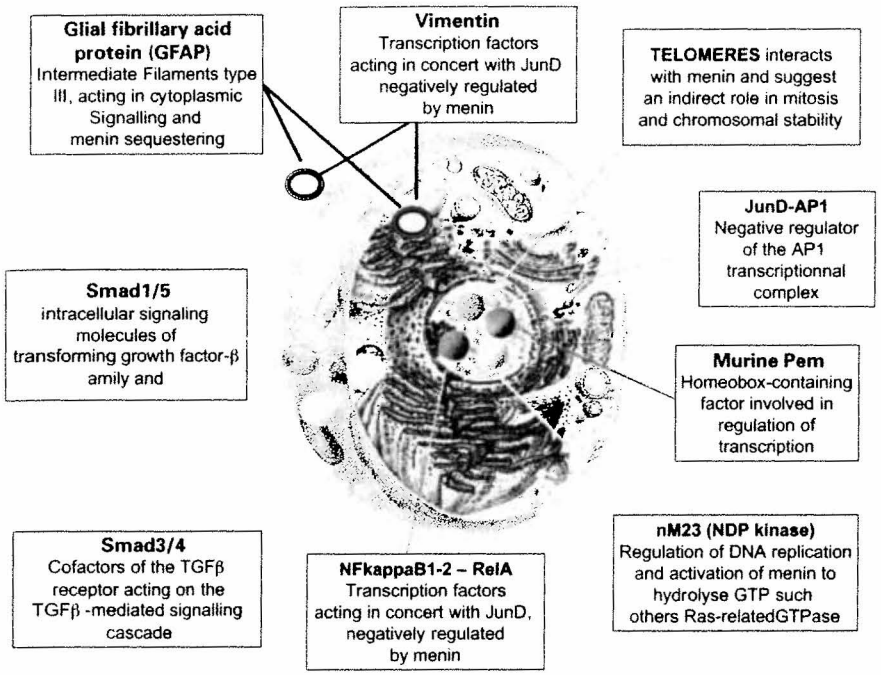

Fig. 5.18 MEN 1. Menin interacting proteins and putative function of the *MEN1* gene.

tiation during embryogenesis and postnatal life. To date, menin has been shown to interact with a subset of proteins involved in regulation of transcription, DNA replication, mitosis, apoptosis, genome integrity, growth factors signalling pathways and extracellular matrix organization. The first discovered was JunD, a transcription factor belonging to the AP1 transcription complex family {12}. Wild-type menin represses transcriptional activation mediated by JunD, may be via a histone deacetylase-dependent mechanism {733}. There may be an antagonistic action of Menin towards JunD with its tumour suppressive function. JunD has a reported effect in the inhibition of cell growth inside the AP1 complex. Menin represses the inducible activity of the c-fos promoter and inhibits Jun N-terminal kinase (JNK)-mediated phosphorylation of both JunD and c-Jun {686}. This occurs through two independent mechanisms uncoupling ERK and JNK activation from phosphorylation of their nuclear targets Elk-1, JunD and c-Jun, hence inhibiting accumulation of active Fos/Jun heterodimers. This provides molecular insights into the tumour suppressor function of menin and suggests a mechanism by which menin may interfere with Ras-dependent cell transformation and oncogenesis. Menin interacts directly with three members of the NF-KappaB family of transcription regulators, NF-KappaB1 (p50), NF-KappaB2 (p52), and RelA (p65) {868}. These proteins are known to play a central role in oncogenesis of various organs, as they modulate the expression of numerous genes. NF-KappaB and JunD cooperate – and interact directly - to activate transcription in rat hepatocytes {1789}. Menin interferes with the TGFß signaling pathway at the level of Smad3 {1021} and probably with Smad1 and Smad5 {2103}. The latter interactions have been implicated in menin-specific inactivation of the commitment of pluripotent mesenchymal stem cells to the osteoblast lineage. Through TGFβ pathways, menin is important for both early differentiation of osteoblasts and inhibition of their later differentiation, and it might be crucial for intramembranous ossification. Smad-mediated TGFß signaling and Ras phosphorylation pathway may be related and lead for instance to activation of AP-1 complexes in which JunD is a primary component. This may be a relevant core action of menin action which has been shown to share an intrinsic GTPase activity {2436}. Last in this series, the rodent protein Pem has been shown to bind Menin directly {1273}. Pem is a homeobox-containing protein, expressed mostly in testis, which plays a role in the regulation of transcription. However, Pem has no known homolog in the human genome. Menin may also be present in the cytoplasm and interact with two intermediate filaments proteins, glial fibrillary acidic protein (GFAP) and vimentin {1353}. These interactions suggest that intermediate filament network may serve as a cytoplasmic sequestering network for menin. The binding of menin to GFAP raises the issue of a putative role of this tumour suppressor in glial cell oncogenesis such as ependymoma. Interestingly, menin interacts with Nm23, a nucleoside diphosphate kinase ß isoform 1 which was first isolated as a metastasis suppressor {1639}. Nm23 associated to GFAP-containing intermediate filaments and enables menin to hydrolyze GTP, hence linking menin to Ras-related GTPases. This suggest again atypical GTPase activity of Menin may play a central role through multiple factors in the cell with specific actions depending the cellular type and physiological context. Menin may play a role in synapse formation and plasticity during embryonical organization. Lastly, menin might control genome stability through interaction with Nm23 which isoform 1 is associated to the centrosomes in dividing cells. Centrosomes regulate chromosome integrity and orchestrate the formation of GFAP and vimentin containing filaments through protein phosphorylations regulated by GTPases. The direct or indirect role of menin in maintaining genome stability and DNA integrity has been assessed by numerous reports which show evidence that normal cells from MEN 1 patients present with an elevated level of chromosome alterations {975, 2251}. These aberrations might be related to the increase of premature centromere division observed in cell lines from patients with a heterozygous MEN 1 gene mutation when compared to normal controls {1906}. Recently, another molecular link between the MEN 1 pathogenic context and cellular DNA replication has been found through the demonstration that menin was found to interact with the 32-kDa subunit (RPA2) of replication protein A (RPA), a heterotrimeric protein required for DNA replication, recombination and repair {2161}. *In vitro* and *in vivo* biological assays have shown that stable overexpression of Menin partially suppresses the *RAS*-mediated tumour phe-

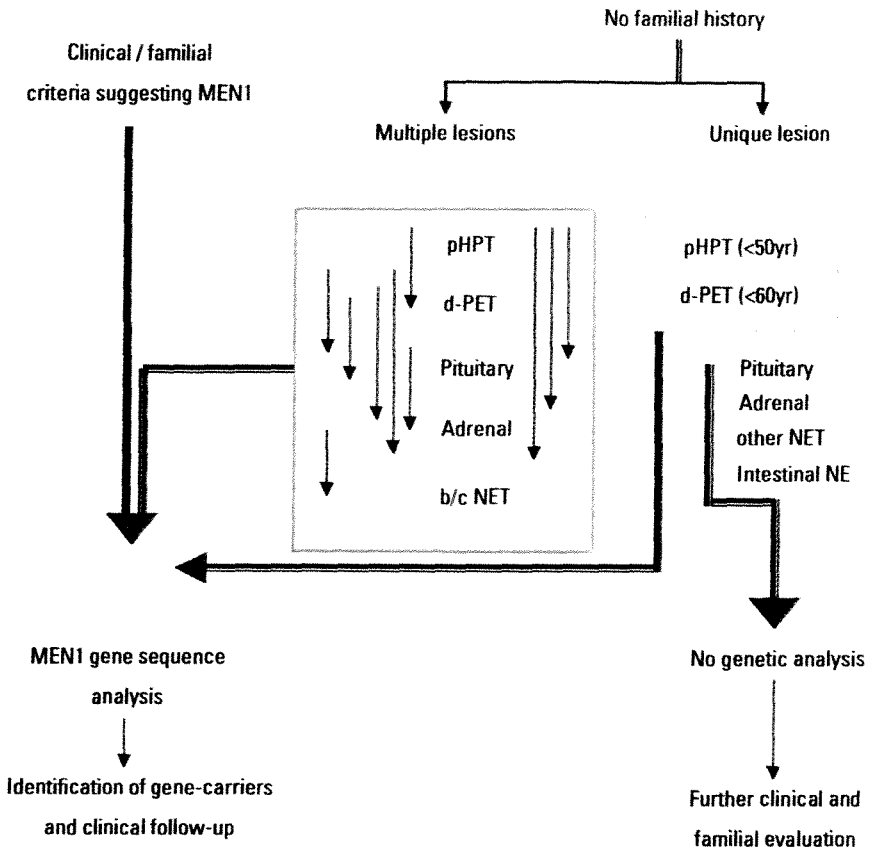

Fig. 5.19 MEN 1. A schematic view of *MEN1* gene testing in clinical practice.

notype directly supporting *MEN1* gene function as a tumour suppressor gene {1078}. In pancreatic islet cells, menin inhibits insulin promoter activity, hormonal secretion and cell proliferation through a mechanism which might involve suppression of AP-1 activation by menin either by direct inhibition on AP-1-mediated transcription and suppression of c-Fos induction {1954,2470}.

Mutation spectrum

Germline and somatic mutations in the *MEN1* gene do not appear to cluster in hot spots and are spread over the entire coding and intronic sequences {2362}. More than 400 different mutations have now been described. Approximatively 60% are truncating mutations, either frameshift (~40%) or nonsense (~20%) mutations, 20% are missense mutations, 10% are in frame deletions or insertions and about 10% are intronic and splice-site mutations. Large germline deletions encompassing the whole *MEN1* locus have also been shown {278,1096}. The frequencies of mutations detected in different studies vary, and range from 75-95% of the MEN 1 kindreds analyzed. Non-familial presentations of at least one or two MEN 1-related endocrine lesions have germline *MEN1* mutations in 5-10% of cases. Taken together, it has been shown that genetic analysis of the *MEN1* gene may be helpful in 8% of all-comers with primary hyperparathyroidism, in those younger than 50 years and in about 6% of subjects affected by duodenal and/or pancreatic endocrine tumours.

Genotype vs phenotype

Penetrance of MEN 1 at age 50 years is about 85% in gene-carriers. To date, there is no clear-cut genotype-phenotype correlation in MEN 1 patients {2362}. The clinical presentation, age of onset and natural history of the disease have been known to vary extensively even among members of the same family. The mutations spread throughout the 9 transcribed exons making any correlation study even more difficult. However, the findings of predominantly missense mutations in familial isolated hyperparathyroidism, especially in large families, may be the exception. For example, in two large autosomal dominant familial isolated hyperparathyroidism (FIHP) families, which are characterized by multiglandular disease (one with 7 affected and the other 14 affected), two distinct missense mutations in close proximity in exon 4, E255K and Q260P were identified {1047, 2206}. Nevertheless, even if specific point mutations might be related to mild phenotype, this has no accurate incidence in clinical follow-up of MEN 1 patients. In the same family, expressivity of MEN 1-related lesions is highly variable {322,723}. Specific mutations have been conversely related to uncommon expression of the disease, such as the Burin-variant of MEN 1, characterized by the absence of pancreatic tumours {1647}. Nevertheless, this correlation may be related to founder effects in related families living in the same region. More than 10% of the mutations arise de novo {130} and despite typical expression of the disease, about 5-10% of MEN 1 patients do not share germline mutations in the coding region or intronic borders of the *MEN1* gene, suggesting that some of the mutations occur in unknown parts or regulatory regions of the *MEN1* gene.

Genetic counselling and preventive measures

Genetic counselling

MEN 1 is inherited in an autosomal dominant manner with an age related penetrance and variable expression. Clinical primary hyperparathyroidism is present in at least 50% of the patients by age 20 years {130,1989}. Penetrance is more than 80% by age 50 years, although blood and urine tests could detect 90% by this age {130,322,1421}. Most individuals with MEN 1 will have an affected parent (90%) although onset of symptoms can be quite variable, even within the same family {350,1421}. DNA-based testing is recommended for index patients and their relatives to establish the diagnosis by molecular means and for medical management rather than to determine major prophylactic interventions {262,350}. DNA-based diagnostic

testing is useful for individuals and families who have some of the components of MEN 1 but are not classic {319,1423}. The appropriate age for offering *MEN1* genetic testing remains controversial, however, given the significant early morbidity that can occur, testing of at-risk children should be strongly considered {241,1308}. Prenatal testing is possible when a *MEN1* mutation has been identified in an affected family member.

Preventive measures

Although early pre-symptomatic biochemical screening for MEN 1 does significantly improve diagnosis, decrease morbidity, and is considered standard of care by most practising clinical cancer geneticists, there is no general consensus as to what management protocol is most beneficial and cost-effective. There is agreement that regular biochemical screening for high-risk individuals should be every 6-12 months, selected imaging studies less often (every 3-5 years), and this should continue for life {241,758, 1421,2358}.

Screening for primary hyperparathyroidism

Blood tests measuring ionized calcium and intact parathyroid hormone (iPTH) levels should be done at 6-12 month intervals, beginning by 8-10 years of age. Elevated ionized calcium and/or elevated parathyroid hormone levels confirm the presence of hyperparathyroidism, at which point surgical resection should be discussed. As parathyroid tumours are multiple in MEN 1 some groups practice complete parathyroidectomy with fresh parathyroid auto-transplantation to the forearm, or cryopreservation of a portion of a parathyroid gland. Others try to leave a parathyroid remnant although the possibility of recurrence remains high. All agree that transcervical thymectomy should be done as part of the initial parathyroidectomy.

Screening for islet cell tumour

Although primary hyperparathyroidism is the usual first presenting sign, this is not always so. Moreover, MEN 1-related islet cell tumours typically present with symptoms of hormone release rather than bulk disease. Thus annual pre-symptomatic screening should include, at a minimum, fasting and secretin-stimulated gastrin levels beginning at age 20. Many practitioners also do fasting glucose, insulin and glucagon, as well as albumin, prolactin and chromogranin-A. Given that one fourth of tumours are non-functional, abdominal imaging studies (CT, MRI or Octreotide scan) should be done every 3-5 years. In general, surgery in MEN 1 is indicated for most symptomatic MEN 1-related islet cell tumours, as these are usually benign. The exception is gastrinoma, which in MEN 1 is usually multiple and/or metastatic, and the role of surgical versus non-surgical management remains controversial.

Screening for pituitary tumour

The management of pituitary tumour in MEN 1 involves annual screening for fasting prolactin levels (PRL) although some advocate monitoring insulin-like growth factor (IGF-1) as well. Most begin regular biochemical screening by age 8-10; some wait until early adulthood. CT scan or gadolinium-enhanced MRI of the pituitary gland is usually not routinely done in the United States, but some physicians will do them every 3 years.

Other screening

Gastroduodenal, thymic and bronchial carcinoid tumours can occur in patients with MEN 1, and are usually more aggressive than sporadic cases. Thus some groups have advocated baseline CT or MRI scan with follow-up imaging studies every 3 years.

Hyperparathyroidism-Jaw tumour syndrome

B.T. Teh
K.M. Sweet
C.D. Morrison

Definition
Hyperparathyroidism-jaw tumour syndrome (HPT-JT) is an autosomal dominant disorder characterised by parathyroid adenoma or carcinoma, fibro-osseous lesions (ossifying fibroma) of the mandible and maxilla, and renal cysts and tumours.

MIM No. 145001

Synonyms
Familial isolated hyperparathyoridism; familial cystic parathyroid adenomatosis

Incidence or prevalence
It is a relatively recently described entity and its incidence or prevalence is unknown.

Diagnostic criteria
Unlike the MEN 1 patients who invariably develop multiglandular disease, the HPT-JT patients present with hereditary solitary (occasionally double) adenoma or carcinoma. The latter is a rare entity and is not associated with other forms of hereditary endocrine neoplasia syndromes and should lead to strong suspicion of this syndrome. About 30% of patients also develop fibro-osseous lesions, primarily in the mandible and maxilla. Kidney lesions have been reported including bilateral cysts, renal adenoma, hamartomas and papillary renal cell carcinoma. It is important to be aware that in some families, only parathyroid lesions are present. As more families are currently being tested genetically, it is expected that the incidence and spectrum of its associated clinical features will be better known in due course.

Hyperparathyroidism

Age distribution/penetrance
About 80% of patients present with hyperparathyroidism, that may develop in late adolescence, similar to the presentation in MEN 1. There is a reduced penetrance in females {2207}, and parathyroid carcinoma occurs in approximately 10-15% of affected individuals {319}.

Clinical features
Compared with MEN 1-related hyperparathyroidism, HTP-JT syndrome appears to run a more aggressive course: the patients tend to have more severe hypercalcemia and some actually present with hypercalcemic crisis. In addition, there appears to be a much higher incidence of parathyroid carcinoma than in other endocrine related disorders.

Pathology
Primary hyperparathyroidism in HPT-JT syndrome is more often one or two gland involvement (adenoma or double adenoma) that may or may not present synchronously {985}. One unique feature of the parathyroid neoplasia is the high incidence of cystic change {1396}, but such changes also occur in sporadic parathyroid adenoma or hyperplasia. The diagnosis of parathyroid carcinoma remains a challenge, and the only indisputable proof for parathyroid malignancy is extensive local invasion and/or metastasis. The finding of parathyroid carcinoma in the small number of reported families with HPT-JT syndrome is significant considering the rarity of parathyroid carcinoma in sporadic parathyroid tumours.

Prognosis and prognostic factors
The majority patients with adenoma can be cured by surgery and recurrence is not as common as in MEN 1 patients. Prognosis is guarded once parathyroid carcinoma is confirmed, but it is unclear whether the prognosis is any different from sporadic parathyroid carcinoma.

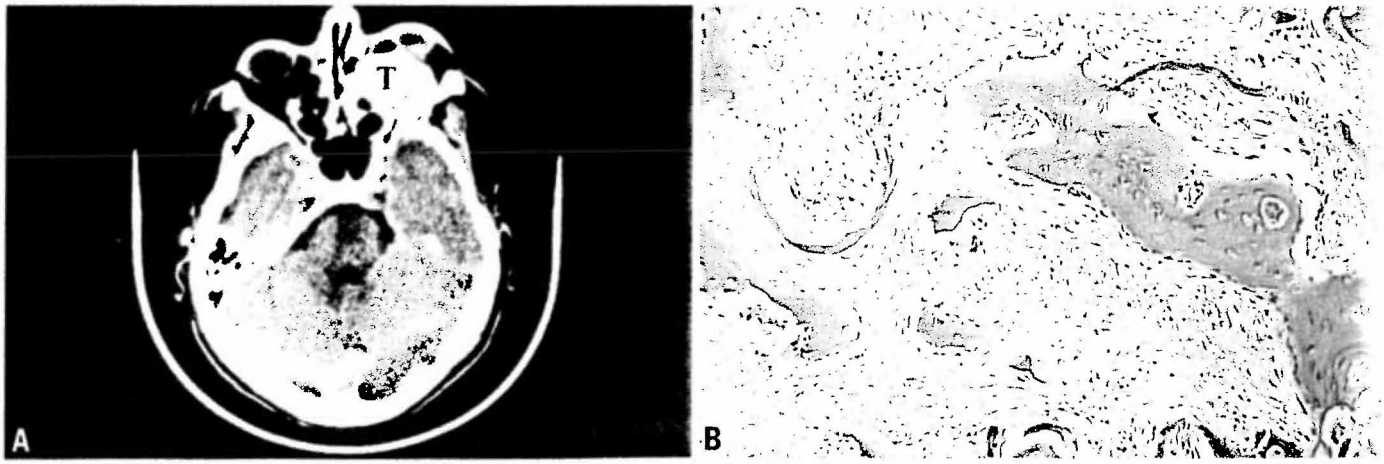

Fig. 5.20 Hyperparathyroidism - jaw tumour syndrome (HPT-JT). **A** CT showing a well demarcated fibroosseus lesion in the maxillary antrum. **B** Typical jaw lesion from a patient with HPT-JT syndrome with a dense, relatively avascular fibroblast-rich stroma and irregular spicules of woven bone, some of which show at least a focal rim of osteoblasts.

Jaw tumours

Clinical features

Some cases are fast growing while some are insidious and slow growing. Radiographic features generally show a well-demarcated osseous lesion of the mandible or maxilla.

Pathology

The usual case is described as having a relatively avascular cellular fibroblast-rich stroma, sometimes with a storiform pattern, admixed with bone trabeculae and/or cementum-like spherules. Some of the bone trabeculae generally show at least focal osteoblastic rimming. It is distinct histologically from the classical bone lesion of hyperparathyroidism, *osteitis fibrosa cystica*, which tends to resolve slowly after correction of the hyperparathyroidism, albeit over a course of months to years {1993}. In contrast, the fibroosseous lesions of HPT-JT do not resolve with correction of the hyperparathyroidism. While most of these lesions involve either the mandible or maxilla of one side, bilateral or multifocal lesions have been described {331, 2354}.

Prognosis and prognostic factors

While none of the described jaw tumours have behaved in a malignant fashion, some have recurred {1864,2207} although it is difficult to discern if this is due to incomplete initial resection or development of a new lesion.

Other features

In addition to cystic adenomatosis and jaw lesions, a wide spectrum of tumours has been associated with HPT-JT syndrome but most notable is the association of various renal lesions, which occurs in approximately 20% of patients {319,1022,2166,2207}. In the two families reported, 5 individuals in one kindred and 2 in the other had renal lesions. In the latter kindred, polycystic kidney disease was the predominant finding, while in the other kindred in addition to renal cysts there were several individuals with renal hamartomas described as cystic tumours with mesenchymal, blastemal, and epithelial elements. Another renal tumour that has been reported with HPT-JT syndrome is Wilms tumour {1022, 2166}, which has been reported in three separate individuals from three separate families.

Table 5.04
Tumours and cysts reported in association with HPT-JT syndrome.

Lesions	Reference
Renal cysts, polycystic kidney disease, renal hamartoma	{2207}
Wilms tumour	{1022,2166}
Renal cysts	{331}
Renal cysts, papillary renal cell carcinoma, multiple renal cortical adenomas, Hurthle cell adenoma, clear cell pancreatic adenocarcinoma	{845}
Adenomyomatous polyps of endometrium	{669}
Papillary thyroid carcinoma, uterine leiomyoma	{965}
Cellular neurofibroma	{1396}

Genetics

Chromosomal location

The *HRPT2* gene is mapped to 1q25-q31 {2166}.

Gene structure

The *HRPT2* gene contains 17 exons spanning 18.5 kb of genomic distance and predicted to express a 2.7kb transcript. It encodes a protein of 531 amino acids {319}.

Gene expression

The gene is ubiquitously expressed including kidney, heart, liver, pancreas, skeletal muscles, brain and lung {319}.

Gene function

The function remains unknown although the gene has moderate (32%) identity to a yeast protein Cdc73p, which is an accessory factor associated with an alternative RNA polymerase II {319}.

Mutation spectrum

The vast majority of mutations are frameshift and nonsense loss-of-function mutations found in several exons. The most common exon involved is exon 1 {319}.

Genotype vs phenotype

To date, it remains unknown if there is a genotype-phenotype correlation.

Genetic counseling

When HPT-JT is suspected in a family, DNA-based testing is recommended for index patients and their relatives to establish the diagnosis and for medical management. As the *HRPT2* gene was recently identified, testing for each of the complex syndromes associated with hereditary primary hyperparathyroidism has become possible {319}.

Screening for primary hyperparathyroidism

Annual blood tests measuring ionized calcium and intact parathyroid hormone (iPTH) levels should begin by 15 years of age. Surgical intervention should occur once serum levels confirm the presence of HPT. Parathyroid disease in HPT-JT is typically represented by asynchronous adenomas although the potential for malignancy needs to be considered {2055,2356}. While some groups advocate removal only of the enlarged parathyroid gland with continued regular monitoring {1423,2055}, the alternative approach would be complete parathyroidectomy with fresh parathyroid autotransplantation to the forearm (or sternocleidomastoid), or cryopreservation of a portion of a parathyroid gland.

Screening for jaw manifestations

Orthopentography of the jaw every three years.

Screening for renal manifestations

At-risk individuals should receive annual abdominal ultrasound or CT scan with and without contrast at least every other year to screen for polycystic disease, Wilms tumour or carcinoma, and renal hamartomas {1993}.

Von Hippel-Lindau syndrome (VHL)

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H.P.H. Neumann
K.H. Pla
T. Bohlin
K. Schneid

Definition

Von Hippel-Lindau (VHL) disease is a dominantly inherited familial cancer syndrome caused by germline mutations in the *VHL* tumour suppressor gene. VHL disease demonstrates marked phenotypic variability and age-dependent penetrance. The most frequent tumours are retinal and central nervous system haemangioblastomas, renal cell carcinoma, phaeochromocytoma and pancreatic endocrine tumours. Families with VHL disease may be subdivided according to clinical phenotype, and this subdivision forms the basis for genotype-phenotype correlations (see later).

MIM No. 193300 {1862}

Synonyms

Von Hippel-Lindau syndrome

Incidence/prevalence

VHL disease was estimated to have an incidence of 1/36000 live births in Eastern England {1383} and a prevalence of 1/39000 in South-West Germany {1590}.

Diagnostic criteria

If there is a confirmed family history of VHL disease, a clinical diagnosis can be made in a relative with a single typical VHL tumour (e.g. retinal or central nervous system haemangioblastoma, clear cell renal cell carcinoma, phaeochromocytoma, pancreatic endocrine tumour or endolymphatic sac tumour). In isolated cases without a family history, two tumours (e.g. two haemangioblastomas or a haemangioblastoma and a visceral tumour) are required for the diagnosis. Genetic studies allow a molecular-based diagnosis of VHL in atypical cases and early diagnosis in patients who do not satisfy clinical diagnostic criteria.

Phaeochromocytoma

Age distribution and penetrance

The association of phaeochromocytoma with VHL is strongly dependant upon genotype (Table 5.06). More than 95% of patients with truncating or null mutations have VHL type 1 (low risk of phaeochromocytoma) {358,433} Patients with VHL type 2 (high risk of phaeochromocytoma) have primarily missense mutations. The penetrance of phaeochromocytoma in those with missense mutations of VHL is high: one large series estimated a 59% risk by age 50 years {1381}. Risks for specific missense mutations vary, with risks of 82% for type 2B codon 167 mutations and 50% for the "Black Forest" c.505 type 2A mutation at 50 years being reported {163,1381}. In series of VHL patients with phaeochromocytomas, the age range of diagnosis is from 5-64 years, starting notably younger and with a younger average at diagnosis than in other hereditary syndromes w phaeochromocytoma (MEN 2, SDH SDHB) {119,244,565,962,1585,15 2291,2348}.

Clinical features

Patients with VHL and phaeochromoc toma commonly have multi-focal d ease, both adrenal and extra-adren Extra-adrenal disease has been parti larly associated with a mutation nucleotide 505 {2348}. As noted abo early age at diagnosis of phaeochron cytoma is a predominant feature, how er, there is some genotype-phenoty correlation with individuals with mu tions at nucleotides 595 and 695 p senting with phaeochromocytomas a younger age than those with other mu tions ($p<0.025$) {2348}. As mutations *VHL* can lead to phaeochromocyto alone, it is difficult to define the freque cy of phaeochromocytoma as the f sign of VHL. However, depending on mode of ascertainment, *VHL* mutatio have been identified from 2-50% of patients with sporadic or isola phaeochromocytoma in hospital bas series {119,244,565,1587,2291}. In recent study of 271 sporadic phaeoch mocytomas in a population-based s ries, mutations in VHL were found in 1 of cases and accounted for almost ha the germline mutations identified (30/ {1585}. The biochemical findings fou in phaeochromocytomas due to V

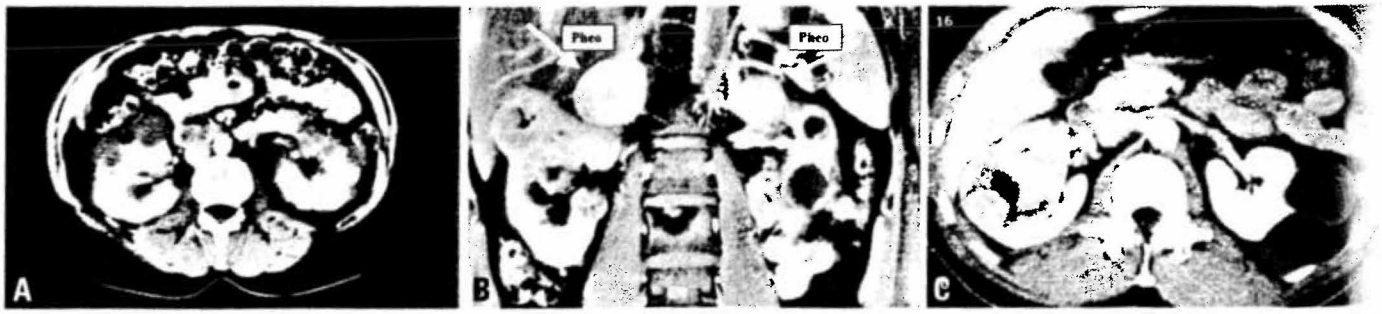

Fig. 5.21 von Hippel-Lindau syndrome. **A** Multiple bilateral renal cysts without tumours in a patient with Von Hippel-Lindau disease. **B** Bilateral multiple renal cys and multiple renal carcinomas and bilateral adrenal phaechromocytomas (arrow and "Pheo") in a 36 year old patient with Von Hippel-Lindau disease; initially t diagnosis was missed and the adrenal tumours summarized among the tumours originating from the kidneys. **C** A 40 year old patient with Von Hippel-Lindau d ease. Note a huge tumour of the right kidney and cysts of the left kidney. The extent of the tumour made nephrectomy necessary.

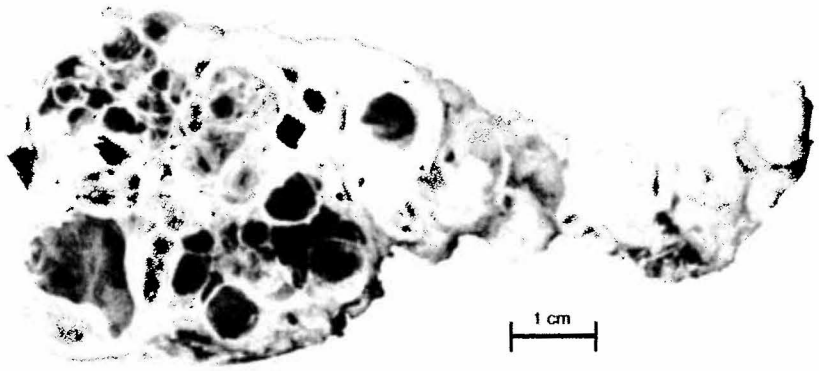

Fig. 5.22 Pancreatic cysts in a patient with von Hippel-Lindau syndrome (VHL).

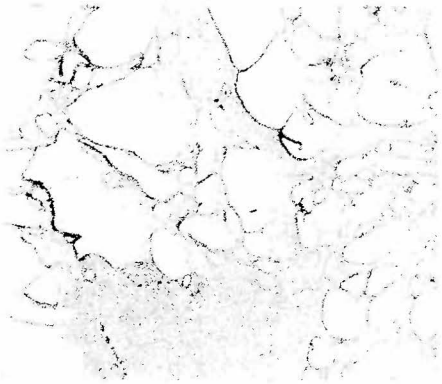

Fig. 5.23 Microscopic aspect of a microcystic adenoma of the pancreas in a VHL patient.

have been elucidated recently. Phaeochromocytomas associated with VHL are noradrenergic due to decreased expression of phenylethanolamine-N-methyltransferase (PNMT) in the tumours, which converts norepinephrine to epinephrine [551]. As a result, patients with phaeochromocytoma due to VHL usually demonstrate an increase only in normetanephrines, reflected in both plasma and urinary measurements.

Pathology

Patients of type 2 families [258] often present with multiple, bilateral phaeochromocytomas, which can be accompanied by extra adrenal chromaffin tumours and parasympathetic paragangliomas [902,937,1564,2480]. A study of 14 phaeochromocytomas in eight patients with VHL disease demonstrated that VHL-associated phaeochromocytomas have a distinct histologic phenotype as compared with phaeochromocytomas in patients with MEN 2. VHL tumours are characterised by a thick vascular tumour capsule; myxoid and hyalinized stroma; round, small to medium tumour cells intermixed with small vessels; cells with predominantly amphophilic and clear cytoplasm; absence of cytoplasmic hyaline globules; and lack of nuclear atypia or mitoses. In contrast to MEN 2 [483], there is frequently no extratumoural adrenomedullary hyperplasia in the VHL adrenal gland [1121]. Another study on 30 VHL-associated phaeochromocytomas revealed lower total tissue contents of catecholamines and expression of TH as well as negligible stores of epinephrine and expression of PNMT when compared to phaeochromocytomas MEN 2 patients [551]. Occasional tumours with melanin-like pigment or lipid degeneration have also been described in VHL patients [1223,1791]. A study has shown that immunohistochemistry is not helpful for the discrimination of VHL-related from sporadic tumours since both tumour types demonstrated positive staining for the VHL protein, suggesting that the antibody also recognizes the mutated VHL protein [1354].

As in sporadic and other familial forms of phaeochromocytomas, malignant transformation may also occur in VHL-associated tumours [1026,1543,1777].

Prognosis and prognostic factors

Phaeochromocytomas associated with VHL are frequently asymptomatic; studies have reported symptoms in 16-30% of patients [551,2348]. While the low frequency of symptoms may in part be because many tumours are detected on routine screening, the frequency is still lower than that in other comparable syndromes, such as MEN 2. The prognosis associated with phaeochromocytoma is quite good, with the rate of malignancy less frequent (<5%) than in sporadic disease [2348]. In one study, 12 VHL patients with 17 adrenal masses were followed for a median of 35 months with no morbidity [2346]. Laparoscopic partial adrenalectomy has been advocated as a means to preserve adrenal function. Partial adrenalectomy has been reported to have similar clinical results to complete adrenalectomy with less morbidity [2346]. Due to the high rate of phaeochromocytoma in patients with VHL type 2, many patients develop contralateral phaeochromocytomas or phaeochromocytoma in remaining adrenal tissue several years after their initial surgery [2348]. However, metastatic disease has not been reported after partial adrenalectomy for phaeochromocytoma [2346].

Renal cell carcinoma

Clinical features

In cross-sectional studies, the frequency of renal cell carcinoma (RCC) in VHL disease is ~35%, although the risk of RCC in type 1 kindreds and type 2B kindreds

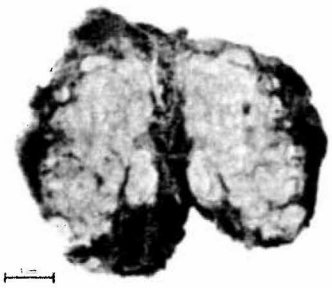

Fig. 5.24 Macroscopic aspect of a clear-cell pancreatic endocrine tumour in a VHL patient.

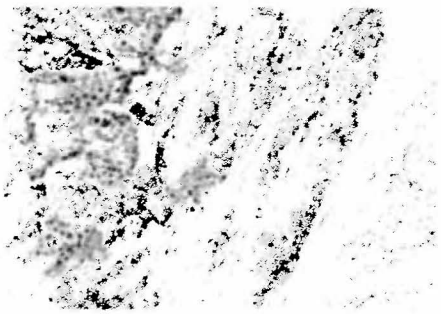

Fig. 5.25 Fat staining of a clear-cell pancreatic endocrine tumour in a VHL patient.

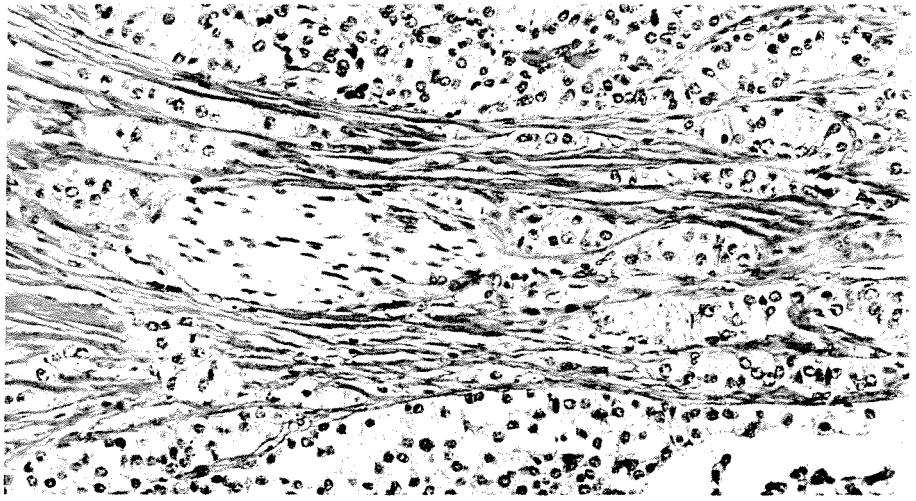

Fig. 5.26 Pancreatic endocrine tumour with clear cell features in a VHL patient.

approaches 70% at age 60 years {1380}. Men and women are equally affected. Mean age of clinical manifestation is 35-45 years. Asymptomatic small RCC have been detected as early as 16 years of age {1061}. Thus VHL-associated RCC is different from sporadic RCC, which is more frequent in males, and detected, on average, about 15 years later than in VHL disease {357,1586}. Similar to patients with sporadic RCC, VHL-associated RCC may become symptomatic with gross hematuria or metastases, but the majority of these tumours are now detected in an asymptomatic stage by renal imaging in VHL patients and at-risk individuals. Renal function is normal in patients with VHL-associated RCC. If one kidney has been already removed, there may be mild impairment of renal function. Acute renal failure can occur in a remnant kidney after hematuria and pelvic clotting. RCC can be the first manifestation of VHL, although this occurs in <20% of cases.

Pathology

VHL-associated RCC is always a clear cell carcinoma {1589}. Sporadic and VHL-associated clear cell tumours are often indistinguishable but certain criteria are frequently observed in VHL.

Macroscopy

VHL-associated RCC can occur as a single carcinoma of a kidney, but multifocal tumours are more frequently observed. Typically, both tiny and large tumours are found. Most tumours are surrounded by a marked pseudocapsule. The tumours are often heterogenous; they comprise cystic areas of serous liquid or blood. In addition to solid tumours, renal cysts are a typical component of VHL-involvement of the kidney {1586}. Both kidneys are usually affected, but the number and size of tumours and cysts differ. Most of these characteristics can be demonstrated in a surgical specimen. However, preoperatively, this can be demonstrated also by CT scanning or MR imaging {379}.

Histology

The dominant finding by conventional haematoxylin and eosin staining is a tumour composed of cells with clear cytoplasm and small nuclei. Most tumours can be classified as grade 1, sometimes, in part, as grade 2, but rarely, as grade 3. Typical is a microcystic growth pattern. The cystic structures contain liquid or blood. Endothelial or epithelial cells are mostly absent. Fibrotic stroma can show incorporation of iron. Necrotic tumour areas are rare. Most tumours have a marked pseudocapsule {1586}. Renal cysts occurring in patients with VHL may contain small foci with atypical epithelial cells or even incipient carcinomas {1765}. By long-term observation, however, most cysts do not transform into cancer. Similar to sporadic RCC, in VHL, RCC tumour invasion into the veins can be observed. This may be evident in small veins of the renal parenchyma or branches of the renal vein. Apparently normal kidney may contain many microfoci of RCC on microscopic examination {2347}.

Histologically, VHL-associated RCC and haemangioblastoma of the CNS can appear with a remarkably similar pattern. In fact, such CNS tumours have been interpreted as metastases of RCCs. Spinal tumours may produce similar diagnostic difficulties. Immunohistochemical staining may be helpful but inconclusive results may be obtained in some instances. It is important to be aware of such problems {1588}.

Prognosis and prognostic factors

Differential diagnosis

Tumourous and cystic involvement of the kidneys can lead to misinterpretations. Tuberous sclerosis complex and autosomal dominant polycystic kidney disease should be excluded. Clinical presentation, radiology and pathology can contribute to the differential diagnosis.

Prognosis and prognostic factors

RCC is the only frequent lesion in VHL that truly constitutes a carcinoma. However, as the diagnosis and treatment of CNS tumours have improved with MR imaging and microsurgical techniques, RCC has emerged as an important cause of morbidity and mortality {1380}. Nevertheless, growth of VHL-associated RCC seems to be slower compared to sporadic RCC. Metastases are associated with large tumours {1586}. One surgical series reported that 25% of VHL patients with a RCC >3 cm at surgery developed metastatic disease, but none with smaller tumours {2344}. Hence, it appears that tumours can be observed up to a diameter of 3 cm, and in many centres nephron sparing surgery is performed in when lesions reach 3 cm. When bilateral nephrectomy has been performed, kidney transplantation is a good option for patients with VHL {737}. So far, there are no clear data demonstrating that specific germline mutations correlate with the prognosis of VHL-associated RCC. However, mutations leading to truncation of the putative VHL protein may be associated with a more aggressive clinical course.

CNS haemangioblastomas

Clinical features

CNS haemangioblastomas are a cardinal feature in VHL disease. The lifetime risk of cerebellar haemangioblastoma is ~70% by age 60 years and symptomatic

spinal cord lesions occur in ~25% of patients [1383]. Supratentorial lesions are rare. Approximately 30% of all patients with cerebellar haemangioblastoma have VHL disease and the mean age at diagnosis of those with VHL disease is considerably younger than in sporadic cases [1382]. Patients with cerebellar haemangioblastomas typically present with symptoms of increased intracranial pressure and limb or truncal ataxia (depending on the location of the tumour).

Pathology

Macroscopically, capillary haemangioblastomas appear as well circumscribed, highly vascularised red tumour nodules, often within the wall of large cysts. Multiple haemangioblastomas occur in patients with VHL disease. This reflects numerous independent tumour sites and is not a sign of metastasis.

Histologically, capillary haemangioblastomas consist of large vacuolated stromal cells embedded in a rich capillary network. The distinction of cellular and reticular variants, based on the abundance of the stromal cells appears not to be of clinical significance.

Current evidence suggests that the stromal cells represent the neoplastic component of the tumour, whereas the capillary network forms as a consequence of aberrant gene expression in stromal cells [2150]. The stromal cell nuclei may vary in size, with occasional hyperchromatic nuclei. The typical "clear cell" morphology of capillary haemangioblastoma is based on numerous lipid-containing cytoplasmic vacuoles. This feature can sometimes lead to differential diagnostic problems between capillary haemangioblastoma and metastatic renal cell carcinoma. Immunohistochemistry for cytokeratins and EMA may facilitate the differential diagnosis, because stromal cells do not express epithelial marker proteins.

Ultrastructurally, the most prominent feature of the stromal cells is an abundant electron-lucent cytoplasm containing lipid droplets. Some studies have demonstrated electron-dense bodies, reminiscent of Weibel-Palade bodies, and small granules, reminiscent of neuroendocrine granules. The histogenetic origin of stromal cells is currently unclear. Sources of origin that have been proposed include microglia, histiocytes, neuroendocrine cells, endothelium, astrocytes, choroid plexus epithelium and haematopoietic cells [1167].

Stromal and capillary endothelial cells differ significantly in their antigen expression patterns. Stromal cells lack endothelial cell proteins, and no consistent antigen expression profile has been established for these cells. The expression of pVHL in stromal and not in endothelial cells, however, supports the notion that only the stromal cells are neoplastic [1354]. Stromal cells may express neuron-specific enolase, neural cell adhesion molecule, transthyretin, epidermal growth factor receptor and ezrin [198]. Vimentin is the major intermediate filament expressed by stromal cells [207].

The stromal cells express high levels of the hypoxia-inducible transcription factors (HIF) -1 and -2 [636]. Constitutive HIF expression in stromal cells is a consequence of increased stability of this protein. HIF-1 and -2 are rapidly degraded by an oxygen-dependent process involving several enzymes, transcription factors and the proteasomal complex [1166,1448]. Proteosomal degradation of HIF-1 and -2 is also dependent on functional pVHL, in stromal cells, the inactivated pVHL leads to HIF-1 and -2 accumulation. As a consequence, HIF target genes such as vascular endothelial growth and erythropoietin are upregulated on the transcriptional level, partly explaining the vascular and cystic phenotype of the tumours and the high incidence of erythocytosis in patients with VHL disease [1167].

The high expression of VEGF in stromal cells and of VEGF-receptors in both vascular endothelial cells and in stromal cells argues for paracrine and autocrine growth mechanisms of haemangioblastomas [207,844,2403].

In accordance with the highly vascular nature of capillary haemangioblastoma, intratumoural haemorrhage may occur. Cysts are common, but necrosis or calcification are usually absent. In adjacent reactive tissues, particularly the cyst walls, gliosis with Rosenthal fibres may occur. The tumour edge is generally well demarcated, and infiltration into surrounding neural tissues rarely occurs. Mitoses are rarely seen and the MIB-1 labelling index is below 1%.

Prognosis and prognostic factors

Haemangioblastomas are benign tumours and the results of surgery for single peripherally located cerebellar lesions are often excellent. However, the treatment of multicentric tumours, and particularly, brain stem and spinal tumours may be difficult, and significant morbidity may result. It is hoped that antiangiogenic therapy may offer a medical approach to the treatment of inoperable CNS and retinal haemangioblastomas and early clinical trials are in progress.

Retinal angiomas

Clinical features

Retinal angiomas are the most common presenting feature of VHL disease and are multiple and bilateral in many cases. Retinal angiomas (mean 1.85 lesions, range 0-15) are found in almost 70% of cases [2366]. Patient management is directed towards identifying asymptomatic angiomas as early treatment reduces the risk of visual loss. Approximately 15% of patients have an optic disc angioma. Intraretinal exudation is a common complication of retinal angioma, but may be transient.

Pathology

The benign vascular lesions characteristic of VHL disease are often referred to as "retinal angiomas", but their histopathological appearance is identical to CNS haemangioblastomas (see above).

Prognosis and prognostic factors

The cumulative risk of visual loss has been estimated as 35% in gene carriers and 55% in patients with retinal angiomas at age 50 years [2366]. Potentially sight-threatening complications such as exudation, retinal traction or hemorrhage tend, on average, to be associated with larger angiomas.

Pancreatic endocrine tumours and pancreatic cysts

Clinical features

Pancreatic cysts and tumours are both features of VHL disease. Multiple cysts are the most frequent pancreatic manifestation but are rarely of clinical significance and impairment of pancreatic function is uncommon. Pancreatic tumours occur in 5-10% of cases and are

usually non-secretory islet cell tumours. A high frequency of malignancies has been reported in VHL associated islet cell tumours. Surgery is indicated in tumours >3 cm while tumours <1 cm may be monitored {1295}.

Pathology

Endocrine pancreatic tumours

Approximately 5-10% of VHL patients develop endocrine pancreatic tumours, which may be multiple. The tumours are typically well circumscribed and vary in size from 0.4 to 8 cm (median 2 cm). They are usually confined to the pancreas and exhibit a tan, red/brown, gray or yellow colour.

Morphologically, the endocrine pancreatic tumours are characterised by solid, trabecular, and/or glandular architecture and prominent stromal collagen bands with no detectable amyloid on congo red stains. Sixty percent of the tumours reveal focal or general clear-cell cytology {896}; however, glycogen is detectable in a minority of cases. There may be marked focal nuclear atypia but mitoses seldom exceed 2 per 10 high-power fields. Immunohistochemically most tumours are positive for panneuroendocrine markers (chromogranin A and/or synaptophysin), cytokeratin and S-100; 35% demonstrate focal positivity for pancreatic polypeptide, somatostatin {1389, 1951}, insulin, and/or glucagon; and no immunostaining for pancreatic and gastrointestinal hormones is observed in the remaining 65% of tumours {1357}. Clinically, the majority of tumours are functionally inactive {811}. Dense core neurosecretory granules are evident by electron microscopic examination, and the clear cells additionally exhibit abundant intracytoplasmic lipid. By molecular analysis, tumours show allelic loss of the second copy of the VHL gene {1357}.

No nesidioblastosis-like proliferations or islet cell hyperplasia are seen in the non-neoplastic pancreatic tissue. Occasionally, VHL patients present with pancreatic masses, which are due to metastases of renal cell carcinoma {340,1125}.

Pancreatic cystic disease

Microcystic adenoma and benign serous cysts of the pancreas may occur in 35-75% of VHL patients {811}, usually with coexisting renal lesions. Virtually all affected individuals develop multiple lesions, which may grossly be seen as cysts and microcystic adenomas as conglomerate of cysts ranging from 0.5 to 18 cm or subtotally replacing the pancreatic parenchyma. A histopathological analysis of 21 cysts and 98 microcystic adenomas in nine VHL patients with a known germline mutation revealed 21 benign serous cysts, 63 microscopic microcystic adenomas (size <0.4 cm), and 35 macroscopic microcystic adenomas (size >0.5 cm) {1523}. The average number of lesions per patient was 2.1 benign cysts (range, 0-8), 7.7 (1-37) microscopic microcystic adenomas, and 3 (0-21) macroscopic microcystic adenomas. All cystic lesions show similar histology. They exhibit prominent fibrous stroma containing small vessels and smooth muscle cells. The epithelial lining of cysts consists of cells with clear and/or amphophilic cytoplasm, abundant intracytoplasmatic glycogen on PAS/PAS-D stain, absence of acidic mucin on mucicarmine stain and positive immunoreactivity for cytokeratin and MAK6.

VHL deletions were detected in all types of pancreatic cystic lesions providing direct molecular evidence of their neoplastic nature and integral association with VHL disease {1523}. Interestingly, *VHL* gene alterations may also be detected in some sporadic microcystic adenomas of the pancreas {2332}.

Other rarely encountered lesions in the pancreas of VHL patients include haemangioblastoma and ductal adenocarcinoma {810}.

Prognosis and prognostic factors

Endocrine pancreatic tumours are usually confined to the pancreas and exhibit a slow growth rate but metastatic tumours have been described {340,416,810, 1125,1357,1523,2332}. Metastasising tumours show a mean diameter of 5 cm. All types of cystic lesions are clinically benign and follow an indolent course. They have an excellent prognosis because malignant transformation is very rare.

Other component features

Papillary cystadenoma of the epididymis

Papillary cystadenoma of the epididymis account for <10% of benign epididymal tumours and an association with VHL disease is well recognised. Although often asymptomatic, they may present as an intrascrotal mass or be detected during investigations for infertility. A survey of 56 male patients with VHL disease revealed evidence of epididymal cystadenomas in 54% and two-thirds of these had bilateral lesions.

Histopathology

Epididymal papillary cystadenomas (ECs) are found in 54% of male VHL patients and 2/3 of all tumours are associated with VHL. They are unilateral in 33% and bilateral in 67% of cases and are mostly located in the head of the epididymis. They usually grow as 1.5 to 2.0 cm solid tan-brown masses with small cystic components {378}. Histologically, the tumours consist of solid cords of cells and dilated ducts outlined with papillae. The cells are cuboidal to low columnar with a ciliated surface and exhibit a glycogen-rich cytoplasm with secretory droplets.

Prognosis

Epididymal cystadenomas are benign and usually do not require treatment. Although no association between epididymal cysts and clinical subtype of VHL disease has been detected, one study {378} found a suggestive (p=0.06) correlation with truncating *VHL* gene mutations.

Endolymphatic sac tumours (ELST)

Endolymphatic sac tumours (ELST) have only been recognized as a specific component of VHL disease in the last decade. In a large survey of VHL patients using MRI and CT scans, Manski et al. {1402} found that 11% patients with VHL disease had an ELST. Hearing loss is the most common symptom of an ELST, but tinnitus and vertigo also occur in many cases. Mean age at onset of hearing loss was 22 years and in 62% of patients with ELSTs, hearing loss was the first manifestation of VHL. Therefore, endolymphatic sac tumours should be considered as a cause of hearing loss in VHL disease. Hearing loss is associated with larger tumours.

Histopathology

Endolymphatic sac tumours (ELST) are rare intracranial tumours originating from the pars rugosa of the endolymphatic sac, which can grow bilaterally in VHL patients and lead to hearing loss {2240}.

However, they also may occur sporadically. Histologically, the tumours exhibit papillary structures containing non-ciliated cuboidal or columnar cells occasionally with PAS positive globules. Glandular structures containing colloid like fluid may also be encountered. Mitoses and necrosis are rare. A thick fibrous stroma with numerous microvessels, haemorrhage and haemosiderin deposits is frequently seen.
The cells show a distinct expression of CKs (CAM 5.2, 34betaE-12, CK7, CK8 and CK19), but not for CK10/13 or CK20. Vascular endothelial growth factor (VEGF) and neuron specific enolase are usually strongly positive and there is also a weak CD34 immunoreactivity. CEA, GFAP, S-100, synaptophysin and thyroglobulin are negative {913}.

Papillary cystadenomas of the broad ligament and mesosalpinx

In female VHL patients, benign papillary cystadenomas of the broad ligament and mesosalpinx have been described {700}. The tumours are probably of mesonephric origin and manifest as cystic lesions up to 3 cm in diameter. They are bilateral in 50% of cases and exhibit complex papillae lined by a mostly single layer of bland cuboidal to columnar, non-ciliated cells. More recently a broad ligament tumour of probable Müllerian origin and papillary tumours in the retroperitoneum have also been reported {2031, 2379}.
A study on allelic deletion of the VHL gene in papillary tumours of the broad ligament, epididymis and retroperitoneum provided evidence that these rare benign neoplasms are a phenotypic manifestation of the VHL disease {2031}.

Haemangioblastomas

Capillary haemangioblastoma are benign, highly vascular tumours limited almost exclusively to the central nervous system, however, they are occasionally also found at other sites such as peripheral nerves {704}, the pancreas {810}, liver {1853} and spinal nerve roots {973}. Morphologically, they are indistinguishable from their CNS counterparts. The tumours are well circumscribed and contain many small calibre vessels lined by endothelial cells and surrounded by pericytes. They are rich in large, often vacuolated stromal cells which stain strongly for vimentin and neuron-specific enolase and only occasionally for S100 protein.

Table 5.05
Comparison of clinical features of VHL disease, tuberous sclerosis (TSC) and autosomal dominant polycystic kidney disease (PKD). SEGA, subependymal giant cell astrocytoma.

	VHL	TSC	PKD
Renal cancer	+++	(+)	(+)
Angiomyolipoma	-	+++	-
Renal cysts	+	+	+++
Renal insufficiency	-	(+)	++
Cranial lesions	Haemangioblastoma	Calcifications Tubera, SEGA	Aneurysms
Liver lesions	Rarely cysts/angioma	Rarely hamartoma	50% cysts
Pancreas lesions	Cysts Infrequently islet	Rare	Rarely cysts

Cysts of the pancreas, kidney, adrenal, testis and ovary

Rarely, benign epithelial cysts are found in locations such as testis {252}, ovary {1564}, adrenal, kidney and pancreas in VHL patients. However, in the latter two locations, lesions have to be separated from microcystic adenomas and cystic clear cell carcinomas, respectively.

Genetics

Chromosomal location

The *VHL* tumour suppressor gene maps to chromosome sub-band 3p25.

Gene structure

The *VHL* coding sequence is represented in three exons and encodes two *VHL* transcripts. The major transcript (isoform I) represents all 3 exons, whereas exon 2 is absent from isoform II {1820}. To date, no isoform 2-encoded protein product has been detected and the identification of VHL patients with germline deletions of exon 2 (resulting in the expression of isoform II only from the mutant allele) suggests that isoform II does not encode a functional gene product. The *VHL* gene specifies two translation products: a full length 213 amino acid protein (pVHL30) which migrates with an apparent molecular weight of ~28-30 KDa, and a shorter protein (~18-19 KDa; pVHL19), which is translated from an internal translation initiation site at codon 54 and produces a 160 amino acid protein. Evolutionary conservation of *VHL* amino acid sequence is very strong over most of the sequence included in pVHL19, but the first 53 amino acids included in pVHL30 are much less conserved {2417}. The primary sequence of pVHL19 shows little homology to any known protein.

Gene expression

The 4.7 kb mRNA is widely expressed in both fetal and adult tissues. In particular, the tissue expression of the *VHL* mRNA and protein does not reflect the limited number of organs affected in VHL disease {415,1241,1354,1822}.

Gene function

The *VHL* gene product appears to have multiple functions, the best characterised of which is the role of pVHL in regulating proteolytic degradation of the a subunits of the HIF transcription factors, HIF-1 and HIF-2. The VHL gene product has two well-defined protein binding domains. The pVHL a domain interacts with the elongin C protein and this interacts with two further proteins, elongin B and cullin-2 to form a tetrameric VCBC complex {1348,1704}. Structural and sequence motif homologies between the VCBC complex and the yeast SCF (Skp1-Cdc53/Cul1-F-box) complex suggested that VCBC might have a SCF-like function and target specific proteins for ubiquitination and protosomal degradation {1348}. In particular, pVHL was predicted to have a "F-box function" and to determine which proteins were targeted. This model was supported by the observation that the Rbx-1 protein, an essential general component of SCF complexes, associated with the VCBC complex {1031}. Furthermore, the elucidation of the crystal structure of the pVHL/elonginB/elonginC complex demonstrated structural analogy between the F-box protein in SCF complexes and the elongin C binding site in pVHL {2118}. Thus, there was strong circumstantial evidence that pVHL might target specific proteins for polyubiquitination.
VHL-related tumours such as RCC, hae-

mangioblastoma and phaeochromocytoma are highly vascular and overexpress a wide range of hypoxia-inducible mRNAs, including VEGF and VEGF receptor, in normoxic conditions {1448, 2050,2403}. Normal oxygen-dependent regulation could be restored by re-introducing wild-type pVHL into VHL null RCC cell lines {731,955,1286,2050}. Many hypoxia-inducible genes are regulated by the HIF-1 and HIF-2 heterodimeric transcription factors. Whilst the β-subunits of HIF-1 and HIF-2 are constitutively expressed, their α-subunits are degraded rapidly by the proteasome under normoxic conditions, but are stabilised by hypoxia. pVHL plays a critical role in regulating proteosomal degradation of HIF-1 and HIF-2α subunits {1448}. Thus, under normoxic conditions, pVHL binds to HIF-1α (or HIF-2α) (via a β-domain surface binding site on pVHL) and promotes polyubiquitylation and proteosomal degradation of HIF-1α {396, 1031,1448,2196}. In the absence of a functioning pVHL, HIF-1α is not destroyed resulting in HIF-1 (and HIF-2)-mediated upregulation of hypoxia-inducible mRNA expression. Thus, the VHL-HIF-α interaction links the SCF-like function of the VCBC complex to the angiogenic phenotype of VHL-associated tumours. 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* may be oncogenic per se {1134,1404}.

The ability of pVHL to bind HIF-1α is dependent on the hydroxylation status of specific proline residues in the HIF-1α protein. Hydroxylation of these prolines is oxygen-dependent and so under hypoxic conditions, the prolines are not hydroxylated and pVHL is unable to bind HIF-1α {977,983}.

In addition to its role in regulation of HIF-1 and HIF-2, pVHL has been implicated in a variety of cellular processes including cell cycle control, regulation of mRNA stability, fibronectin metabolism and microtubule stability {872,1637,1703, 1747}. In view of the role of pVHL in targeting HIF-1 α-subunits for ubiquitylation and proteolysis, it might be expected that further such targets would be identified. However, although some additional targets for pVHL targeted ubiquitylation have been suggested (e.g. a novel deubiquitinating enzyme and an atypical protein kinase C (PKClambda) {1294, 1645}, the relevance of these to VHL tumour suppressor activity has not been established. In particular, it may be difficult to elucidate whether pVHL functions are independent of HIF dysregulation. Thus, while cyclin D1 transcription appears to be regulated by pVHL, the evidence that this is HIF-independent is equivocal {194,2481,2482}. Although a 1999 report suggested that folding and assembly of pVHL into a complex with elongin B and C is directly mediated by the chaperonin TRiC/CCT {612}, it has since been suggested that retention of TriC/CCT binding is not of primary importance in VHL tumour suppressor activity {816}.

Table 5.06
Clinical subtypes of VHL disease and their association with *VHL* gene mutations and pVHL function.

Subtype	Tumour frequency			Mutations	Effect on HIF
	HAB	RCC	PCC		
Type 1	High	High	Rare	Mainly deletions, truncations or missense mutations that affect protein folding	Upregulation of *HIF-α* and *HIF-1* target genes
Type 2A	High	Rare	High	Missense mutations	Upregulation of *HIF-α* and *HIF-1* target genes
Type 2B	High	High	High	Mainly missense mutations	Upregulation of *HIF-α* and *HIF-1* target genes
Type 2C	Nil	Nil	High	Missense mutations	No evidence of HIF dysregulation

Key: HAB = haemangioblastoma, RCC = renal cell carcinoma, PCC = phaeochromocytoma

Mutation spectrum

Germline *VHL* gene mutations have been identified in >500 kindreds {358,433, 1381,1821,2483} (http://www.umd.necker.fr). Although a wide variety of mutations have been described, no mutations have been reported in the first 53 amino acids of pVHL30. Germline *VHL* mutations may be divided into three broad groups. Large genomic deletions account for up to 40% of all mutations and the rest are divided approximately equally between intragenic missense mutations and protein truncating mutations (nonsense, frameshift insertions and deletions, splice site mutations). Molecular genetic analysis of the complete *VHL* coding region by direct sequencing and large deletion detection (e.g. quantitative Southern blot and FISH analyses) has been reported to detect mutations in up to 100% of cases {1672,2137}. VHL patients without detectable mutations may be mosaic {2013}. Recurrent *VHL* gene mutations (e.g. C694T, C712T, G713A) mostly represent multiple *de novo* mutations at hypermutable sequences (e.g. CpG dinucleotides, small repeats) {1821}. However, the T505C (Y98H) "Black Forest" mutation common in Southwest Germany and in North American kindreds of German origin is a founder mutation in these communities {245}.

Genotype vs phenotype

VHL disease kindreds have been divided into type 1 (no [or rare] phaeochromocytoma) and type 2 (phaeochromocytoma+) subsets. Large deletions and truncating mutations typically predispose to haemangioblastomas and RCC but not phaeochromocytomas (PCC) (type 1 phenotype) {433,1381,2483}. Certain missense mutations that are predicted to disrupt pVHL protein folding may produce a type 1 phenotype, but the majority of type 2 kindreds have a missense mutation. Type 2 families are further subdivided according to the presence or absence of RCC and haemangioblastomas {245,1589,2418}. These genotype-phenotype correlations may be helpful in clinical management with regard to the risk of phaeochromocy-

oma, but missense mutations are heterogeneous, the tumour risks associated with specific mutations may differ markedly and so risk estimates should be used with caution. Patients presenting with a familial phaeochromocytoma only history (type 2C) may have a mutation that has been detected in other type 2 subsets (e.g. R167Q which is seen in type 2B) suggesting that these kindreds are also at risk for haemangioblastomas and RCC. However, some missense mutations are restricted to type 2C kindreds suggesting that they do not predispose to other VHL tumours. These complex correlations are consistent with the hypothesis that the *VHL* gene product has multiple and tissue specific functions.

Structural analysis of the VCBC complex {2118} demonstrated that missense mutations associated with a type 1 phenotype often occur at codons within the hydrophobic core mutations and are predicted to cause complete disruption to the pVHL structure. In contrast, type 2A/2B mutations show a trend against hydrophobic core mutations, causing mostly local effects, suggesting that type 2 mutations have a strong bias against total loss of function {2118}. These observations are consistent with the high frequency of loss of function deletion (and truncating mutations) in type 1 families and the rarity of such mutations in type 2 kindreds. Furthermore, *in vitro* analysis of pVHL function demonstrates partial retention of pVHL binding to elongin C or HIF-1 with phaeochromocytoma-associated missense mutations {393,394}. 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 Type 1, 2A and 2B associated mutations, but Type 2C mutants retained the ability to regulate HIF-1 (although fibronectin binding was impaired) {393,394,901}. These findings suggest that HIF-1 dysregulation is not necessary for phaeochromocytoma development in VHL disease.

Further evidence of the complexity of genotype-phenotype correlations has emerged with the demonstration that homozygous missense VHL mutations may cause congenital polycythaemia syndromes without evidence of VHL disease {58,1700}.

In addition to the phenotypic variability associated with allelic heterogeneity, genetic modifiers may influence the phenotypic expression of VHL disease. Thus, one series suggested that patients with retinal angiomas had a higher risk of cerebellar haemangioblastoma and RCC than those without retinal involvement {2367}. Evidence for genetic modifiers was provided by the observation that there was a significant correlation between numbers of retinal angiomas in close relatives but not between distant relatives. Recently, allelic variants in the cyclin D1 gene have been reported to influence haemangioblastoma development {2481}.

Genetic counselling

VHL disease is a pleotropic autosomal dominant disorder with age-dependent penetrance (penetrance is >95% at age 60 years). Individuals known or suspected of having VHL should be provided genetic counselling. Genetic counselling consists of collecting a detailed family medical history, providing a risk assessment, and arranging genetic testing {1732,1977}. The genetic testing process involves discussing the implications of test results to the patient and other family members, and facilitating appropriate follow-up care {696}. Providers should be sensitive to the possible emotional sequelae of positive test results and the burden of sharing this information with other at-risk relatives {136,781,1016}.

Clinical *VHL* gene testing is performed with Southern blot analysis to identify large deletions and sequence analysis to identify intragenic mutations. This combined approach is estimated to detect the underlying mutation in greater than 95% of individuals with VHL {2137}. Once the mutation or deletion has been identified in the affected individual, other blood relatives can undergo targeted genetic testing which will reveal that the familial DNA alteration is either present (yielding a positive result) or absent (a true negative result).

First-degree relatives of an affected individual should be offered genetic testing. More distant relatives can also be offered testing if the intervening relative declines testing or is not available. Because screening for VHL-associated tumours begins in childhood, it is reasonable to offer predictive genetic testing to individuals under age 18 (usually from age 5 years) {4}. Couples at risk for having a child with VHL can elect to have prenatal testing, but uptake of prenatal testing is low. Preimplantation testing for VHL has also been reported {1799}.

Preventive measures

Because of the diverse organs at risk, coordination of clinical care is challenging for individuals with VHL. At-risk individuals should be entered into a comprehensive screening programme in childhood (unless VHL is excluded by molecular genetic testing) {1308,1380}. Recommendations for detection of specific tumours are outlined below {1308, 1380,1972}.

Screening for renal cell carcinoma

Individuals at risk for VHL-associated tumours should have ultrasound examinations or MRI scans of the abdomen every 12 months beginning in adolescence.

Screening for retinal angioma

At-risk individuals should undergo careful ophthalmic examinations every 12 months beginning in infancy or early childhood.

Screening for haemangioblastoma

It is suggested that individuals at risk for VHL-associated tumours should have MRI scans of the head (+spine) every 12-36 months beginning in adolescence.

Screening for phaechromocytoma

At-risk individuals should undergo yearly screening for phaeochromocytoma beginning in early childhood. This consists of blood pressure monitoring and 24-hour urine studies to measure catecholamine metabolites. Measurement of plasma normetanephrine levels is reported to be the most sensitive test for detecting phaeochromocytoma in VHL disease {551}, but is not yet in widespread use.

Familial paraganglioma-phaeochromocytoma syndromes caused by *SDHB*, *SDHC* and *SDHD* mutations

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Definition
Syndromes characterised by susceptibility to phaeochromocytoma and paraganglioma resulting from germline mutations in *SDHB*, *SDHC* and *SDHD*.

MIM Numbers
MIM numbers were assigned according to location prior to gene identification. Therefore, both the locus and gene names and their MIM numbers are given. *See:* http://www.ncbi.nlm.nih.gov/omim {1468}.

Paragangliomas, familial nonchromaffin,1 (*SDHD*) 168000 (602690)
Paragangliomas, familial nonchromaffin, 3 (*SDHC*) 605373 (602413)
Carotid body tumours and multiple extra-adrenal phaeochromocytomas (*SDHB*) 115310 (185460)

Synonyms
Hereditary paraganglioma syndrome; hereditary phaeochromocytoma-paraganglioma syndrome.

Incidence / prevalence
The overall incidence of tumours of the autonomic nervous system including phaeochromocytomas, and sympathetic and parasympathetic paraganglioma has been estimated at ~1/300,000 {138}. The proportion of those tumours due to familial paraganglioma/phaeochromocytoma varies depending both on tumour type and population of ascertainment. The best estimate for isolated phaeochromocytoma comes from a population based study in Germany and Poland and in which 8.5% of patients without family history or other syndromic features had mutations in *SDHD* or *SDHB* {1585}. The rate of *SDHX* mutations in patients with parasympathetic paraganglioma is higher, ranging from 20% in a US clinic based population to over 50% in a Dutch clinic population {142,455}.

Diagnostic criteria
The finding of a mutation in *SDHB*, *SDHC*, or *SDHD* confirms the molecular diagnosis of a hereditary paraganglioma/phaeochromocytoma syndrome. Currently, there are no clinical criteria for the diagnosis.

Age distribution and penetrance
Males and females are affected equally. Age at diagnosis is known only from a limited member of germline mutation carriers. Mean age of diagnosis of phaeochromocytomas and paragangliomas for *SDHB* and *SDHD* mutation carriers in the population based study of phaeochromocytoma was 26 (range, 12-48) and 29 (range, 5-59) years, respectively {1585}. The mean age at diagnosis is, thus, 15-20 years younger than that for sporadic phaeochromocytoma and paraganglioma.

Sympathetic paraganglioma

Sympathetic paragangliomas are derived from the sympathetic chain, and usually located in the chest, abdomen or pelvis.

Clinical features
As described for phaeochromocytoma, the clinical features of sympathetic paraganglioma are a consequence of either the secretion of catecholamines or the size of the tumours with consequent impingement on other structures.

Pathology
The morphology of adrenal phaeochromocytomas and extra-adrenal paragangliomas is usually very similar and there is no reliable pattern to discriminate between them. Finding an attached adrenal remnant or an uninvolved adrenal may be helpful. It can be difficult to separate metastasising paragangliomas from multicentric paragangliomas. As in the adrenal medulla, there are no reliable criteria for predicting malignancy. However, the frequency of malignancy is significantly higher in sympathetic tumours with extra-adrenal location. Indicators of malignancy are the same as described for phaeochromocytomas in the adrenal.

Parasympathetic paragangliomas of head and neck

Parasympathetic paragangliomas are tumours of the parasympathetic ganglia, usually found in the head and neck region, arising from the cell nests located adjacent to blood vessels such as the carotid body or the ganglion jugulare, vestibulare and aortae. Various terms have been used in the past to describe paragangliomas of the head and neck region, including glomus tumours, chemodectomas, carotid body tumours, and nonchromaffin tumours. As glomus tumour is not a specific term and is more often used to describe a histologically different, benign, nonendocrine cutaneous tumour arising from neuromyoepithelial cells surrounding the cutaneous arteriovenous anastomosis {203}, it is best to define these tumours as paraganglioma based on the anatomical location (e.g. carotid paraganglioma, jugulotympanic paraganglioma or vagal paraganglioma).

Clinical features
The symptoms and signs in head and neck paragangliomas (HNP) depend on the anatomical locations of the tumours {2478}. The carotid body is the most common location of the HNPs followed by vagal, jugular, tympanic and laryngeal paragangliomas. The most common presentation of HNPs is a slow-growing painless mass in the neck and a visible bulge in the oropharynx. The neck mass in carotid body paragangliomas is movable from side to side but not up and down and may also have a pulsatile character. A thorough examination for a primary tumour of thyroid, oropharynx and nasopharynx is necessary, since metastasis to a cervical node is a much more frequent cause of a neck mass than HNPs. The HNPs may cause cranial nerve defects that may present as weakness of tongue and as shoulder drop. Vocal cord paralysis is a common presenting sign of laryngeal paragangliomas. Jugular paragangliomas may

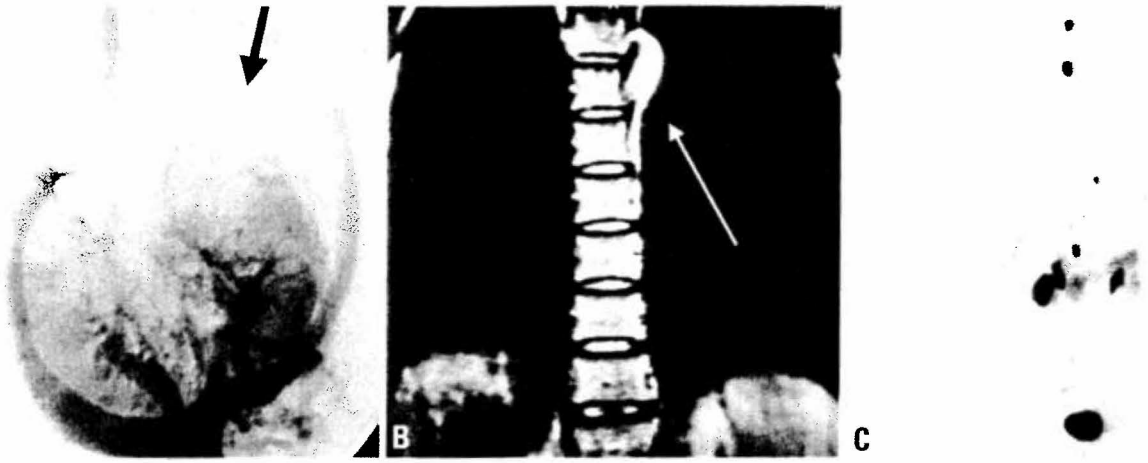

Fig. 5.27 SDHX. **A** MRI angiography demonstrating an intercarotid well vascularized tumour (carotid parasympathetic paraganglioma or glomus carotid tumour) in a carrier of a SDHD mutation. **B** Coronal thoracic MRI demonstrating a tumour of the upper thoracic part of the sympathetic trunk. **C** 18-Fluoro DOPA positron emission tomography (DOPA PET) of a carrier of SDHD mutation. Uptake is visible in the left skull base, the right carotid body, after parasympathetic paraganglioma resection in remnant of the contralateral carotid body, in projection of the cardiac atria, and the right adrenal gland. Uptake in all locations has been confirmed by MRI.

cause defects in cranial nerves IX, X and XI and may lead to hearing loss, tinnitus and balance abnormalities. Intracranial involvement is also possible and may lead to signs of increased intracranial pressure {1565}. Unlike paragangliomas in the abdomen, the HNPs are usually biochemically silent. Malignancy as indicated by local invasion or metastases is seen in less than 10% of the cases {1193}. The number and location of the tumours can be reliably identified using computerised tomography (CT) and magnetic resonance imaging (MRI). Conventional angiography or MR angiography can be of confirmatory value in the differential diagnosis because HNPs are extremely vascular. Similar to phaeochromocytoma, 18-fluoro dopa PET is highly sensitive for the diagnosis of HNPs and an ideal method for documentation of PGL syndromes with multiple tumours in more than one region of the body. Multiple HNPs strongly suggest a genetic predisposition.

Pathology

Morphologically, all paragangliomas are closely related to one another and to phaeochromocytomas of the adrenal gland. Hereditary paragangliomas are frequently bilateral/multicentric, exhibit an equal sex ratio and occur in a slightly younger age group than sporadic tumours {1584}. The rate of multifocal disease in patients with familial paragangliomas was reported to be about 40-50% reaching 78% in vagal paragangliomas {455,1583}. The most frequently occurring familial paragangliomas are carotid body paragangliomas, followed by jugular and vagal paragangliomas {455,2288}. Furthermore, sympathetic paragangliomas also can occur in association with familial paragangliomas of the head and neck region {1379}.

Grossly, paragangliomas are fairly well circumscribed, rubbery-firm tumours with a fibrous pseudocapsule and tan to purple colour. The histological and immunohistochemical features of familial paragangliomas are similar to those of sporadically occurring tumours and as described for sympathetic tumours {994,1249}. However, in general, parasympathetic paragangliomas usually exhibit a pronounced „Zellballen" appearance and sheets of cells or spindle cell patterns are less commonly seen. Furthermore, intracytoplasmic hyaline globules are rare and some tumours may show prominent perivascular sclerosis or fibrosis. In other cases, marked dilation of the tumour vessels creates an angiomatous appearance. Nuclear pleomorphism and cellular hyperchromatism may be seen and should not be considered evidence of malignancy.

Several malignant parasympathetic paragangliomas in patients with inherited tumour forms have been reported {460, 1249,1499,1999}. As with tumours of the sympathetic paraganglia, there are no reliable histological criteria for predicting malignant behaviour. The malignant potential of the tumour is determined by the presence of local, regional or distant metastases. Numerous mitoses, extensive vascular invasion and central necrosis of cell nests have been associated with malignancy.

Prognosis and prognostic factors

The head and neck paragangliomas grow slowly, metastasise rarely and may remain asymptomatic in certain individuals; the autopsy incidence of HNPs is much higher than their clinical incidence {138}. Surgery is the main mode of clinical management for symptomatic HNPs and leads to a complete cure if there is no metastasis or residual disease. However, surgical management of HNPs can result in mortality in less than 2% of the cases and in morbidity in approximately 40% of the cases, which is often caused by cranial nerve injury and cerebral ischaemic events. The benefits of pre-operative embolisation to reduce blood loss during surgery and the irradiation therapy are controversial {1702}.

Phaeochromocytoma

Clinical features

Patients with germline mutations of the *SDHB*, *SDHC*, or *SDHD* gene may develop one or multiple tumours in the abdomen or the thorax in addition to paragangliomas of the neck and skull base. Additional types of tumours have not been reported to be associated with *SDHX* mutations at this point. As mutations in these genes have been only recently identified as associated with cancer susceptibility, this may change in

the future. The clinical features of phaeochromocytoma and paraganglioma are a consequence of their growth, along with the production, storage and release of catecholamines.

Phaeochromocytoma (including paraganglioma) has been called the 'great actor', as the presenting symptoms can be misinterpreted as signs of other diseases. The three classic symptoms of phaeochromocytoma are headache, palpitations and sweating {1400}. Hypertension is the major finding associated with phaeochromocytoma, and the blood pressure can be permanently elevated. However, more typical of phaeochromocytomas, and diagnostic, is intermittent hypertension. Hypertensive crises can occur and may result in intracranial haemorrhage or left ventricular failure. Episodes of catecholamine release lead to the sensation of palpitations resulting from paroxysmal tachycardia. During the periods of catecholamine release, patients often complain of headaches. The third classic symptom is increased perspiration, which may result in profuse sweating attacks without a clear etiology. Symptoms can vary and rarely give hints to tumour location; crises that happen after micturition can be due to paraganglioma of the urinary bladder {1705}.

Endocrine diagnosis

The release of catecholamines into the circulation and excretion of catecholamines and their metabolites can be used to make the diagnosis of phaeochromocytoma. Epinephrine, norepinephrine, vanillylmandelic acid, metanephrines and normetanephrines can be assayed in the plasma or the urine. It has been demonstrated that plasma free metanephrines are the single best test for detecting phaeochromocytoma {1277}. For hereditary cases of phaeochromocytoma, urinary fractionated metanephrines provide similar sensitivity (96% vs. 97%) but decreased specificity (82% vs. 96%).

Imaging

Conventional CT scan, MR imaging and MIBG scintigraphy are the classical diagnostic tools for the diagnosis of phaeochromocytomas and paragangliomas. Recently 18-fluoro dopa (or dopamine) positron emission tomography (PET) has been shown to be highly sensitive for abdominal phaeochromocytoma and neck paragangliomas {900, 1668}.

Table 5.07
Summary of 14 different germline mutations of the *SDHB* gene as published in literature or found in the Freiburg Phaeochromocytoma Study

Mutation (cDNA Nucleotide)	Exon	Consequence (Amino Acid)	Adrenal PHEO	Abdominal Sympathetic Paraganglioma	Head/Neck Parasympathetic Paraganglioma	Reference
213 C/T	2	R27X		+		{746}
221 ins CAG	2	Ins Q30	+			{746}
270 C/G	2	R46G		+	+	{746}
309 C/T	2	Q59X			+	{138}
345-347 ins C	3	M71 frameshift			+	{138}
402 C/T	3	R90X		+	+	{97}
436 G/A	4	C101Y		+		{746}
526 C/G	4	P131R			+	{138}
708 T/C	6	C192R	+			{746}
721 G/A	6	C196Y		+		{746}
724 C/G	6	P197R		+		{97}
847/849 del TCTC	7	F238 frameshift	+	+		{746}
859 G/A	7	R242H		+		{746}
881 C/A	7	C249X	+			{746}

Location

Carriers of germline mutations of the *SDHB* and *SDHD* genes can develop tumours of the sympathetic trunk, including the adrenal medulla, as well as of the parasympathetic ganglia. In contrast, a *SDHC* germline mutation has so far only been found in one small family with paragangliomas of the neck {1599}. *SDHB* and *SDHD* germline mutations have been identified both in patients who have only one adrenal or extra-adrenal tumour and in patients with multiple tumours either in one or several parts of the body, e.g. abdomen, thorax, and neck {139, 717,746}.

The tumours can be multicentric and/or bilateral and may be associated with extra-adrenal chromaffin tumours (sympathetic paragangliomas). In a recent study of sporadic phaeochromocytoma patients with no family history or features of associated syndromes, carriers of *SDHD* and *SDHB* mutations were found to present with isolated adrenal phaeochromocytoma (60% with *SDHD* germline mutations and 50% with *SDHB* germline mutations) {1585}.

Histopathology

The histological structure of paragangliomas occurring in patients with familial paraganglioma syndromes is similar to that of sporadic counterpart tumours. However, no detailed comparative studies addressing this subject are available. Phaeochromocytomas usually are not demarcated by a fibrous capsule and exhibit a prominent vascular network. They show a mixture of alveolar („Zellballen") and trabecular patterns and may contain areas of spindle cells or a diffuse growth pattern. Nests of tumour cells may vary considerably in size and are surrounded by sustentacular cells that probably play a paracrine role. Tumour cells are usually polygonal with sharply defined cell borders, the cytoplasm is finely granular and may be lightly eosinophilic, amphophilic or basophilic and intracytoplasmic. PAS-positive, diastase-resistant hyaline globules may be observed. Tumour cell nuclei may be vesicular with prominent nucleoli and occasionally contain nuclear pseudoinclusions. Some tumours contain cells with moderate to marked nuclear enlargement and hyperchromasia as well as occasional mitotic figures, features which have no impact on prognosis.

Immunohistochemistry

Neuroendocrine cells of paragangliomas are negative for cytokeratins, express general neuroendocrine markers such as chromogranin A, PGP9.5 and synaptophysin and occasionally neurofilaments, neuropeptides such as enkephalin, neuropeptide Y and peptides derived from proopiomelanocortin.

Synaptophysin is not an ideal marker t

separate adrenocortical from medullary tumours since focal immunoreactivity for synaptophysin may also be seen in the normal and neoplastic adrenal cortex. Furthermore, enzymes involved in the synthesis of catecholamines such as tyrosine hydroxylase (TH), phenylethanolamine N-methyltransferase (PNMT) and dopamine-beta-hydroxylase (DBH) can be localized in the tumour cells. Sustentacular cells are immunoreactive for S100 protein and sometimes for glial fibrillary acidic protein (GFAP).

Similar to sporadically occurring counterpart tumours, there are no reliable criteria to predict clinical tumour behaviour. However, in general, malignant tumours are larger, exhibit a higher mitotic count, local or vascular invasion, coarse nodularity, confluent necrosis and absence of hyaline globules. They also contain a smaller number of sustentacular cells and express fewer neuropeptides on immunohistochemical study than benign tumours {1307}.

Precursor lesions. The precursor lesions of paragangliomas in the setting of paraganglioma syndromes have not been as well characterised as those in MEN 2.

Prognosis and prognostic factors

Prognosis of phaeochromocytoma and paraganglioma depends on timely performed diagnostic imaging and measurement of catecholamines. The vast majority of these tumours are benign. The accepted criterion for malignancy is distant metastases, as local invasion is not always a sign of malignant phaeochromocytoma. In particular, the multifocal nature of the tumours associated with the SDHX syndromes may be confusing and may lead to these separate primary tumours being misinterpreted as metastases and hence, malignant. Whether carriers of mutations in *SDHB* have a different aggressive disease and prognosis compared to *SDHD* is unknown. Truly malignant phaeochromocytoma has been observed in a few *SDHB* mutation carriers but not in *SDHD* mutation carriers (Neumann et al, unpublished data).

Table 5.08
Summary of 29 different germline mutations of the *SDHD* gene as published in literature or found in the Freiburg Phaeochromocytoma (PCC) Study

Mutation (cDNA nucleotide)	Exon	Consequence (Amino acid change)	Adrenal PCC	Abdominal Sympathetic Paragang-lioma	Thoarcic Sypathetic Paragang-lioma	Head/Neck Parasympathetic Paragang-lioma	Reference
3 G/C	1	M1I				+	{107}
14 G/A	1	W5X	+		+	+	{746}
33 C/A	1	C11X	+		+	+	{746}
36,37 del T	1	Frameshift	+	+		+	{746}
52+2 T/G (IVS1+2)	Intron	Splice defect	+			+	{139,746}
54 ins C	2	Frameshift				+	{2201}
64 C/T	2	R22X				+	{715}
94 del TC	2	Frameshift	+	+			{97}
95 C/G or 95 C/A	2	S32X				+	{1504}
106 C/T	2	Q36X				+	{139}
112 C/T	2	R38X	+		+	+	{139,717,746}
120 ins C	2	Frameshift				+	{2201}
129 G/A	2	W43X				+	{325}
191,192 del TC	3	Frameshift				+	{107}
208 A/G	3	R70G				+	{2201}
242 C/T	3	P81L				+	{139}
274 G/T	3	D92Y	+	+		+	{139,455,746}
277 del TAT	3	del Y93				+	{107}
284 T/C	3	L95P				+	{455,2201}
305 A/T	3	H102L				+	{139}
325 C/T	4	Q109X				+	{138}
337 ins T	4	Frameshift				+	{1504}
337-340 del GACT	4	Frameshift				+	{325}
341 A/G	4	Y114C				+	{1504}
361 C/T	4	Q121X	+		+		{746}
381-383 del G	4	Frameshift				+	{139}
416 T/C	4	L139P				+	{455,2201}
443 del G	4	Frameshift				+	{1504}

Genetics

Chromosomal location

SDHB maps to chromosome 1p35-36.1 {1260}, *SDHC* maps to chromosome 1q21-23 {555} and *SDHD* maps to chromosome 11q23 {890}. Pseudogenes also have been identified for each gene.

Gene structure

SDHB, *SDHC* and *SDHD* genes are composed of eight, six and four exons, respectively and span approximately 40 kilobases (kb), 50 kb and 8 kb of genomic distances, respectively {99,555,889}.

Gene expression

SDHB, *SDHC* and *SDHD* are housekeeping genes with TATA-less promoters. Their expression of the SDH subunits may be coordinated by transcription factors, such as the nuclear respiratory factors NRF-1 and NRF-2 {1955}. NRF-1 binding sites have been found upstream of *SDHB*, *SDHC* and *SDHD* {100,555, 889}. NRF-2 binding sites have also been found upstream of these genes. Promoter analysis of the *SDHB* gene indicates that both NRF-1 and NRF-2 are required for normal gene expression. This suggests that NRF-1 and, to a lesser extent, NRF-2 coordinate the expression of the complex II genes.

Gene function

SDHB, *SDHC* and *SDHD* encode three of the four distinct subunits of mitochondrial complex II (succinate dehydrogenase; succinate-ubiquinone oxidoreductase). *SDHB*, *SDHC* and *SDHD* encode 280 amino acids (aa), 159 aa and 169 aa and correspond to 30 kDa, 15kDa and12 kDa proteins, respectively. *SDHA* and *SDHB* gene products constitute the hydrophilic catalytic part of the complex. *SDHC* and

SDHD gene products are hydrophobic integral membrane proteins, which form the cytochrome b and link the catalytic subunits to the mitochondrial inner membrane. Complex II is involved both in the Krebs (tricarboxylic acid) cycle and in aerobic electron transport chain {140}. Succinate dehydrogenase catalyzes the oxidation of succinate to fumarate in the Krebs cycle. The extracted electrons are transferred via FAD (Flavin adenine dinucleotide) and iron-sulfur clusters in the catalytic subunits to ubiquinone and enters the electron transport chain {1958}. The mechanism of tumourigenesis in PGL is unknown. Because sporadic carotid body paragangliomas develop in increased frequency among high altitude dwellers, it has been hypothesised that mitochondrial complex II may play an important role in oxygen sensing. This hypothesis is partly supported by the finding of increased expression of hypoxia-inducible genes in a phaeochromocytoma tumour with an *SDHD* mutation {715}.

Table 5.09
Cancer susceptibility syndromes associated with the pathogenesis of phaeochromocytoma and paragangliomas.

Syndrome	Gene	Chromosome	Adrenal Pheo	Abdominal Sympathetic Paraganglioma	Thoracic Sympathetic Paraganglioma	Head/Neck Parasympathetic Paraganglioma
PGL 1	*SDHD*	11q23	++	++	+	++
PGL 2	Unknown	11q13				++
PGL 3	*SDHC*	1q21-23				++
PGL 4	*SDHB*	1p36	++	++	++	++
VHL	*VHL*	3p25-26	++	++	+	ER
MEN 2	*RET*	10q11.2	++	ER		ER
NF 1	*NF1*	17q11	+			
Carney triad	Unknown	unknown				+

++/+: Observed in normal/decreased frequency, ER: exceptionally rare in association with this syndrome.

Mutation spectrum

Germline heterozygous, inactivating mutations in *SDHB*, *SDHC* and *SDHD* genes cause hereditary paraganglioma (PGL) {97,107,139,325,1504,1599}. Mutations in these three genes account for at least 70% of familial, 8% of non-familial HNPs and 8% of non-syndromic, non-familial adrenal phaeochromocytomas and extra-adrenal paragangliomas {142}. Amongst mutations in the three genes, *SDHD* mutations are the leading cause of HNPs, whereas *SDHD* and *SDHB* mutations may contribute equally to the adrenal phaeochromocytomas and extra-adrenal paragangliomas. Only one *SDHC* coding region mutation has been reported. Paragangliomas are transmitted only through fathers in PGL1 (*SDHD*) families suggesting a role for genomic imprinting in the regulation of *SDHD* gene expression {2293}. *SDHB* and *SDHC* genes do not display parent of origin effects. There are strong founder effects in the etiology of HNPs in the Netherlands, where three *SDHD* mutations account for nearly all of their heritable HNPs {455,2201}. Currently, there is no evidence for an increased risk of developing other tumour types in individuals with *SDHB*, *SDHC* and *SDHD* mutations.

In *SDHD*, mutations are observed throughout the four exons, whereas in *SDHB*, mutations have been identified in exons 2, 3, 4, 6 and 7, but not in exons 1, 5, and 8. The data are currently too scarce to make inferences about possible hot spots and genotype-phenotype correlations.

Genetic counselling and preventive measures

In the absence of clinical or molecular features of MEN 2, neurofibromatosis type 1 or von Hippel-Lindau syndrome, the presence of a mutation in the *SDHX* genes should be considered upon presentation of isolated or familial paraganglioma or phaeochromocytoma {717, 1585}. In other words, based on the population-based study on apparently isolated phaeochromocytoma {1585}, all presentations of phaeochromocytoma or paraganglioma, irrespective of syndromic features of family history, should be offered genetic testing for the *SDHX*, *VHL* and *RET* genes.

Clinical testing is currently available for mutations in the *SDHD* and *SDHB* genes and should start with an individual who has had a phaeochromocytoma or paraganglioma in order to identify the familial mutation (See GeneTests for a list of laboratories http://www.genetests.org/-servlet/access). A negative test result does not rule out a hereditary syndrome and family members may still be at risk. Once a familial mutation is identified, subsequent predictive testing is possible with high accuracy. Development of paragangliomas occurs primarily in adulthood, however, there are published reports of individuals diagnosed as young as age 5, indicating that testing of minors is reasonable {197,1453,1585, 2288}.

Because phenotypic expression of the *SDHB* gene does not demonstrate parent-of-origin effect, families can be counselled regarding classic autosomal dominant inheritance {97}. Families with *SDHD* mutations must be counselled regarding autosomal dominant inheritance of the mutant allele, but that individuals who inherit the allele from their mother are unlikely to develop tumours {2293}. It must be stressed that the children of men who have inherited the mutant allele from their mother are at 50% risk of carrying the mutant allele and at risk for tumour development. The exact penetrance of mutations in *SDHD* and *SHDB* is not known.