Genetic testing for hereditary mutations in the *VHL* gene that cause von Hippel-Lindau syndrome

MSAC application no 1153

Assessment report

September 2011

Online ISBN: 978-1-74241-782-0

Online ISSN: 1443-7139

Publication approval number: D0895

Electronic copies of the report can be obtained from the Medical Service Advisory Committee's Internet site at http://www.msac.gov.au/

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MSAC's advice does not necessarily reflect the views of all individuals who participated in the MSAC evaluation.

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This report should be referenced as follows:

Morona J.K., Newton S., Wang S., Tamblyn D., Ellery B., Merlin T. (2012). *Genetic testing for hereditary mutations in the* VHL *gene that cause von Hippel-Lindau syndrome*. MSAC Application 1153, Assessment Report. Commonwealth of Australia, Canberra, ACT.

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Purpose of Application

An application requesting the Medicare Benefits Schedule (MBS) listing of genetic testing for hereditary mutations in the *VHL* gene was received from the Pathology Services Table Committee (PSTC) by the Department of Health and Ageing in November 2010. Testing was requested for (i) patients with symptoms of VHL syndrome and (ii) family members of a patient with a known VHL mutation.

VHL syndrome is an autosomal dominant neoplastic disease caused by germ-line mutations or deletions in one copy of the *VHL* tumour suppressor gene located on chromosome 3p25. Tumours arise when spontaneous mutations occur in the second copy of the *VHL* gene in individual cells of affected organs. It is suggested that patients presenting with one or more characteristic tumours or a positive family history of VHL syndrome should be screened to determine if there is a germ-line mutation in the *VHL* gene.

As the result is definitive, VHL genetic testing would only need to be performed once for each patient. However, the two following different types of delivery of VHL genetic tests would need to occur:

(i) *Diagnostic VHL genetic testing* of patients suspected of having VHL syndrome would be used in addition to the existing clinical diagnostic service during the non-acute stage of patient management, that is, after the initial presentation, diagnosis and treatment of the presenting complaint. The genetic test predicts a patient's risk of VHL syndrome but must be used in conjunction with routine clinical screening in order to provide a disease-specific diagnosis. A positive VHL genetic test will not affect the requirement for annual screening, and there would be no change in the use of co-administered screening interventions for patients with confirmed VHL syndrome.

(ii) *Pre-symptomatic or predictive VHL genetic testing* would be performed as a non-urgent test once a VHL mutation has been identified in a family. Pre-symptomatic testing can be offered, after accredited genetic counselling, to first-degree family members (mother, father, offspring and sibling) and, as appropriate, second-degree family members (grandparent, half-sibling, aunt, uncle, niece, nephew and cousin). Individuals who have inherited the VHL mutation would be offered a lifelong screening program and early intervention to reduce the risk from, or severity of, VHL-associated neoplasms. However, if accurate, a negative VHL genetic test would eliminate the requirement for

annual screening. Thus, the test will replace the routine clinical screening interventions for these patients.

A summary of the screening procedures for individuals at risk of VHL syndrome, adapted from the VHL Family Alliance screening guidelines (VHL Family Alliance 2005), is provided in Table 1.

Table 1 Australian VHL screening protocol

Age	Screening test
Birth – 4 years	*Annually:* - Eye review by ophthalmologist
Ages 5–14 years	*Annually:* - Eye review by ophthalmologist - Medical specialist review: check of blood pressure, urine test or blood test to check for elevated catecholamines and metanephrines (phaeochromocytoma screen)
Age 15 years and older	*Annually:* - Eye review by ophthalmologist - Medical specialist review: check of blood pressure, urine test or blood test to check for elevated catecholamines and metanephrines (phaeochromocytoma screen) - Ultrasound of abdomen (kidneys, pancreas and adrenals) *Every 2 years:* - MRI with gadolinium of brain and entire spine cord (performed yearly if abnormality detected) *Every 2–3 years:* - CT of abdomen (instead of that year's ultrasound)

Source: adapted from the VHL Family Alliance screening guidelines (VHL Family Alliance 2005)

Proposal for public funding

The proposed MBS items are summarised in Table 2. The ordering of these tests would be restricted to specialised genetic services. It is expected that the MBS item for the testing of relatives would primarily be used for first- and second-degree relatives, but the proposed listing has been kept broad to allow for exceptional circumstances where wider use may be required.

Table 2 Proposed MBS item descriptor for VHL genetic testing

Category 6–Pathology services
MBS [item number] **(proposed MBS item 1)** Detection of germ-line mutations of the *VHL* gene in: (i) Patients with a clinical diagnosis of VHL syndrome: • a family history of VHL and a haemangioblastoma (retinal or CNS), phaeochromocytoma or renal cell carcinoma • two or more haemangioblastomas, or one haemangioblastoma and a tumour or cyst of the adrenal gland, kidney, pancreas, epididymis, and broad ligament (with the exception of epididymal and renal cysts, which are frequent in the general population)

(ii) Patients presenting with one or more clinical features suggestive of VHL syndrome:

- haemangioblastomas of the brain, spinal cord, and retina
- phaeochromocytoma or functional extra-adrenal paraganglioma

Fee: $600

Prior to ordering these tests, the ordering practitioner should ensure that the patient has given informed consent. Testing can only be performed after genetic counselling. Appropriate genetic counselling should be provided to the patient by a genetic counselling service or a clinical geneticist on referral. Further counselling may be necessary upon receipt of the test results.

MBS [item number] **(proposed MBS item 2)**

Detection of germ-line mutations of the *VHL* gene in:

(i) Biological relatives of patients with a known mutation in the *VHL* gene

Fee: $340

Prior to ordering these tests, the ordering practitioner should ensure that the patient has given informed consent. Testing can only be performed after genetic counselling. Appropriate genetic counselling should be provided to the patient by a genetic counselling service or a clinical geneticist on referral. Further counselling may be necessary upon receipt of the test results.

A team from Adelaide Health Technology Assessment (AHTA), School of Population Health and Clinical Practice, University of Adelaide, was engaged to conduct a systematic review of the literature and an economic evaluation of VHL genetic testing for patients with a clinical diagnosis of VHL syndrome or presenting with one or more clinical features suggestive of VHL syndrome, and for biological relatives of patients with a known mutation in the *VHL* gene.

Current arrangements for public reimbursement

Currently, there is no MBS listing for any test that detects germ-line mutations in the *VHL* gene. Patients may have their blood sample collected through a public hospital, in which case that facility may be charged for the genetic testing. Alternatively, when patients are referred by a private facility, they are billed directly.

Only three pathology laboratories offer VHL genetic testing in Australia, using assays developed in house. In New South Wales one laboratory offers direct DNA sequencing of the polymerase chain reaction (PCR) amplified exons 1, 2 and 3 of the *VHL* gene, with a turnaround time of 3 months. This test detects point mutations and frame-shift mutations but not large deletion mutations, and therefore does not identify all patients with VHL syndrome. One laboratory in South Australia and one in Western Australia offer both DNA sequencing and multiplex ligation-dependent probe amplification (MLPA), which is based on the semi-quantitative PCR principle and is used to detect large deletions of the *VHL* gene, for patients referred through a clinical genetic service, with a turnaround time of 2 months. Diagnostic VHL genetic testing is also commercially available overseas using DNA sequencing with or without MLPA.

Predictive testing is also available from the Australian laboratories and is cheaper than diagnostic testing as laboratories are identifying a specific abnormality in family members that was first identified in the index case.

Background

There have been no previous MSAC considerations of the VHL genetic test.

Prerequisites to implementation of any funding advice

VHL genetic testing is currently classified as a Class 3 in vitro diagnostic (IVD) by the Therapeutic Goods Administration (TGA). Laboratories offering the test in house must have National Association of Testing Authorities (NATA) accreditation, with demonstrated compliance with the suite of standards on the validation of in-house IVDs, as published by the National Pathology Accreditation Advisory Committee, for each test manufactured.

Consumer impact statement

The public was invited to provide feedback on the draft protocol for undertaking this evaluation of VHL testing during March 2011. The responses were from specialists and a researcher. The perceived benefits and disadvantages arising from genetic testing for the presence of germ-line VHL mutations in symptomatic patients and their asymptomatic at-risk relatives are summarised below.

Benefits of diagnostic and predictive VHL testing

Providing equity of access to VHL genetic testing across the country avoids local variations in funding arrangements for genetic testing provided by the states.

Patients will no longer be affected by limited annual genetic testing budgets. Medicare listing will permit more patients with suspected VHL syndrome to be identified, with the attendant benefits to themselves and their asymptomatic family members, through cascade testing.

Patients desire clarity in their diagnosis and the VHL genetic test would allow this.

For a patient or family member that tests positive, it will provide confirmation of a VHL syndrome clinical diagnosis. It may also facilitate patient compliance with the intense surveillance that is necessary with the condition.

For a patient or family member that tests negative, it would provide confidence that they do not have undiagnosed VHL syndrome. In patients with clinical symptoms, this exclusion of VHL would allow a differential diagnosis to be undertaken. A negative test result would also exclude the necessity for intense long-term surveillance for neoplasms,

reduce the associated stress on the individual/family, and limit any possible impact on reproductive choices.

Disadvantages of diagnostic and predictive VHL testing

Testing must be done in the setting of a clinical genetics unit for adequate management of expectations regarding sensitivity/specificity of testing and implications of results.

There may be family pressure to be tested; hence, genetic counselling is essential.

For a patient with a clinical diagnosis of VHL and a positive genetic test result, there would be little change to circumstances as it is simply a confirmation or genetic explanation for a condition already known to be present.

For asymptomatic family members with a positive genetic test result, certain knowledge of a known predisposition to VHL syndrome could be overwhelming, causing psychological harm—although, with pre-test counselling from a clinical genetics unit or similar service, there are seldom major long-term problems.

Clinical need

VHL syndrome affects approximately 1 in 91,000 people worldwide, with a birth incidence of 1 in 36,000 live births. It is characterised by both benign and malignant tumours in specific organs of the body, including the central nervous system (CNS), eye, inner ear, kidney, pancreas, adrenal gland, and epididymis in the male and broad ligament in the female.

The mean age of onset of VHL disease is 26 years, and 90% of affected individuals will show signs of the disease by age 65 years. Before routine comprehensive screening, the median survival of patients with VHL syndrome was less than 50 years (Lonser et al 2003; Maher et al 1990). Today, the life expectancy is similar to the norm due to improved screening guidelines (Nordstrom-O'Brien et al 2010). Mortality is mostly due to metastases of renal cell carcinoma (clear-cell) and complications of haemangioblastomas of the CNS (Barontini & Dahia 2010; Nordstrom-O'Brien et al 2010).

There is an association between genotype and phenotype that forms the basis of the clinical classification of VHL syndrome. Type 1 VHL disease does not include phaeochromocytoma, whereas phaeochromocytoma is a common feature of type 2 disease. Type 2 disease can be further separated into three categories: type 2A disease is associated with a low risk of renal cell carcinoma and pancreatic cysts, type 2B has an increased risk of renal cell carcinoma and pancreatic cysts, and type 2C is characterised by phaeochromocytoma only (Barontini & Dahia 2010).

The genetic defects of these subgroups are also distinct. Whereas type 2 disease is caused almost exclusively by missense mutations, type 1 disease can result from deletions and truncations in addition to missense mutations. Knowing the type of VHL disease could aid medical practitioners in targeting screening towards the most likely manifestations of the syndrome in that patient.

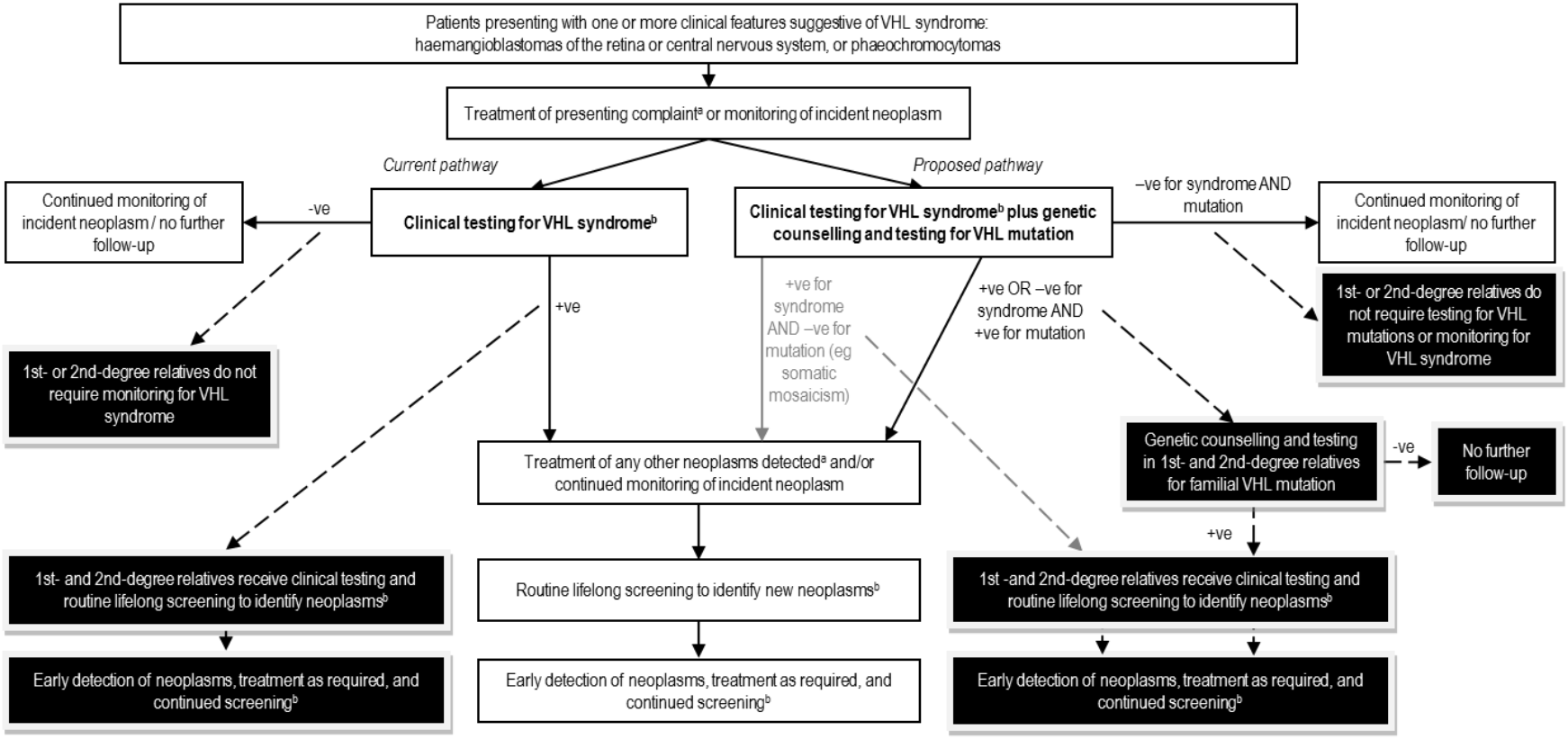
Predictive VHL genetic testing would allow triaging of first- and second-degree family members of patients with confirmed mutations in the *VHL* gene, providing a mechanism for identifying the individuals that require lifelong routine screening. Those who do not have the mutation do not need to undergo unnecessary lifelong screening procedures, and those that do have the mutation can receive screening targeted according to their VHL disease subtype.

There will be a small number of patients who receive a negative VHL genetic test despite having a range of VHL-type tumours. These patients and their close family members would still require lifelong monitoring, as it is possible that they have a VHL mutation, such as a splicing mutant or a mutation in the promoter region, which lies outside the area tested. Additionally, a small number of patients may have somatic genetic mosaicism, which occurs when the somatic cells of an individual are of more than one distinct genotype due to mutations occurring during embryonic development (De 2011). It is therefore possible to have a genetic mutation within cells of one part of the body, resulting in VHL syndrome, that is undetectable by testing the peripheral blood supply. The real incidence of somatic mosaicism in VHL patients is unclear (Santarpia et al 2007).

In Australians with familial cancer, there are approximately 11.5 first- and second-degree relatives per patient with a documented heritable mutation. Of these, approximately 40% take up the offer of pre-symptomatic genetic testing (Pathology Services Table Committee 2010).

A management algorithm is provided below for both the diagnostic and predictive use of VHL genetic testing (Figure 1).

Figure 1 Management algorithm for use of VHL genetic testing in patients who present with clinical features suggestive of VHL syndrome, as well as their first- and second-degree relatives



Outcomes

Direct effectiveness :	Primary = mortality, overall/progression-free survival, quality of life, incidence and severity of life-threatening events Secondary = incidence of symptoms, cancer detection rates, tumour stage, age at diagnosis
Predictive accuracy:	Sensitivity and specificity (and therefore rates of false positives and negatives), positive and negative likelihood ratios, positive and negative predictive values (and therefore false alarm and reassurance rates), diagnostic odds ratios, receiver operator characteristic curves, area under the curve, accuracy
Change in management :	Rate and type of referral, frequency and compliance with clinical screening, rate and type of treatment, hospital separations and re-admissions, length of hospital stay
Safety:	Psychological and physical harms from testing and clinical screening

Notes:
1st-degree relatives are parents, offspring and siblings that share 50% of their genes; 2nd-degree relatives are grandparents, grandchildren, uncles, aunties, nephews, nieces and half-siblings that share 25% of their genes
[a] Surgical resection, radiotherapy, laser therapy, anti-VEGF therapy; [b] Clinical testing = computed tomography, magnetic resonance imaging, ultrasound, urine and blood tests, family history, clinical history and other tests as appropriate to identify any signs of disease other than presenting complaint; biopsy and histopathology of any neoplasms; [c]Screening = CT, MRI, ultrasound, urine and blood tests; CNS=central nervous system

Comparator to the proposed intervention

Diagnosis of VHL syndrome is currently based on clinical criteria. Patients with a family history and a haemangioblastoma (including retinal), phaeochromocytoma or renal cell carcinoma are diagnosed with the disease. Those with no relevant family history must have two or more haemangioblastomas, or one haemangioblastoma and a visceral tumour (with the exception of epididymal and renal cysts, which are frequent in the general population), to meet the diagnostic criteria (Nordstrom-O'Brien et al 2010).

The healthcare resources required to clinically diagnose and monitor patients with VHL syndrome and asymptomatic family members with a confirmed VHL mutation would be the same for both intervention and comparator. Only family members with no pathogenic mutations in the *VHL* gene do not require clinical screening.

Scientific basis of comparison

No studies were identified that met the inclusion criteria on which to judge the safety of genetic testing for VHL mutations. A total of 14 case series (level IV interventional evidence) reported on the likelihood of VHL mutation positive patients developing various VHL-associated neoplasms, and 5 of these studies provided health outcome data following VHL genetic testing. However, these uncontrolled studies did not provide any useful information on the effectiveness of using VHL genetic testing compared with clinical testing alone. A linked evidence approach was therefore taken, resulting in the inclusion of 71 level III-2 diagnostic accuracy studies, 51 level IV case series providing diagnostic yield data, and 5 case series providing non-comparative data on patient management following VHL genetic testing. Data on the effectiveness of different treatment strategies following VHL genetic testing were not evaluated, as the Protocol Advisory Standing Committee advised that the currently available treatment strategies were unlikely to differ based on the method of diagnosis.

Comparative safety

Key results

Genetic testing requires sampling of the patient's blood, generally from veins in the upper limbs. Rarely, this may result in physical harms such as pain, bruising, nerve damage, arterial puncture or infection of the puncture site (Lavery & Ingram 2005; Scales 2008). However, no studies were identified that could inform an assessment of the safety of genetic testing in the diagnosis of VHL syndrome or for identification of family members with a VHL mutation.

Comparative effectiveness

Direct evidence

No comparative direct evidence was identified that reported a change in patient health outcomes following genetic testing either i) in addition to usual clinical diagnosis when compared with usual clinical diagnosis alone in patients suspected of having VHL syndrome or ii) when used as a triage test for lifelong screening of their family members.

Ten case series reported on the likelihood of VHL mutation positive patients developing various VHL-associated neoplasms (Table 3).

Table 3 Summary of prevalence/incidence and mean age of onset of VHL-related neoplasms

Neoplasm	Mean age at onset	Prevalence	Incidence from 1971 to 2008	Incidence as first manifestation
CNS haemangioblastoma	31 years (range 8–57)		77.8%	31.5%
Retinal haemangioblastoma	20–24 years (range 5.5–62)	48.8–67.8% 10% at age 9.3 years 95% at age 37.5 years 100% at age 55.3 years	53.7%	27.8%
Phaeochromocytoma	20.5 years (range 8–36)		11.1%	3.7%
Endolymphatic sac tumour		4.5–16.3%		0%
Renal cysts	32 years (range 12–57)		46.3%	0%
Renal cell carcinoma	31.5 years (range 23–55)		18.5%	7.4%
Pancreatic cysts	29 years (range 12–63)	35.3–55.5%	35.2%	3.7%
Pancreatic tumours	41 years (range 24–57)	**PNETs** 10.7–19.1% **Malignant PNETs** 2.1–-2.7%	9.3%	1.9%
Liver cysts or tumours	39 years (range 27–45)		9.3%	0%
Cysts of the broad ligament	23 years		1.9%	0%
Epididymal cystoadenoma	21 years (range 10–37)	41.2%	11.1%	1.9%

CNS = central nervous system; PNETs = pancreatic neuro-endocrine tumours

Three studies examined the likelihood of patients with a VHL mutation suffering from vision loss or blindness due to the presence of, or treatment for, retinal haemangioblastomas. The overall probability of a VHL mutation positive patient incurring vision loss was 24–35% (Niemela et al 2000; Webster et al 1999b), but this increased to

55–71% if the haemangioblastoma was symptomatic at the time of diagnosis and decreased to only 3% of eyes with asymptomatic haemangioblastomas (Kreusel et al 2006; Webster et al 1999b). The risk of blindness varied greatly, with Niemela et al (2000) reporting that 18.2% of their patients lost sight in an eye, and Kreusel et al (2006) finding that only 5.3% of their patients did. This variability could be explained by the difference in sample sizes.

Neumann et al (1999) reported that adrenal-sparing surgery in treating VHL mutation positive patients with symptomatic phaeochromocytomas was usually successful, and that only 1 patient out of 33 became steroid dependent, due to loss of adrenal function, over a 6-year follow-up period.

Two studies reported on health outcomes in VHL mutation positive patients with renal cell carcinoma. Neumann et al (1998) found that the overall 10-year survival rate for VHL patients with renal cell carcinoma was 86%, even though 36% of patients with tumours larger than 7 cm developed metastatic disease. Joly et al (2011) found that 17.7% of VHL patients with renal cell carcinoma required haemodialysis and 8% required renal transplantation. Both studies found similar VHL-associated mortality rates (15.0% and 20.6%, respectively).

Key results

The data obtained highlighted the health benefits resulting from annual screening but provided no information on the direct effectiveness of genetic testing in addition to current clinical management. Any health benefits would stem from early detection and treatment of newly developed VHL-associated neoplasms and thus reduced morbidity and mortality. As the annual screening protocol is identical for all VHL syndrome patients, irrespective of their VHL mutation status, and their at-risk family members, the lack of comparative data was predictable.

Linked evidence

Diagnostic accuracy of VHL genetic testing in patients suspected of having VHL syndrome

Eighty-one studies met the inclusion criteria outlined *a priori* and reported on the analysis of VHL mutations in the diagnosis of VHL syndrome in patients presenting with one or more VHL-associated neoplasms.

Fifty-six comparative studies provided data on the diagnostic accuracy (level III-2 diagnostic evidence) of genetic testing alone, compared with current clinical diagnosis alone for patients who could potentially have VHL syndrome. The pre-specified reference standard, of clinical diagnosis in the long term, was not available. In the absence of long-term clinical diagnostic data, these studies were included, although the estimates of

sensitivity and specificity of the genetic test were imperfect. The studies were grouped according to the study population and the genetic testing methodology used.

Twenty-four studies only included patients who had all been clinically diagnosed with VHL syndrome. Although this is representative of a large proportion of patients expected to undergo genetic testing, the absence of patients with a negative clinical diagnosis meant that test specificity could not be determined. Fifteen studies included patients presenting with one or more neoplasms associated with VHL syndrome. The remaining studies included patient groups that had a specific VHL-associated neoplasm, with or without a clinical diagnosis of VHL syndrome, and thus are representative of the type of patients expected to undergo genetic testing to diagnose VHL syndrome.

Studies that used DNA sequencing methodologies were divided into two groups, depending on whether or not the PCR products obtained from the patient's *VHL* gene were pre-screened prior to sequencing. Pre-screening methods detect differences in the physical properties of PCR products derived from a mutant *VHL* gene compared with a normal control *VHL* gene; only the PCR products that have different properties to the normal PCR products are sequenced. Alternatively, all PCR products can be directly sequenced without using any pre-screening methods; direct DNA sequencing is the current standard used by laboratories offering VHL genetic testing (Gene Tests 1993). Large deletions or rearrangements, involving part, or all, of the *VHL* gene cannot be detected by DNA sequencing if a second normal copy of the *VHL* gene is present. These are detected using methods such as MLPA and Southern blotting. Many studies used both a DNA sequencing method to detect small mutations and a large deletion detection method. These studies were separated into two groups depending on whether or not a pre-screening methodology was used.

The sensitivity of the VHL genetic test depended heavily on the different genetic testing methodologies used, and varied greatly between patient groups except those involving phaeochromocytoma patients. In these studies VHL mutations were detected by DNA sequencing (with or without pre-screening), and 7 out of 8 studies had a sensitivity of 100%, compared with a sensitivity of 44.4–91.4% for DNA sequencing in studies involving other VHL patient groups. Due to the homogeneous nature of the VHL mutations in this patient group, a higher sensitivity for DNA sequencing is expected. Thus, the median sensitivity was calculated using all studies except those involving phaeochromocytoma patients.

The diagnostic accuracy results for different genetic testing methodologies are summarised in Table 4. Direct DNA sequencing of the *VHL* gene seems more successful at identifying small errors than sequencing of pre-screened PCR products. The false negative rate of 24.9% for direct DNA sequencing studies correlates with the 20–30% of

the VHL families that have large germ-line deletions of all or part of the *VHL* gene that are not detectable by DNA sequencing (Ciotti et al 2009). This also explains the low median sensitivity for studies that used methodologies to detect large deletions of the *VHL* gene. When DNA sequencing with pre-screening was combined with a deletion detection methodology, the sensitivity improved to 74.6% (range 14.3–100). However, when direct DNA sequencing and a deletion detection methodology were both used, the median sensitivity improved even further to 100% (range 70–100). Currently, all laboratories that offer genetic testing of the *VHL* gene offer direct DNA sequencing, and most combine this with a deletion detection methodology such as MLPA, suggesting that most diagnostic laboratories should be able to correctly identify nearly all patients that carry a germ-line VHL mutation. However, the false negative rate of 10.2% suggested that germ-line VHL mutations are not yet detectable in all patients with VHL syndrome.

Table 4 Median and range of diagnostic accuracy data from studies with a low–medium risk of bias for different genetic testing methodologies

Genetic testing methodology	Sensitivity[a]	Specificity[b]	PPV[b]	NPV[b]
Pre-screened DNA sequencing	66.9% (51.8–87.5) FN = 40.5% [37.5, 43.6] k = 8 (2 Q1)	95.0% (88.9–100) FP = 3.4% [1.1, 9.0] k = 5 (3 Q1)	97.8% (85.7–100) k = 5 (3 Q1)	72.2% 30.3–100) k = 5 (3 Q1)
Direct DNA sequencing	76.9% (44.4–91.4) FN = 24.9% [21.5, 28.6] k = 13 (5 Q1)	100% (57.1–100) FP = 5.2% [3.3, 8.1] k = 8 (2 Q1)	100% (36.0–100) k = 8 (2 Q1)	80.9% (14.3–100) k = 8 (2 Q1)
Deletion detection (DD) methods	17.4% (3.9–36.6) FN = 85.7% [83.6, 87.5] k = 18 (5 Q1)	100% (100–100) FP = 0% [0, 10.0] k = 5 (0 Q1)	100% (100–100) k = 5 (0 Q1)	17.1% (4.8–52.4) k = 5 (0 Q1)
Pre-screened DNA sequencing plus DD	74.6% (14.3–100) FN = 27.4% [25.2, 29.8] k = 15 (6 Q1)	94.9% (50.0–100) FP = 5.1% [3.9, 6.5] k = 9 (4 Q1)	97.1% (54.2–100) k = 9 (4 Q1)	80.0% (12.5–100) k = 9 (4 Q1)
Direct DNA sequencing (no pre-screening) plus DD	100% (70.0–100) FN = 10.2% [7.8, 13.0] k = 17 (7 Q1)	100% (50.0–100) FP = 4.2% [1.6, 10.1] k = 8 (1 Q1)	100% (77.8–100) k = 9 (1 Q1)	100% (33.3–100) k = 8 (1 Q1)

[a] Median and range measured using all studies except those involving phaeochromocytoma patients; [b] Median and range measured using all studies including those involving phaeochromocytoma patients, except those involving only patients with a clinical diagnosis of VHL syndrome; the median values for all groups did not vary significantly if only studies with a low risk of bias were included in the analysis; low-quality studies with a high risk of bias were also excluded from the calculations; the 95% CI for false positives and false negatives are within square brackets

CI = confidence interval; FN = false negatives; FP = false positives; k = number of studies; NPV = negative predictive value; PPV = positive predictive value; Q1 = high-quality study with a low risk of bias

The median specificity for all studies that involved patients with both a positive and negative clinical diagnosis of VHL syndrome was uniformly high (94.9–100) across genetic test methodologies, with a false positive rate between 0% and 5.2%. It is highly likely that the few patients with a false positive result actually had the first manifestations of VHL syndrome but their disease had not yet progressed sufficiently to

obtain a positive clinical diagnosis. The high positive predictive value indicates that a patient with a positive test result has a very high probability of having a true germ-line VHL mutation. The negative predictive value for direct DNA sequencing plus a deletion detection methodology, corresponding to current laboratory standard for VHL genetic testing, had a median negative predictive value of 100%, indicating that patients with a negative test result are unlikely to have an undetected VHL mutation.

Twenty-three case series reported on the diagnostic yield (level IV diagnostic evidence) of genetic testing for VHL mutations when used to diagnose patients presenting with clinical signs of disease. Sixteen studies, divided into three groups, provided diagnostic yield data for VHL genetic testing of patients diagnosed with phaeochromocytomas. A group of 3 studies involving phaeochromocytoma patients with or without VHL, and other syndromic diseases such as multiple endocrine neoplasia type 2 (MEN 2), had 10.2% of patients overall with a VHL mutation.

Four studies provided diagnostic yield data for VHL genetic testing of patients with familial phaeochromocytomas but no other symptoms for syndromic diseases such as VHL or MEN 2. Overall, VHL mutations were detected in 45.8% of patients with familial phaeochromocytomas. This suggests that approximately half of all patients who present with phaeochromocytomas and have a family history of only this type of neoplasm carry a VHL mutation, corresponding to type 2C VHL syndrome, which is characterised by phaeochromocytomas in the absence of other clinical manifestations.

The overall probability of patients with sporadic CNS and retinal haemangioblastomas, pancreatic neuro-endocrine tumours, and renal cell carcinomas having a germ-line mutation in the *VHL* gene were 5.1% (5/98), 0% (0/27), 1.0% (1/101) and 1.6% (3/187), respectively. As retinal haemangioblastomas are a common first manifestation of VHL disease (according to Poulsen et al (2010) in 27% of patients), the lack of VHL mutations identified in patients with sporadic retinal haemangioblastomas is probably due to the small size of the 2 studies.

Diagnostic accuracy of VHL genetic testing in family members of patients with a known VHL mutation

Forty-one studies met the inclusion criteria outlined *a priori* and reported on the analysis of VHL mutations in the pre-symptomatic genetic testing of close relatives of index patients (or probands) that carry a known *VHL* gene mutation. Fifteen studies provided comparative data (level III-2 diagnostic evidence) reporting on the predictive accuracy of genetic testing compared with clinical diagnosis in first-degree relatives (4 studies) or a combination of first- and second-degree relatives (12 studies). Twenty-six studies reported on the diagnostic yield (level IV diagnostic evidence) of pre-symptomatic

genetic testing of first-degree relatives (12 studies), second-degree relatives (2 studies), and a combination of first- and second-degree relatives (17 studies).

The median and range of diagnostic accuracy data from the studies with a low–medium risk of bias for first-degree and first- and second-degree relatives of a known VHL mutation carrier are shown in Table 5. As anticipated, all relatives that showed symptoms of VHL syndrome were found to have inherited the familial germ-line VHL mutation, resulting in a sensitivity of 100% and no false negatives. Conversely, as VHL genetic testing can identify relatives who have inherited the familial germ-line VHL mutation before the manifestation of clinical signs of disease, a high level of 'false positives' was anticipated. Indeed, the median specificity for first-degree relatives was 78% (range 50.0–100) and first- and second-degree relatives 85.0% (range 42.9–100), with a false positive rate of 23.5% and 16.9%, respectively.

Table 5 Median and range of diagnostic accuracy data from studies with a low–medium risk of bias for relatives of a known VHL mutation carrier

Relatives	Sensitivity	Specificity	PPV	NPV
First-degree relatives	100% (100–100) FN = 0% [0, 11.4] k = 4 (1 Q1)	83.3% (50–100) FP = 23.5% [7.8, 50.2] k = 3 (0 Q1)	69.4% (33.3–100) k = 4 (1 Q1)	100% (100–100) k = 3 (0 Q1)
First- and second-degree relatives	100% (100–100) FN = 0% [0, 8.0] k = 10 (5 Q1)	85.0% (42.9–100) FP = 16.9% [10.9, 25.2] k = 5 (3 Q1)	47.8% (20.0–100) k = 10 (5 Q1)	100% (100–100) k = 5 (3 Q1)

[a] Median (range) for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) measured using all studies except those with a high risk of bias; the 95% CI for false positives and false negatives is within square brackets

CI = confidence interval; FN = false negatives; FP = false positives; k = number of studies; Q1 = high quality study with a low risk of bias

The specificity and false positive rates are dependent on the age of the relatives being tested. The younger the relative, the more likely it is that genetic testing has occurred before any signs of VHL syndrome could be detected. The mean age of onset for most VHL-associated neoplasms is 20–40 years (Table 3). The median positive predictive values for first-degree relatives (69.4% (range 33.3–100)) and both first- and second-degree relatives (47.8% (range 20.0–100)) reflect the prevalence of the condition in each subgroup, and also indicate that a positive genetic test result will not always correlate with a positive clinical test result—a consequence of the difference in the timeframe required for a clinical versus a genetic diagnosis.

All studies involving VHL genetic testing of first- and/or second-degree relatives of a known VHL mutation carrier had a negative predictive value of 100%. This was predictable, as relatives with a negative VHL genetic test result will only develop neoplasms associated with VHL syndrome at the same rate as the rest of the Australian population.

The 12 studies that reported on the likelihood of first-degree relatives inheriting a germ-line VHL mutation had an overall diagnostic yield of 36.0% (41/114) for all first-degree relatives and 26.8% (19/71) for asymptomatic first-degree relatives. This is lower than the 50% of first-degree relatives expected to inherit the VHL mutation, and is probably due to the absence of the mutation in parents and siblings of patients with *de novo* VHL syndrome, other symptomatic members of the family being tested previously or older family members having already died due to complications arising from VHL-related neoplasms.

The 17 studies that involved a combination of first- and second-degree relatives had an overall VHL mutation yield of 38.1% (203/533) for all relatives and 22.4% (93/415) for asymptomatic relatives. The similar yields for studies involving either first-degree or both first- and second-degree relatives is likely due to the larger representation of siblings, parents and children (first-degree relatives) compared with grandparents, aunts, uncles, nieces, nephews and cousins (second-degree relatives).

Changes in patient management

Only 5 studies (level IV interventional evidence) provided evidence regarding a change in patient management following diagnosis of VHL syndrome using genetic testing in combination with clinical diagnosis, but none provided a direct comparison between patients who had been genetically tested and those who had not.

A systematic review presented a narrative report on genotype–phenotype correlations in patients with VHL syndrome and their relatives (Ho et al 2003). Knowledge of a specific germ-line VHL mutation in a patient with a clinical diagnosis of VHL syndrome is not expected to alter patient management significantly. For example, VHL mutation status would not affect patient management for patients presenting with the same VHL-associated neoplasms. However, identifying VHL mutations in patients presenting with their first neoplasm and no family history of VHL syndrome may mean that routine screening would be offered earlier, leading to better long-term patient outcomes. The type of VHL mutation may provide some information about the types of neoplasms that are likely to develop in a particular patient, such that annual screening could be tailored to ensure early detection of those neoplasms most likely to occur.

Conversely, the VHL genetic test is expected to change patient management for asymptomatic relatives when used as a triage test for lifelong screening. Relatives with a negative genetic test result would not require lifelong screening.

One study reported that 88.0% (91/103) of VHL patients with retinal manifestations and 97.0% (105/108) of VHL patients without retinal manifestations agreed to genetic testing (Dollfus et al 2002). This rate is quite high when compared with the number of at-risk relatives of VHL patients with a known VHL mutation who agreed to genetic

testing. Rasmussen et al (2010) and Evans et al (1997) reported that 58.5% (92/157) and 65.8% (48/73), respectively, of at-risk relatives agreed to genetic testing. Evans et al (1997) found that relatives aged over 20 years (94.9%; 37/39) were more likely to undergo genetic testing than children aged less than 5 years (0%; 0/6). This suggests that parents are reluctant to have very young children genetically tested.

Rasmussen et al (2010) also found that only 38.9% (14/36) of patients with a VHL mutation continued screening after 5 years. Symptomatic patients (57.9%; 11/19) were significantly more likely to continue screening after 5 years than asymptomatic patients (17.6%; 3/17; OR = 5 [95% CI 1.2, 20.3]; p = 0.02), which the authors suggest was due to complacency of the asymptomatic patients.

Change in management affects patient health outcomes

Although a search on treatment effectiveness was not undertaken, 2 studies were identified that investigated the health outcomes for patients with both a clinical and a genetic diagnosis of VHL syndrome presenting with neoplasms detected by annual screening, compared with detection due to case finding (ie presenting with symptoms). Kreusel et al (2006) found that eyes treated for symptomatic retinal haemangioblastomas had adverse visual outcomes in 71.4% of cases, compared with only 3.0% for asymptomatic eyes. Rasmussen et al (2010) found a small decrease in the mortality rate for the group that had annual screening compared with those that did not (7.1% versus 9.1%), although this difference was not statistically significant (p>0.05).

Key results

Diagnostic accuracy of VHL genetic testing in patients suspected of having VHL syndrome

The current standard VHL genetic testing methods of direct DNA sequencing of PCR products from all three exons of the *VHL* gene, plus a method to detect large deletions of the *VHL* gene such as MLPA, should be highly accurate. The median sensitivity, specificity, and positive predictive and negative predictive values, for these genetic tests were uniformly high. However, the false negative rate of 10.2% suggests that detection of a germ-line mutation is not yet possible for some patients with VHL syndrome. Thus, VHL genetic testing should not be used as a standalone test for the diagnosis of VHL syndrome. Clinical diagnosis of VHL syndrome is still required for patients presenting with VHL-related neoplasms.

The false positive rate of 4.2% was expected, as there will always be a few patients who do not currently meet the criteria for clinical diagnosis of VHL syndrome but have an underlying VHL mutation. In these patients the disease would be expected to progress such that a positive clinical diagnosis would be made in the future.

Patients with familial phaeochromocytomas have a 50% probability of having a VHL mutation that is indicative of type 2C VHL syndrome.

Diagnostic accuracy of VHL genetic testing in family members of patients with a known VHL mutation

Once an index case has a pathogenic VHL mutation identified, their close relatives need only be tested for that specific mutation, using a testing methodology known to be able to detect that type of mutation. Thus, every included study reporting accuracy data for relatives of a patient with a known VHL mutation reported a sensitivity of 100%. The median specificity of 83.3–85.0% and the false positive rates of 16.9–23.5% reflect the difference in the timeframe required for a positive clinical diagnosis compared with a positive genetic test. Younger relatives are more likely to receive a positive genetic test before any clinical signs of disease can be detected by clinical screening.

Approximately 4 out of 10 of all first- and second-degree relatives, and 2–3 out of 10 asymptomatic first- and second-degree relatives that undergo VHL genetic testing were identified as carriers of the familial VHL mutation.

Changes in patient management

Some evidence was identified regarding patient management following diagnosis of VHL syndrome using genetic testing in combination with clinical diagnosis, but none provided a direct comparison between patients who had been genetically tested and those who had not been tested. Due to the lack of an appropriate comparator group, no conclusions can be made about the change in patient management (ie the clinical impact) from genetic testing.

Interestingly, only 38.9% of patients with a VHL mutation continued screening after 5 years. Symptomatic patients were more likely to continue than asymptomatic patients. Patients who have symptoms or have a neoplasm detected early are more aware of the personal risks involved than patients who have not developed any detectable neoplasms.

While 88.0–97.0% of clinically diagnosed VHL patients agreed to genetic testing, only 58.5–65.8% of at-risk relatives agreed. Additionally, relatives aged over 20 years were more likely to undergo genetic testing than children aged less than 5 years, suggesting that parents are reluctant to have very young children genetically tested.

Change in management affects patient health outcomes

The Protocol Advisory Sub-Committee (PASC) made the decision that it was not necessary to conduct a separate literature search to assess the effectiveness of treatments in patients with VHL-associated neoplasms, as these were well established. Data obtained supported this assumption and highlighted that health benefits are

derived from annual screening for early detection of newly developed neoplasms, through reduced morbidity and mortality. Annual screening of clinically diagnosed VHL syndrome occurs irrespective of VHL mutation status. In at-risk relatives, VHL testing acts to triage candidates for annual screening.

Economic evaluation

Cost comparison

In the absence of direct evidence for the increased effectiveness of the addition of genetic testing to clinical testing, at least equal effectiveness was assumed and a cost comparison was performed. The assumption of equal effectiveness is a conservative one.

The analysis considered the costs associated with an individual suspected of having VHL syndrome (the index case) and the costs associated with testing and monitoring (annual screening) their first- and second-degree relatives (who are at risk of having the VHL mutation). The first part of the analysis delivers individuals or family members into either monitoring or no-monitoring health states based upon the best information known from either genetic and clinical testing or clinical testing alone. A proportion of family members are assumed to refuse genetic testing (40%) and a proportion to refuse monitoring (60%). This non-compliance is a more realistic situation than 100% adoption of either testing or monitoring, and is important to consider because it will tend to dilute the cost savings associated with the genetic testing arm. Those who are genetically positive (whether this status is known or unknown) but refuse monitoring will transit to a monitoring state once they become symptomatic.

Due to the high sensitivity and specificity of the genetic test compared with a clinical diagnosis, there was very little difference in costs associated with managing the index case between the two arms, except for the cost of the VHL diagnostic test and the genetic counselling. However, when applied to family members, who have an assumed likelihood of carrying the VHL mutation of 26%, there is a marked decrease in monitoring among those who do not require monitoring (22.1%).

Costs of monitoring are assumed to be accrued over a lifetime, with mortality estimated from the Australian life tables (Australian Bureau of Statistics 2010a). Treatment costs are assumed to be equivalent in both arms.

The overall cost saving (through avoided inappropriate monitoring) of a single index case and their family over their lifetimes is $7,749 in discounted costs and $20,783 in undiscounted costs. As there are many uncertainties in the analysis, several sensitivity analyses have been performed. The cost comparison is most sensitive to the prevalence of VHL syndrome among patients who are suspected of having it, and the uptake of

genetic testing and monitoring among family members. In most sensitivity analyses, a cost saving remains following the introduction of VHL genetic testing. Furthermore, if monitoring and genetic testing rates among family members increase, the cost saving associated with genetic testing will markedly increase. The cost comparison is not sensitive to moderate changes in the proposed MBS reimbursement for VHL genetic testing.

Overall conclusion with respect to comparative cost-effectiveness

It is likely that the costs associated with managing individuals suspected of having VHL syndrome and their families are fewer when genetic and clinical testing is available, compared with clinical testing alone. This is largely driven by the reduction of monitoring in family members who are not at risk of developing VHL syndrome.

Financial/budgetary impacts

Current usage of the VHL diagnostic test is estimated at 80 tests per year. This is based on data from 2006 and 2007 and may be a high estimate if this was a period of testing a 'backlog' of patients. It has been assumed that the number of tests will increase to 160 per year over 5 years following the listing of VHL genetic testing on the MBS. Again, this may be high and therefore represents a conservative estimate.

Usage of VHL predictive tests are assumed to be 30 per year and will not increase because the numbers rely upon the identification of a VHL mutation rather than any increase in the use of the VHL genetic test.

The costs included in the financial impact analysis are those of the genetic tests, genetic counselling and the cost savings due to averted monitoring.

If VHL genetic testing is listed on the MBS, the total cost for testing and counselling to the Australian healthcare system will be between $86,100 (based on 80 diagnostic tests) and $154,400 (based on a doubling of diagnostic tests) per year.

The costs borne by the MBS for these scenarios will be $64,600 and $115,800, respectively.

The costs avoided through improvements in targeted monitoring will increase annually as an increasing number of people are spared lifelong monitoring. In 5 years, based on sparing monitoring for 10 people per year, the saving to the Australian healthcare system will be $34,300, of which $25,800 will be saved by the MBS.

Table 6 Annual costs to the MBS, other governments and patients of genetic testing and genetic counselling, with cost savings from avoided monitoring

Total costs (50% reduction in monitoring)	2012	2013	2014	2015	2016
MBS	$59,445	$67,103	$74,760	$82,417	$90,074
Other government	$14,861	$16,776	$18,690	$20,604	$22,518
Patient/insurer	$4,954	$5,592	$6,230	$6,868	$7,506
Total	**$79,261**	**$89,470**	**$99,680**	**$109,889**	**$120,099**

Overall, the cost of genetic testing to the Australian healthcare system, accounting for savings associated with avoided monitoring, will be between $79,300 and $120,100 (Table 6).

Importantly, the cost of VHL genetic testing will plateau, given that the incidence of VHL syndrome is unlikely to increase, whereas the savings associated with avoided lifelong monitoring will continue to increase for many years, eventually resulting in a net cost saving to the Australian healthcare system. It should be recognised that this financial impact analysis is flawed because the test is already being utilised. Therefore, the listing of the genetic test on the MBS may not result in a substantial decrease in cost to the Australian healthcare system, but rather a shift of costs from the state/territory governments or the individual to the MBS. As VHL genetic testing is already being done, the MBS is currently receiving a cost saving from avoided monitoring, for which it would bear the majority of the costs.

Key uncertainties

Key uncertainties for safety of VHL genetic testing

No studies were identified that could inform an assessment of the safety of genetic testing in the diagnosis of VHL syndrome, or for identification of family members with a VHL mutation.

Overall conclusion with respect to comparative safety

Even with a lack of evidence, the likelihood of adverse events as a consequence of VHL genetic testing are low, but it is recognised that there are some risks associated with genetic testing. These relate to minor injuries associated with venepuncture, as well as psychological harms from a positive VHL diagnosis. False negative or false positive test results may also cause psychological harms, and possibly physical harms as well due to delayed or inappropriate treatment. However, in the case of VHL genetic testing, patients with a false positive test result are probably true carriers of a VHL mutation, in whom a positive clinical diagnosis could not be given at the time. Few patients should receive a false negative test result when tested using dual test methods, and they will

still receive annual screening upon becoming symptomatic, minimising any potential harms.

Key uncertainties for effectiveness of VHL genetic testing

No comparative direct evidence was identified that could inform an assessment of the effectiveness of VHL genetic testing in addition to usual clinical diagnosis in patients suspected of having VHL syndrome, or when used as a triage test for lifelong screening of family members.

The *a priori* reference standard of clinical diagnosis in the long term was not reported in any studies. Hence, diagnostic accuracy of VHL genetic testing was measured against the imperfect standard of current or short-term clinical diagnosis.

Similarly, routine clinical screening provided an imperfect reference standard against which the accuracy of pre-symptomatic genetic testing was measured.

It is uncertain as to what proportion of patients and at-risk relatives would agree to genetic testing, as well as the proportion who would comply with annual screening for VHL-associated neoplasms (which could vary over different time horizons).

Minimal evidence was identified regarding a change in patient management following diagnosis of VHL syndrome using genetic testing in combination with clinical diagnosis; and, due to the lack of a comparative group using clinical diagnosis alone, no comments can be made about the incremental clinical impact of genetic testing on patient management.

Overall conclusion with respect to effectiveness of VHL genetic testing

There were no data available to determine the direct health impact of including genetic testing as part of the current diagnostic strategy for patients suspected of VHL syndrome and their relatives. However, by linking evidence on the accuracy of VHL testing in individuals with change in management data, it is clear that most of the benefits from testing will accrue from reducing the need to screen for VHL-associated neoplasms in asymptomatic family members who test negative for the mutation.

Other relevant factors

Counselling services

Listing genetic counsellor services on the MBS may reduce the overall cost of genetic testing to the government by reducing the use of specialists for all genetic counselling associated with VHL genetic testing.

Australian VHL registry

Currently, there is no Australian registry for patients with VHL syndrome and their relatives that carry a VHL mutation. A registry would provide important data for the management of patients with VHL, while maintaining the individual's privacy and confidentiality.

Quality assurance and molecular methodologies

Three laboratories accredited by the National Association of Testing Authorities (NATA) currently conduct genetic testing to identify mutations in the *VHL* gene (Royal College of Pathologists of Australasia 2008).

Previously, there has not been a quality assurance program (QAP) to monitor the performance of laboratories providing VHL genetic testing (RCPA Quality Assurance Programs Pty Ltd 2009). However, the RCPA/HGSA Molecular Genetics QAP Committee has commenced monitoring the performance within and between the three laboratories that offer VHL genetic testing using in-house methodologies from 2010.

Additional applications for VHL genetic testing

Somatic VHL genetic testing of CNS haemangioblastomas

Currently, patients presenting with isolated CNS haemangioblastomas are routinely tested for both germ-line VHL mutations and somatic VHL mutations in the tumour itself.

However, the proposed MBS items do not allow for reimbursement for somatic VHL genetic testing as the descriptor has been limited to the 'detection of germ-line mutations of the *VHL* gene'.

Prenatal and pre-implantation VHL genetic testing

With increased understanding of the consequences and likelihood of having children affected by a familial VHL mutation, parents are looking for ways to ensure that their offspring are unaffected. They have two main options.

- Prenatal diagnostic tests such as chorionic villus sampling and amniocentesis, which are only useful if the parents are willing to abort the affected foetus. Amniocentesis also has an associated risk of miscarriage. Prenatal predictive VHL genetic testing would not be reimbursed under Medicare as the foetus is not considered an 'eligible person' for health insurance.

- Pre-implantation genetic diagnosis, which is performed on the embryo prior to implantation and is only offered in the private setting in Australia. The Victorian Assisted Reproductive Treatment Authority lists the *VHL* gene as one of the single gene disorders that were tested using pre-implantation genetic diagnosis in 2010.

Targeted therapies

The elucidation of how pVHL functions in tumour suppression has increased our understanding of how cancer develops. This has led to the development of targeted therapies that target proteins that are overexpressed due to the loss of the suppressor function of pVHL. Disrupting the function of these proteins interferes with tumour progression. Tumours that develop due to the loss of pVHL activity may respond to treatment using angiogenic inhibitors (sorafenib, sunitib, pazopanib and axitinib) or vascular epidermal growth factor receptor (VEGF) antibodies (bevacizumab) to slow down the rate of angiogenesis, thus inhibiting growth of the tumour. Mammalian target of rapamycin (mTOR) inhibitors (temsirolimus and everolimus) or the histone deacetylase inhibitor (sodiumbutyrate), which act on the overexpressed HIF protein in tumour cells, may offer other treatment options for patients with renal cell cancer.

As our understanding of the differences in activation of the various pathways affected by pVHL due to different VHL mutations increases, therapies may be developed that can be targeted to counteract the neoplastic effects caused by specific VHL mutations.

Ethical considerations

All things considered, genetic testing appears ethically acceptable provided that it is both preceded and followed by adequate counselling to ensure informed consent and minimise risks of harm, both psychological and, in the longer term, physical. Counselling should include, among other things, the limitations and significance of test results, including possible ramifications for family members, and the possible courses of effective treatment should a test result be positive.

Test results should remain confidential, although the patient should be counselled on the benefit of sharing information with family members who may benefit from knowledge of VHL mutation status. As always, confidentiality should be broken only if risks to others are serious, imminent, certain and unavoidable, and attempts at encouraging voluntary disclosure have been exhausted.

There should be equitable access to genetic testing for all who might benefit from it, and it should not be a financial burden.

Glossary and abbreviations

AHTA	Adelaide Health Technology Assessment
aPKC	atypical protein kinase C
AR-DRG	Australian Refined Diagnosis Related Group
CI	confidence interval
c-MET	receptor tyrosine kinase c-Met
CNS	central nervous system
CSGE	conformation-sensitive gel electrophoresis
CT	computed tomography
ddNTP	dideoxynucleotide triphosphate
DGGE	denaturing gradient gel electrophoresis
DHPLC	denaturing high-performance liquid chromatography
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide
ECM	extracellular matrix
EPO	erythropoietin
EGFR	epidermal growth factor receptor
FISH	fluorescence in-situ hybridization
GFR	glomerular filtration rate
Glut1	glucose transporter type 1
GT	genetic test
HB	haemangioblastoma
HIF	hypoxia inducible factor
IVD	in vitro diagnostic medical device
IMVS	Institute of Medical and Veterinary Science
MLPA	multiplex ligation-dependent probe amplification
MBS	Medicare Benefits Schedule
MEN 2	multiple endocrine neoplasia type 2
MESP	Medical Expert Standing Panel
MMP	matrix metalloproteinase
MRI	magnetic resonance imaging
MSAC	Medical Services Advisory Committee
mTOR	mammalian target of rapamycin

NATA	National Association of Testing Authorities
NF1	neurofibromatosis type 1
NHMRC	National Health and Medical Research Council
NPAAC	National Pathology Accreditation Advisory Committee
NPV	negative predictive value
OM	ocular manifestations
p53	tumour suppressor p53
PASC	Protocol Advisory Sub-Committee
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
PDGFR	platelet-derived growth factor receptor
PGL	paraganglioma
PNET	pancreatic neuro-endocrine tumour
PPV	positive predictive value
PSTC	Pathology Services Table Committee
pVHL	the VHL protein
QAP	quality assurance program
Q-PCR	quantitative PCR
RCC	renal cell carcinoma
RCPA	Royal College of Pathologists of Australasia
RR	relative risk
SB	Southern blotting
SNAIL	(transcription factor) SNAIL
SSCP	single-strand conformational polymorphism
TCF–LEF	T-cell factor–lymphoid enhancer factor
TGA	Therapeutic Goods Administration
TGFα	transforming growth factor α
UB	ubiquitin
UPQFM-PCR	universal primer quantitative fluorescent multiplex PCR
VEGF	vascular endothelial growth factor
VEGFR	vascular epidermal growth factor receptor
VHL	von Hippel-Lindau

Introduction

A rigorous assessment of evidence is the basis of decision-making when funding is sought under Medicare.

The Medical Services Advisory Committee (MSAC) evaluates new and existing health technologies and procedures for which funding is sought under the Medicare Benefits Schedule (MBS), in terms of their safety, effectiveness and cost-effectiveness, while taking into account other issues such as access and equity. The MSAC adopts an evidence-based approach to its assessments, based on reviews of the scientific literature and other information sources, including clinical expertise.

The MSAC is a multidisciplinary expert body, comprising members drawn from such disciplines as diagnostic imaging, pathology, surgery, internal medicine, and general practice, clinical epidemiology, health economics, consumer health and health administration.

A team from Adelaide Health Technology Assessment (AHTA), School of Population Health and Clinical Practice, University of Adelaide, as part of its contract with the Department of Health and Ageing, was engaged to conduct a systematic review of the literature on VHL testing in order to inform MSAC's decision-making regarding public funding of the intervention.

Input and advice from members of a Medical Expert Standing Panel (MESP; see Appendix B) was sought, and the current evidence was reviewed for genetic testing for mutations in the *VHL* gene to diagnose VHL syndrome in patients with symptoms of the disease, and in family members of a patient with a known VHL mutation.

This report summarises the current evidence for the safety, effectiveness and cost-effectiveness of VHL genetic testing in patients suspected of having VHL syndrome, and in family members of patients with a confirmed VHL mutation.

Rationale for assessment

In November 2010 an application from the Pathology Services Table Committee (PSTC) was received by the Department of Health and Ageing, requesting an MBS listing for genetic testing for hereditary mutations in the *VHL* gene that cause VHL syndrome for (i) patients with symptoms of VHL syndrome and (ii) family members of a patient with a known VHL mutation. Currently, there is no MBS listing for any test that detects germ-line mutations in the *VHL* gene.

Background

Clinical need and burden of disease

Von Hippel-Lindau (VHL) syndrome is an autosomal dominant neoplastic disease with a worldwide birth incidence of 1 in 36,000 (Barontini & Dahia 2010; Kim et al 2010; Lonser et al 2003; Maher et al 1991; Nordstrom-O'Brien et al 2010). Specific data for the prevalence of VHL syndrome in Australia are not available. One recent study conducted in the United Kingdom (UK) found that the prevalence of VHL syndrome was 1 in 91,111 people, with a birth incidence of 1 in 42,987 and a 21% *de novo* (or spontaneous) mutation rate (Evans et al 2010). The decreased birth incidence found in Evans et al (2010) may reflect a combination of increased understanding of the inheritance of genetic diseases and improved reproductive technology, such as prenatal and pre-implantation genetic diagnosis. One study involving adults aged 20–40 years with a known VHL mutation found that 25% of individuals did not want to have children and an additional 50% planned to use prenatal diagnosis and termination of an affected pregnancy (Levy & Richard 2000). The disease is characterised by the development of both benign and malignant tumours, in particular haemangioblastomas of the retina and central nervous system (CNS), especially the cerebellum and spinal cord; endolymphatic sac tumours; phaeochromocytomas; renal cell carcinomas; and cysts in various organs including the kidney, pancreas and liver (Barontini & Dahia 2010; Poulsen et al 2010). VHL syndrome can be subdivided into types 1, 2A, 2B and 2C, depending on the specific neoplasms that manifest within the family (see Table 8).

The mean age of onset of VHL disease is 26 years, and 90% of affected individuals will show signs of the disease by age 65 years. Before routine comprehensive annual screening, the median survival of patients with VHL disease was less than 50 years (Karsdorp et al 1994; Lonser et al 2003; Maher et al 1990). Today, the average life expectancy of a patient with VHL syndrome is not significantly shorter than people without the disease due to improved screening guidelines (Nordstrom-O'Brien et al 2010).

Haemangioblastomas of the CNS

Haemangioblastomas of the CNS are the most common lesion associated with VHL disease (Andrews 2011). They are highly vascular benign tumours, but they may cause important neurological deficits and have been associated with a significant mortality rate (Poulsen et al 2010). In early studies 53% of patients with VHL syndrome died due to complications of cerebellar haemangioblastomas (Karsdorp et al 1994). As surgical techniques have improved, the death rate has fallen dramatically. Haemangioblastomas can occur sporadically, but in about 20–30% of cases they are a component tumour of

VHL disease (Andrews 2011). Cerebellum and spinal cord tumours are the major CNS manifestations and affect 60–84% of patients with type 1 or type 2A and 2B VHL disease. Tumours develop from childhood (less than 10 years of age) but are more common in the third decade of life (Andrews 2011; Barontini & Dahia 2010; Shuin et al 2006).

Retinal haemangioblastomas

Retinal capillary angiomas are non-malignant and are often the first sign of VHL disease (Kreusel et al 2006; Poulsen et al 2010). They can lead to retinal detachment, blindness, cataract and (secondary) glaucoma (Koch et al 2008). They occur less frequently as a sporadic tumour, and usually in older patients. They predominantly appear in the third decade of life, but individuals of any age from early childhood (less than 10 years of age) and older can be affected (Barontini & Dahia 2010; Dollfus et al 2002; Poulsen et al 2010).

Renal cell carcinoma

Approximately two-thirds of patients with VHL disease develop multiple renal cysts and renal cell carcinoma. They develop with increasing frequency over 20 years of age, and it has been reported that by the age of 60 years about 70% of patients with type 1 and type 2B VHL disease have developed a renal cell carcinoma (Barontini & Dahia 2010; Joly et al 2011; Neumann et al 1998).

Phaeochromocytomas

Phaeochromocytomas are frequently benign and are a hallmark of type 2 VHL disease. They appear in younger patients, mostly before the age of 40 years, and paediatric cases are very common. They are catecholamine-producing neuro-endocrine tumours or intra-adrenal paragangliomas, which are embryologically derived from the extra-adrenal chromaffin tissue, the same cells that give rise to the sympathetic nervous system (Donckier & Michel 2010; Waguespack et al 2010). Germ-line mutations in the susceptibility genes responsible for hereditary phaeochromocytoma (*VHL*, *RET*, *SDHB*, *SDHC*, *SDHD*, *NF1*) can be detected in more than 25% of all cases and 40% of paediatric cases (Donckier & Michel 2010; Waguespack et al 2010). *VHL* gene mutations associated with phaeochromocytoma are predominantly missense mutations (Barontini & Dahia 2010).

Pancreatic tumours

Pancreatic tumours or cysts, most of which are benign, develop in 35–77% of VHL patients. They include cystoadenomas (12%), haemangioblastomas (< 1%), adenocarcinomas (< 1%) and neuro-endocrine tumours (5–27%) (Kim et al 2009a; Lombardi et al 2009). The mean age at diagnosis is 29–38 years (Lombardi et al 2009).

Malignant tumours occur in 8–50% of patients and can metastasise to the liver (Barontini & Dahia 2010).

Endolymphatic sac tumours

Tumours of the endolymphatic sac are locally invasive papillary cystoadenomas arising within the posterior temporal bone of the inner ear (Choo et al 2004). Although they can occur sporadically, they are rare in the general population but are frequently associated with VHL disease (Barontini & Dahia 2010; Choo et al 2004).

Cystoadenomas of the adnexal reproductive organs

Papillary cystoadenomas arising from the epididymal duct are usually benign and occur in 25–60% of males with VHL disease, often in their teenage years (Koch et al 2008). Papillary cystoadenomas arising from the broad ligament in females is rare; thus, the frequency and age of usual onset are unknown. Both the epididymis in males and the broad ligament in females are derived from the embryonic mesonephric duct (Lonser et al 2003).

Although, due to improved screening guidelines, the life expectancy of a patient with VHL syndrome is currently not significantly shorter than people without the disease, morbidity is still a significant problem (Nordstrom-O'Brien et al 2010). Physical disability in VHL disease is mostly due to CNS haemangioblastomas and associated postoperative complications (Shuin et al 2006). Retinal haemangioblastomas and endolymphatic sac tumours can also result in physical disability via loss of vision and hearing, respectively (Manski et al 1997; Niemela et al 2000; Webster et al 1999b). Mortality is mostly due to metastases of renal cell carcinoma and complications of CNS haemangioblastomas (Barontini & Dahia 2010; Nordstrom-O'Brien et al 2010).

The Australian Institute of Health and Welfare (AIHW) National Hospital Morbidity Database (by principal diagnosis in ICD-10-AM) for 2007–08 provides data on the number of hospital separations for disease types that would include VHL-associated neoplasms. It also provides data based on age group, which enables the number of separations for patients of a relevant age to be determined. However, the cause of the neoplasm is not defined, and in many cases the specific disease is not differentiated; that is, haemangioblastomas are not separated from other benign neoplasms of the CNS. The total number of hospital separations for relevant principal diagnoses and appropriate age groups is shown in Table 7.

Table 7 Number of hospital separations for disease types and specific age groups that would include VHL-associated neoplasms in Australia in 2007-2008

Principle Diagnosis	Number of total separations	Separations	Age group
C64 Malignant kidney neoplasm	3,980	1,264	20–59 years
D33 Benign neoplasms of the central nervous system	551	79	< 30 years
D31 Benign neoplasms of the eye and adnexia	400	222	< 30 years
D35.0 Benign neoplasms of the adrenal gland	330	17	< 20 years
D13.6 Benign neoplasms of the pancreas	127	13	25–39 years
C25.4 Malignant neoplasm of endocrine pancreas	33	2	25–39 years
D29.3 Benign neoplasms of the epididymus	13	0	10–19 years
D28.2 Benign neoplasms of the uterine tubes and ligaments	125		N/A

In Australians with familial cancer, there are approximately 11.5 first- and second-degree relatives per patient with a documented heritable mutation. Of these, approximately 40% take up the offer of pre-symptomatic genetic testing (Pathology Services Table Committee 2010).

Accredited VHL genetic testing was offered in three Australian states (New South Wales (NSW), South Australia (SA), Western Australia (WA)) based on a survey of genetic testing conducted in 2006 and 2007 (Royal College of Pathologists of Australasia 2008). Over these 2 years, 159 diagnostic tests of patients with clinical signs of disease and 49 predictive tests of family members were conducted.

Mutations in *VHL* gene

VHL syndrome is caused by germ-line mutations or deletions in one copy of the *VHL* tumour suppressor gene located on chromosome 3p25. The second copy of the *VHL* gene is fully functional. The tumours that develop conform with Knudson's 2-hit hypothesis, and arise when spontaneous mutations occur in the second copy of the *VHL* gene in individual cells of affected organs (Clark & Cookson 2008; Kim et al 2010; Knudson 1986).

The prevalence of VHL syndrome is similar in both genders and in all ethnic backgrounds (Nordstrom-O'Brien et al 2010). Most of the mutations are missense mutations[1], but VHL disease can also be caused by nonsense[2] or deletion[3] mutations of the *VHL* gene

[1] A missense mutation occurs when a single nucleotide is changed in the DNA sequence, resulting in a change in the amino acid sequence of the encoded protein that alters its properties or function.

[2] A nonsense mutation occurs when a single nucleotide is changed in the DNA sequence, resulting in a premature stop codon that shortens the encoded protein, making it non-functional.

(Barontini & Dahia 2010; Kim et al 2010). To date, 1,548 germ-line and somatic VHL mutations have been identified, and detailed phenotype and gene mutation information is available for 945 VHL families (Nordstrom-O'Brien et al 2010).

Function of the VHL tumour suppressor protein

The *VHL* gene encodes the VHL tumour suppressor protein (pVHL), which is important for regulating several cellular mechanisms including the oxygen-sensing pathway, the maintenance of primary cilium, the organisation of the extracellular matrix (ECM), and cell apoptosis and senescence, as shown in Figure 2 (Barontini & Dahia 2010; Calzada 2010). The pVHL has two domains: the alpha domain forms a multi-subunit complex with elongin B, elongin C, Cul2 and RBx1, which has E3 ubiquitin ligase activity and functions in proteosomal degradation, while the beta domain functions as the substrate-docking site (Kim et al 2010; Calzada 2010). The best-known VHL-complex substrate is hypoxia inducible factor (HIF). Loss of functional pVHL due to mutations in the *VHL* gene results in the deregulation of several HIF-dependent and HIF-independent pathways, as illustrated in Figure 2. This leads to increased angiogenesis[4], increased cell survival and proliferation, and increased cell migration and invasion. These processes are all important for tumour development and are explained in greater detail below.

HIF-dependent pathways

The pVHL regulates the oxygen-sensing pathway via its interaction with the HIF transcription factor. In the presence of normal oxygen levels, the VHL-complex binds to and targets hydroxylated HIF-α subunits for degradation (Lonser et al 2003). Under hypoxic conditions, HIF does not become hydroxylated, and HIF dimers bind to specific promoter elements of target genes in the nucleus. In target tissues with mutant pVHL, HIF activity is enhanced due to reduced degradation, which leads to transcriptional activation of several HIF target genes—including enzymes involved in glucose metabolism—and critical angiogenic growth and mitogenic factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor β polypeptide (PDGFβ), erythropoietin (EPO) and transforming growth factor α (TGF-α) (Kim et al 2010; Calzada 2010; Clark & Cookson 2008; Lonser et al 2003). HIF also acts as a transcriptional repressor for genes encoding E-cadherin, matrix metalloproteinase 1 (MMP1) and other proteins involved in adherent and tight junction formation (Kim et al 2010; Calzada 2010).

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[3] A deletion mutation occurs when there has been a rearrangement of the DNA such that all or part of the coding sequence for the protein is missing, and only a small part or no protein is synthesised.

[4] Angiogenesis is the physiological process of developing new blood vessels.

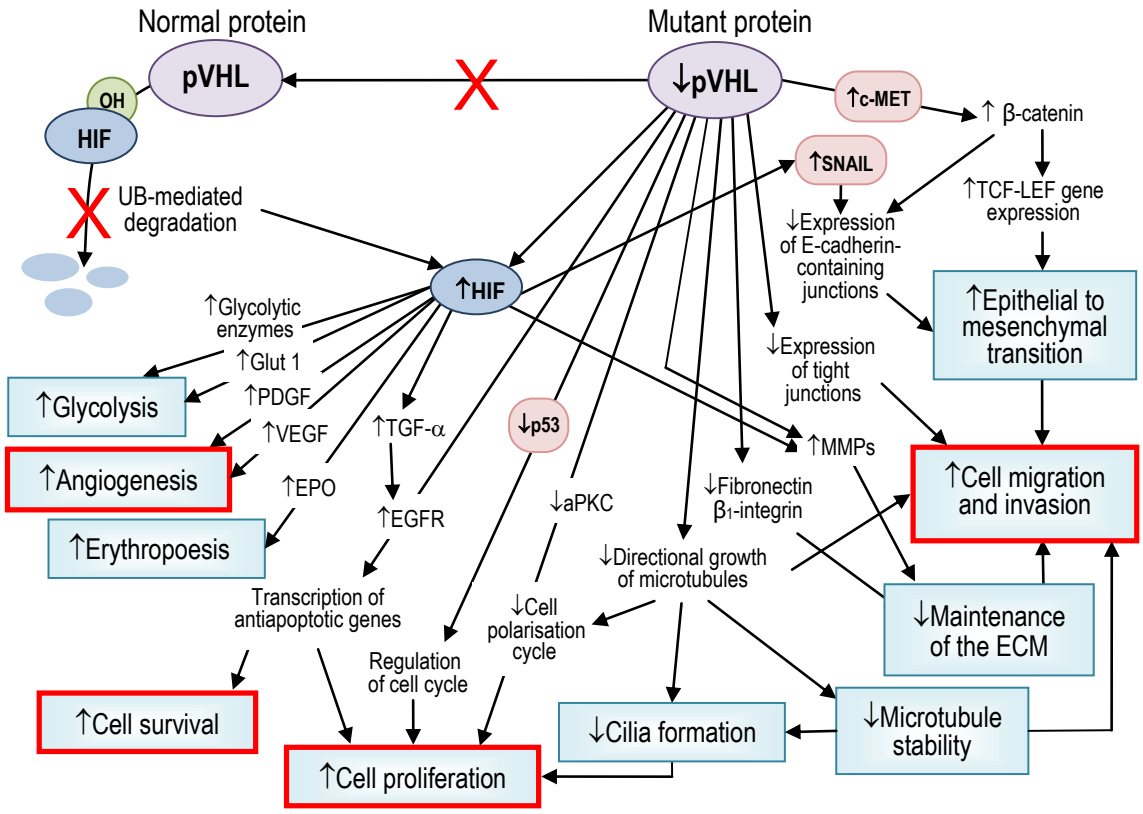


Figure 2 Diagrammatic representation of the HIF-dependent and HIF-independent functions of pVHL

aPKC = atypical protein kinase C; c-MET = receptor tyrosine kinase c-Met; ECM = extracellular matrix; EGFR = epidermal growth factor receptor; EPO = erythropoietin; Glut1 = glucose transporter type 1; HIF = hypoxia-inducible factor; MMP = matrix metalloproteinase; PDGF = platelet- derived growth factor; pVHL = von Hippel-Lindau protein; SNAIL = transcription factor SNAIL; TCF–LEF = T-cell factor–lymphoid enhancer factor; TGF-α = transforming growth factor α; p53 = tumour suppressor p53; UB = ubiquitin; VEGF = vascular epidermal growth factor

Angiogenesis, which is important for the persistence of tumours, is stimulated by HIF transcriptional activation via increased expression of PDGFβ and VEGF; these growth factors are important for proliferation of endothelial cells (Kim et al 2010). This may also explain the highly vascular nature of neoplasms associated with VHL disease. Overexpression of TGF-α, a potent mitogenic factor, especially for renal epithelium, may also play a role in tumour formation (Lonser et al 2003). TGF-α can stimulate cellular overexpression of epidermal growth factor receptors (for TGF-α), creating an autocrine loop. Reduced expression of E-cadherin and related proteins affects formation of adherent tight junctions between cells, and MMP1 and lysyl oxidase affect ECM remodelling; both of these may increase cell migration and support progression to metastases (Kim et al 2010; Lonser et al 2003). Thus, VHL mutations may disrupt tumour suppression indirectly through HIF-mediated effects.

HIF-independent pathways

The pVHL has also been shown to be involved in regulating several cellular mechanisms that are not related to HIF function (Figure 2). The maintenance of primary cilium, a

specialised structure involved in chemical and mechanical sensing, is dependent on the pVHL's ability to stabilise microtubules via two microtubule-binding domains (Calzada 2010; Kim et al 2010). Kinesin-2, a protein known to be involved in cilia regulation, interacts with the acidic domain of the pVHL and influences its binding to microtubules. Thus, in kidney epithelium with mutant pVHL that has lost the ability to stabilise microtubules, the integrity of the primary cilium is affected. The primary cilium responds to urine flow, and triggers calcium-dependent signals that affect the cyto-architecture and cell proliferation of the kidney epithelium. The loss of integrity causes uncontrolled kidney epithelial cell proliferation with the formation of renal cysts (Calzada 2010).

The mechanism by which pVHL regulates the ECM assembly is still unknown. However, pVHL's interaction with intracellular fibronectin seems critical for the proper extracellular assembly of both fibronectin and collagen (Calzada 2010; Kim et al 2010; Lonser et al 2003). Renal cell carcinomas and angiogenic tumours with mutant pVHLs do not properly assemble fibronectin and collagen into fibrils, and therefore fail to organise a normal ECM. This loss of integrity favours angiogenesis, progression to malignancy and cell migration or metastasis. Normal pVHL inhibits the expression of metastasis-associated genes *MMP2* and *MMP9*, which encode matrix metalloproteinases that are important for ECM turnover (Calzada 2010). Thus, expression of the MMP2 and MMP9 proteins in the absence of functional pVHL encourages progression to malignancy and metastasis.

The pVHL has been shown to have an important role in apoptosis (cell death)—the pVHL associates with p53 (a tumour suppressor) and promotes its stability by suppressing its Mdm2-mediated degradation, and p53 limits cellular proliferation by inducing cell cycle arrest and apoptosis in response to cellular stresses. This is thought to be important for the development of phaeochromocytomas. Primitive sympathetic neuroblasts compete for survival factors such as nerve growth factor (NGF) during development, and normally more than 50% die during this time. Notably, like the *VHL* gene, the other genes linked to familial paraganglioma and phaeochromocytoma (*NF1*, *RET*) and three genes encoding succinate dehydrogenase (*SDHB*, *SDBC* and *SDHD*) also affect apoptosis. The reduction in embryonic neuroblast cell death promotes formation of neuro-endocrine tumours or paragangliomas.

Genotype–phenotype associations in VHL disease

The associations between genotype and phenotype form the basis of the clinical classification of VHL disease, as shown in Table 8. Type 1 VHL disease does not include phaeochromocytoma, whereas phaeochromocytoma is a common feature of type 2 disease. Type 2 disease can be further separated into three categories: type 2A disease is associated with a low risk of renal cell carcinoma and pancreatic cysts, type 2B has an

increased risk of renal cell carcinoma and pancreatic cysts, and type 2C is characterised by phaeochromocytoma only (Barontini & Dahia 2010).

The genetic defects of these subgroups are also distinct. Whereas type 2 disease is caused almost exclusively by missense mutations, type 1 can result from deletions and truncations in addition to missense mutations. The mutant pVHL is believed to function differently in these subforms, accounting for the clinical variability of the disease. Although there are two known isoforms of the *VHL* gene, mutations that cause VHL disease target a common region shared by the two isoforms (Barontini & Dahia 2010; Ho et al 2003).

Table 8 Clinical and molecular subclassification of VHL disease

VHL subtype	Type 1	Type 2A	Type 2B	Type 2C
Mutation type	Large deletions—nonsense or missense	Missense	Missense	Missense
HIF activation	Prominent	Prominent	Prominent	Less prominent or absent
Risk of developing:				
Renal cell carcinoma	High	Low	High	Low
Haemangioblastoma	High	High	High	Low
Phaeochromocytoma	Low	High	High	High
Pancreatic lesions	High	Low	High	Low

Note: Endolymphatic sac tumours and cystoadenomas of the epididymis and broad ligament have not been assigned to specific VHL types

Genetic testing for VHL mutations

Diagnosis

Patients presenting with one or more characteristic lesions or a positive family history of VHL disease may be screened genetically to identify a germ-line mutation in the *VHL* gene. Currently, most laboratories that offer VHL genetic testing use a combination of direct deoxyribonucleic acid (DNA) sequencing and multiplex ligation-dependent probe amplification (MLPA) (Gene Tests 1993; Royal College of Pathologists of Australasia 2008). *De novo* mutations in the *VHL* gene occur in 21% of cases, and occasionally patients may have somatic mosaicism that makes diagnosis more difficult (Barontini & Dahia 2010; Evans et al 2010). Genetic mosaicism occurs when the somatic cells of an individual are of more than one distinct genotype. It is therefore possible to have a genetic mutation within cells of one part of the body, resulting in VHL syndrome, that is undetectable by testing the peripheral blood (De 2011; Santarpia et al 2007).

DNA sequencing

Direct sequencing of the polymerase chain reaction (PCR) amplified exons 1, 2 and 3 of the *VHL* gene remains the gold standard for detecting small germ-line VHL mutations such as small deletions, and missense and nonsense mutations (Nordstrom-O'Brien et al 2010). DNA sequencing reactions are similar to PCR reactions, both requiring template DNA, specific primers, DNA polymerase and deoxynucleotides (dNTPs). Fluorescent labelled dideoxynucleotide triphosphates (ddNTPs) are also added to sequencing reactions. When a ddNTP is incorporated into the DNA strand, further elongation is prevented due to its inability to form phosphodiester bonds with a subsequent nucleotide. Random incorporation of ddNTPs results in DNA fragments of varying length, which are separated by electrophoresis according to their length and size. Fluorescent labelling of ddNTPs enables determination of the DNA sequence by detection of the specific fluorescent label that corresponds to a particular ddNTP (Watson et al 1992; Sanger et al 1977; Smith et al 1986).

Direct sequencing is not suitable for identification of partial and complete *VHL* gene deletions.

Multiplex ligation-dependent probe amplification

MLPA is based on the semi-quantitative PCR principle and can be applied to detect large deletions of the VHL gene, as shown in Figure 3.

. The primers or probes for MLPA have two parts—a hybridisation sequence (shown in black) that binds to the target DNA and a primer sequence (grey) that is used to amplify the final product. The MLPA probe mix is added to denatured genomic DNA and allowed to hybridise to the target sequence. The probes that are bound to the target sequence are then ligated using a thermostable ligase (unbound single-stranded probes cannot be ligated). PCR using primers complementary to the green primer sequence of the probes then amplifies the ligated probes. Probes for multiple target sequences can be combined in a single reaction. When the PCR products are run on a gel, differences in gene copy number can be detected by the intensity of the PCR product when compared with a 'normal' standard. Thus, MLPA can be used for the detection of large deletions or duplications in the *VHL* gene (Schouten et al 2002).

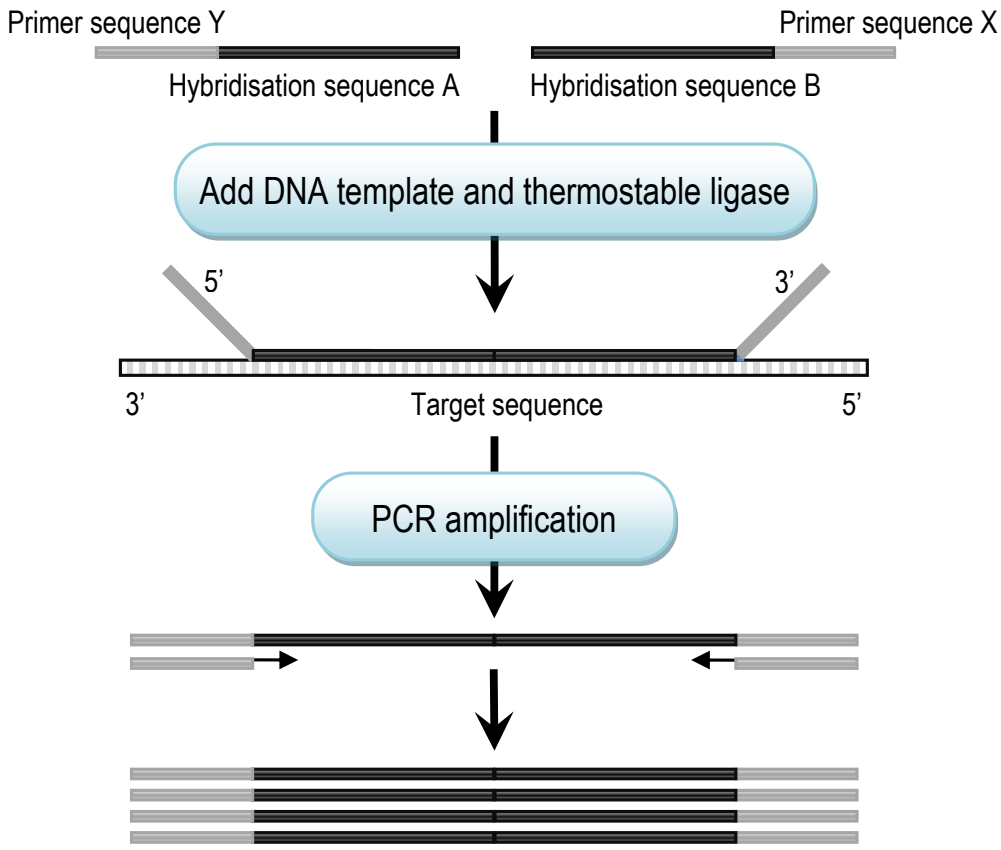


Figure 3 The principles of multiplex ligation-dependent probe amplification (MLPA)

The MLPA primers are mixed with the target DNA and the hybridisation sequences bind to the complementary sequence, if it is present in the target DNA. The two primers are joined together with the ligase (the ligase will not join unbound primers), and are then PCR amplified using primers complementary to the primer sequence (single primers will not amplify). Multiple primer pairs of different lengths can be combined in a single reaction. The PCR products are then visualised on a gel.

Intended purpose

VHL mutation testing will be used in patients suspected of having VHL syndrome and in close family members of those who are confirmed as having VHL syndrome or a pathogenic VHL mutation.

For diagnostic purposes, genetic testing of patients suspected of having VHL syndrome is likely to be used in addition to the existing clinical diagnostic procedures. The presence of clinically relevant mutations in the *VHL* gene does not provide a diagnosis of the specific pathology; rather, this information indicates the likely presence of a VHL-associated neoplasm. Thus, molecular testing must be used in conjunction with routine screening in order to provide a disease-specific diagnosis.

For predictive testing of relatives who have not inherited the family's VHL mutation, the genetic test will be used instead of lifelong screening. Individuals who have inherited the VHL mutation will be offered a lifelong screening program and early intervention to reduce the risk or severity of VHL-associated neoplasms.

Existing procedures for the diagnosis of VHL syndrome and screening for associated neoplasms

Diagnosis of VHL syndrome is currently based on clinical criteria. Patients with a family history and a haemangioblastoma (CNS or retinal), phaeochromocytoma or renal cell carcinoma are diagnosed with the disease. Those with no relevant family history must have two or more haemangioblastomas, or one haemangioblastoma and a visceral tumour (with the exception of epididymal and renal cysts, which are frequent in the general population), to meet the diagnostic criteria (Barontini & Dahia 2010; Kim et al 2010; Nordstrom-O'Brien et al 2010; VHL Family Alliance 2005).

VHL syndrome is a progressive disease of diverse nature, with a high frequency of multiple neoplastic lesions in various organ systems. Thus, patients with VHL syndrome, as well as their first- and second-degree family members, require annual routine screening to detect new neoplasms. Routine screening of patients with a family history of VHL syndrome should begin in infancy. Young children should have annual physical and ophthalmologic examinations, with imaging of the abdominal organs and the CNS beginning in the teenage years. Renal cysts and tumours should be monitored by computed tomography (CT) annually. A summary of the screening procedures, divided by age, is provided in Table 9. This screening protocol is an adapted and simplified version of the VHL Family Alliance suggested screening guidelines for individuals at risk of VHL (VHL Family Alliance 2005).

Table 9 Australian VHL screening protocol

Age	Screening test
Birth – 4 years	*Annually:* - Eye review by ophthalmologist
Ages 5–14 years	*Annually:* - Eye review by ophthalmologist - Medical specialist review: check of blood pressure, urine test or blood test to check for elevated catecholamines and metanephrines (phaeochromocytoma screen)
Age 15 years and older	*Annually:* - Eye review by ophthalmologist - Medical specialist review: check of blood pressure, urine test or blood test to check for elevated catecholamines and metanephrines (phaeochromocytoma screen) - Ultrasound of abdomen (kidneys, pancreas and adrenals) *Every 2 years:* - MRI with gadolinium of brain and entire spinal cord (performed yearly if abnormality detected) *Every 2–3 years:* - CT of abdomen (instead of that year's ultrasound)

Source: adapted from the VHL Family Alliance guidelines (VHL Family Alliance 2005)

A positive VHL genetic test will not affect the requirement for annual screening, and there would be no change in the use of co-administered screening interventions for

patients with confirmed VHL syndrome. However, it is proposed that a negative VHL genetic test will eliminate the requirement for annual screening. Thus, the test will replace routine screening interventions for these patients.

The clinical diagnostic procedures used to monitor and detect specific VHL-associated neoplasms are described below.

CNS haemangioblastomas

Haemangioblastomas are diagnosed by magnetic resonance imaging (MRI) of the brain and the spinal cord. The clinical features of haemangioblastomas depend on the localisation of the tumours and the degree of invasion in the CNS. These include features such as headaches, numbness, dizziness, weakness or pain in the arms and legs, sensory deficits, gait or spinal ataxia, dysmetria, nystagmus, hydrocephalus and incontinence (Andrews 2011; Kim et al 2010; Barontini & Dahia 2010).

Patients diagnosed with VHL syndrome, as well as their first- and second-degree family members, are recommended to have an MRI with gadolinium of the brain and spine every 2 years after the onset of puberty (VHL Family Alliance 2005).

Retinal haemangioblastomas

In their early stages, retinal haemangioblastomas are detectable only by examination of the dilated eye. Clinically, patients usually present with a painless loss of visual acuity or visual field or both. Advanced cases can present with haemorrhage, leading to secondary glaucoma and loss of vision. Peripheral retinal angioma is easily diagnosed by its typical fundoscopic aspect (Kim et al 2010; Barontini & Dahia 2010).

Patients diagnosed with VHL syndrome, as well as their first- and second-degree family members, are recommended to have an annual eye/retinal examination with indirect ophthalmoscope by an ophthalmologist informed about the VHL history, and using a dilated examination (VHL Family Alliance 2005).

Renal cell carcinoma

Renal cell carcinomas are the most common cause of death for patients with VHL syndrome, and are detected using CT, MRI and ultrasound. They often remain asymptomatic for a long time; thus, diagnosis during pre-symptomatic screening is likely to improve patient outcomes. More advanced cases can present with haematuria, flank pain or a flank mass. Although renal cysts may be benign, they are considered to be premalignant lesions (Kim et al 2010; Barontini & Dahia 2010).

Phaeochromocytoma

Phaeochromocytomas in VHL disease tend to be benign (less than 5% are malignant). In affected patients, hypertension is the most common symptom, followed by headache

and sweating. Other symptoms include palpitations, tachycardia, pallor and nausea. Nevertheless, when associated with VHL disease, about 30% of patients can be normotensive and asymptomatic. Phaeochromocytomas in VHL patients display a distinctly and consistently noradrenergic phenotype, with norepinephrine concentrations representing 98% and epinephrine concentrations only 1.5% of the total catecholamine content (Barontini & Dahia 2010; Donckier & Michel 2010; Kim et al 2010; Waguespack et al 2010).

The diagnosis is based on measuring the free metanephrine level in plasma. MRI, CT, ^{131} or ^{123}I(iodine)-methyl benzyl guanidine or octreotide scintigraphy, ^{18}F(fluoride)-DOPA-positron emission tomography, ^{18}F-dopamine- and ^{18}F-deoxyglucose scans are used for tumour localisation. Two imaging methods are necessary to document the tumour (Barontini & Dahia 2010; Kim et al 2010; Waguespack et al 2010).

Pancreatic tumours

Most pancreatic neuro-endocrine tumours are detected by CT imaging. Patients rarely present with symptoms due to secreted peptides, like diarrhoea or hypoglycaemia, and most neuro-endocrine tumours are non-functional and asymptomatic. However, these tumours can cause pancreatitis or pain (Barontini & Dahia 2010; Kim et al 2010; Kim et al 2009a).

Patients diagnosed with VHL syndrome, as well as their first- and second-degree family members, are recommended to have an annual ultrasound of the abdomen (with and without contrast) to assess their kidneys, pancreas and adrenals, and the uterus in females. This should be replaced with a CT scan every 2–3 years. They should also have an annual blood test for elevated metanephrine levels (VHL Family Alliance 2005).

Endolymphatic sac tumours

Endolymphatic sac tumours are detected by MRI or CT. Clinical symptoms include hearing loss, tinnitus, vertigo or disequilibrium, aural fullness and, less frequently, facial paresis. Any hearing loss is irreversible. These tumours usually occur early in life with a mean age of onset of 22 years (Barontini & Dahia 2010; Kim et al 2010). These symptoms are investigated as they occur but are not screened for in Australia (expert advice of MESP clinical expert).

Cystoadenomas of the adnexal reproductive organs

Epididymal cystoadenomas in men are usually asymptomatic, and are diagnosed by palpation and confirmed by ultrasound. Papillary cystoadenomas arising from the broad ligament in females are diagnosed by CT or ultrasound. The tumours in both males and females are grossly and histologically alike (Lonser et al 2003).

Marketing status of the technology

Currently, the Royal College of Pathologists of Australasia (RCPA) genetic testing website (Appendix F) lists only two pathology laboratories that offer VHL genetic testing using assays developed in house, and they offer two different levels of service. The Cancer Genetics Diagnostic Laboratory, PaLMS-RNSH, in NSW offers DNA sequencing of all three exons of the *VHL* gene with a turnaround time of 3 months. This test detects point mutations and frame-shift mutations but not large deletion mutations, and therefore does not identify all patients with VHL syndrome. On the other hand, the Molecular Pathology Division of the Institute of Medical and Veterinary Science (IMVS) in Adelaide, SA, offers both DNA sequencing and MLPA analysis of the *VHL* gene for patients referred through a clinical genetic service, with a turnaround time of 2 months for A$600. This enables the detection of point mutations, frame-shift mutations and large deletions of *VHL* gene, and identifies virtually all cases of VHL syndrome. The Australian Genetic Testing Survey 2006 reported that VHL genetic testing using both DNA sequencing and MLPA analysis was also conducted in WA, but no information about the laboratory was available (Royal College of Pathologists of Australasia 2008).

Diagnostic VHL genetic testing is also commercially available. A Swiss company (Diagnogene) offers DNA sequencing for approximately A$687. However, this does not include analysis for gene deletions (ie MLPA). A Belgian firm, GenDia, offers both sequencing and MLPA analysis for around A$1,223. Predictive testing is cheaper than diagnostic testing as laboratories are identifying a specific abnormality in family members that was first identified in the index case. The cost of predictive testing through Diagnogene is not known, while GenDia charge approximately A$440. The IMVS charge is $340 for predictive testing.

As the national demand for VHL genetic testing is likely to be low, it is probable that it would be undertaken by a small number of laboratories to ensure that they have sufficient throughput to maintain training and procedural quality. Testing of the *VHL* gene can be completed using conventional methods and instrumentation in a genetic pathology laboratory. The staffing required will depend on the caseload, throughput and infrastructure of the laboratories that provide testing.

Regulatory status

In vitro diagnostic medical devices (IVDs) are, in general, pathology tests and related instrumentation used to carry out testing on human samples, where the results are intended to assist in clinical diagnosis or in making decisions concerning clinical management (Therapeutic Goods Administration 2009).

The Therapeutic Goods Administration (TGA) regulatory framework for IVDs changed in July 2010, such that in-house laboratory tests now receive a similar level of regulatory scrutiny as commercial kits. As testing for VHL is currently only provided by laboratories, it would be classified as a Class 3 in-house IVD (Box 1).

Box 1 Classification of Class 3 in vitro diagnostic medical devices

Therapeutic Goods (Medical Devices) Regulations 2002 – Schedule 2A

1.3 Detection of transmissible agents or biological characteristics posing a moderate public health risk or high personal risk

1. **An IVD is classified as a Class 3 IVD medical device or a Class 3 in-house IVD if it is intended for any of the following uses**:
   a. detecting the presence of, or exposure to, a sexually transmitted agent;
   b. detecting the presence in cerebrospinal fluid or blood of an infectious agent with a risk of limited propagation;
   c. detecting the presence of an infectious agent where there is a significant risk that an erroneous result would cause death or severe disability to the individual or foetus being tested;
   d. prenatal screening of women in order to determine their immune status towards transmissible agents;
   e. determining infective disease status or immune status where there is a risk that an erroneous result will lead to a patient management decision resulting in an imminent life-threatening situation for the patient;
   f. the selection of patients for selective therapy and management, or for disease staging, or in the diagnosis of cancer;
   g. human genetic testing;
   h. to monitor levels of medicines, substances or biological components, when there is a risk that an erroneous result will lead to a patient management decision resulting in an immediate life-threatening situation for the patient;
   i. the management of patients suffering from a life-threatening infectious disease;
   j. screening for congenital disorders in the foetus.

Note: For paragraph (f) An IVD medical device would fall into Class 2 under clause 1.5 if:

   a. a therapy decision would usually be made only after further investigation; or
   b. the device is used for monitoring.

2. Despite subsection (1), an IVD is classified as a Class 3 IVD medical device or a Class 3 in-house IVD if it is used to test for transmissible agents included in the Australian National Notifiable Diseases Surveillance System (NNDSS) list as published from time to time by the Australian Government.

Source: http://www.tga.gov.au/ivd/ivd-classification.htm [accessed January 2011]

Laboratories that manufacture in-house Class 3 IVDs are required to notify the TGA of the types of IVDs manufactured in their laboratory for inclusion on a register. These laboratories must have NATA accreditation, with demonstrated compliance with the suite of standards on the validation of in-house IVDs, as published by the National Pathology Accreditation Advisory Committee (NPAAC), for each test manufactured (Therapeutic Goods Administration 2011).

Current reimbursement arrangement

A list of the MBS numbers associated with the existing clinical diagnostic and screening procedures is provided in Appendix C. Currently, there is no MBS listing for any test that detects germ-line mutations in the *VHL* gene.

There are, however, MBS items that allow reimbursement for molecular tests that detect specific genetic mutations (Table 10). The range of MBS fees associated with these items is indicative of the range of molecular methodologies used to detect the relevant mutations. Quantitative or semi-quantitative assays will incur greater costs than methods that are simply qualitative.

Table 10 Current MBS items related to detection of genetic mutations

Item number	Description
Item 73308	Characterisation of the genotype of a patient for Factor V Leiden gene mutation, or detection of the other relevant mutations in the investigation of proven venous thrombosis or pulmonary embolism - 1 or more tests Fee: $36.70
Item 73311	Characterisation of the genotype of a person who is a first-degree relative of a person who has proven to have 1 or more abnormal genotypes under item 73308 - 1 or more tests Fee: $36.70
Item 73317	Detection of the C282Y genetic mutation of the HFE gene and, if performed, detection of other mutations for haemochromatosis where: (i) the patient has an elevated transferrin saturation or elevated serum ferritin on testing of repeated specimens; or (ii) the patient has a first-degree relative with haemochromatosis; or (iii) the patient has a first-degree relative with homozygosity for the C282Y genetic mutation, or with compound heterozygosity for recognised genetic mutations for haemochromatosis (Item is subject to rule 20) Fee: $36.70
Item 73320	Detection of HLA-B27 by nucleic acid amplification includes a service described in 71147 unless the service in item 73320 is rendered as a pathologist determinable service (Item is subject to rule 27) Fee: $40.80
Item 73305	Detection of genetic mutation of the *FMR1* gene by Southern blot where the results in item 73300 are inconclusive Fee: $204.00
Item 73314	Characterisation of gene rearrangement or the identification of mutations within a known gene rearrangement, in the diagnosis and monitoring of patients with laboratory evidence of: (i) acute myeloid leukaemia; or (ii) acute promyelocytic leukaemia; or (iii) acute lymphoid leukaemia; or (iv) chronic myeloid leukaemia; Fee: $232.50

Source: Department of Health and Ageing (2011)

Proposal for public funding

Based on the predicted patient population and the proposed intervention, the proposed MBS items are suggested as:

1. A diagnostic test to detect germ-line mutations in the *VHL* gene
2. A predictive test to detect mutations in the *VHL* gene in family members of a proband.

The proposed MBS items are summarised in Table 11. The ordering of these tests would be restricted to specialised genetic services. It is expected that the MBS item for the testing of relatives would primarily be used for first- and second-degree relatives, but the proposed listing has been kept broad to allow for exceptional circumstances where wider use may be required.

Table 11 Proposed MBS item descriptor for VHL genetic testing

Category 6–Pathology services
MBS [item number] **(proposed MBS item 1)** Detection of germ-line mutations of the *VHL* gene in: (iii) Patients with a clinical diagnosis of VHL syndrome: • a family history of VHL and a haemangioblastoma (retinal or CNS), phaeochromocytoma or renal cell carcinoma • two or more haemangioblastomas, or one haemangioblastoma and a visceral tumour (with the exception of epididymal and renal cysts, which are frequent in the general population) (iv) Patients presenting with one or more clinical features suggestive of VHL syndrome: • haemangioblastomas of the brain, spinal cord, and retina • phaeochromocytoma or functional extra-adrenal paraganglioma Fee: $600 Prior to ordering these tests, the ordering practitioner should ensure that the patient has given informed consent. Testing can only be performed after genetic counselling. Appropriate genetic counselling should be provided to the patient by a genetic counselling service or a clinical geneticist on referral. Further counselling may be necessary upon receipt of the test results.
MBS [item number] **(proposed MBS item 2)** Detection of germ-line mutations of the *VHL* gene in: (ii) Biological relatives of patients with a known mutation in the *VHL* gene Fee: $340 Prior to ordering these tests, the ordering practitioner should ensure that the patient has given informed consent. Testing can only be performed after genetic counselling. Appropriate genetic counselling should be provided to the patient by a genetic counselling service or a clinical geneticist on referral. Further counselling may be necessary upon receipt of the test results.

Approach to assessment

Objective

The objective of this assessment is to determine whether there is sufficient evidence in relation to clinical need, safety, effectiveness and cost-effectiveness to recommend public funding for genetic testing for hereditary mutations in the *VHL* gene for (i) patients with symptoms of VHL syndrome and (ii) a family member of a patient with a known VHL mutation.

Questions for public funding

In the event that **direct evidence**[5] was available to assess the safety, effectiveness and cost-effectiveness of genetic testing for mutations in the VHL gene, the following questions were to be addressed by this evaluation:

1. Is VHL genetic testing safe, effective and cost-effective when used in addition to clinical diagnostic approaches in the diagnosis of patients presenting with symptoms suggestive of VHL syndrome?

2. Is VHL genetic testing safe, effective and cost-effective when used as a triage test for lifelong screening of family members of patients who are positive for a VHL mutation?

In the event that **linked evidence** was the only evidence available to assess the safety, effectiveness and cost-effectiveness of genetic testing for mutations in the VHL gene, the following questions were to be addressed by this evaluation:

Linkage 1 – Test accuracy

1. Is genetic testing for mutations in the VHL gene, in addition to usual diagnostic assessment, as accurate as, or more accurate than, usual clinical diagnosis in diagnosing patients with suspected VHL syndrome?

2. Is genetic testing for mutations in the VHL gene, plus annual screening, as accurate as, or more accurate than, annual screening for diagnosing relatives of patients with a known VHL mutation?

[5] For a description of direct evidence and linked evidence see section on Diagnostic Assessment Framework (page 50)

Linkage 2 – Change in patient management

3. Does genetic testing for mutations in the VHL gene, in addition to usual diagnostic assessment, change patient management compared with usual clinical diagnosis in patients with suspected VHL syndrome?

4. Does genetic testing for mutations in the VHL gene, in addition to usual diagnostic assessment, change patient management compared with usual clinical diagnosis for relatives of patients with a known VHL mutation?

Linkage 3 – Likely impact of change in patient management from VHL genetic testing on patient health outcomes

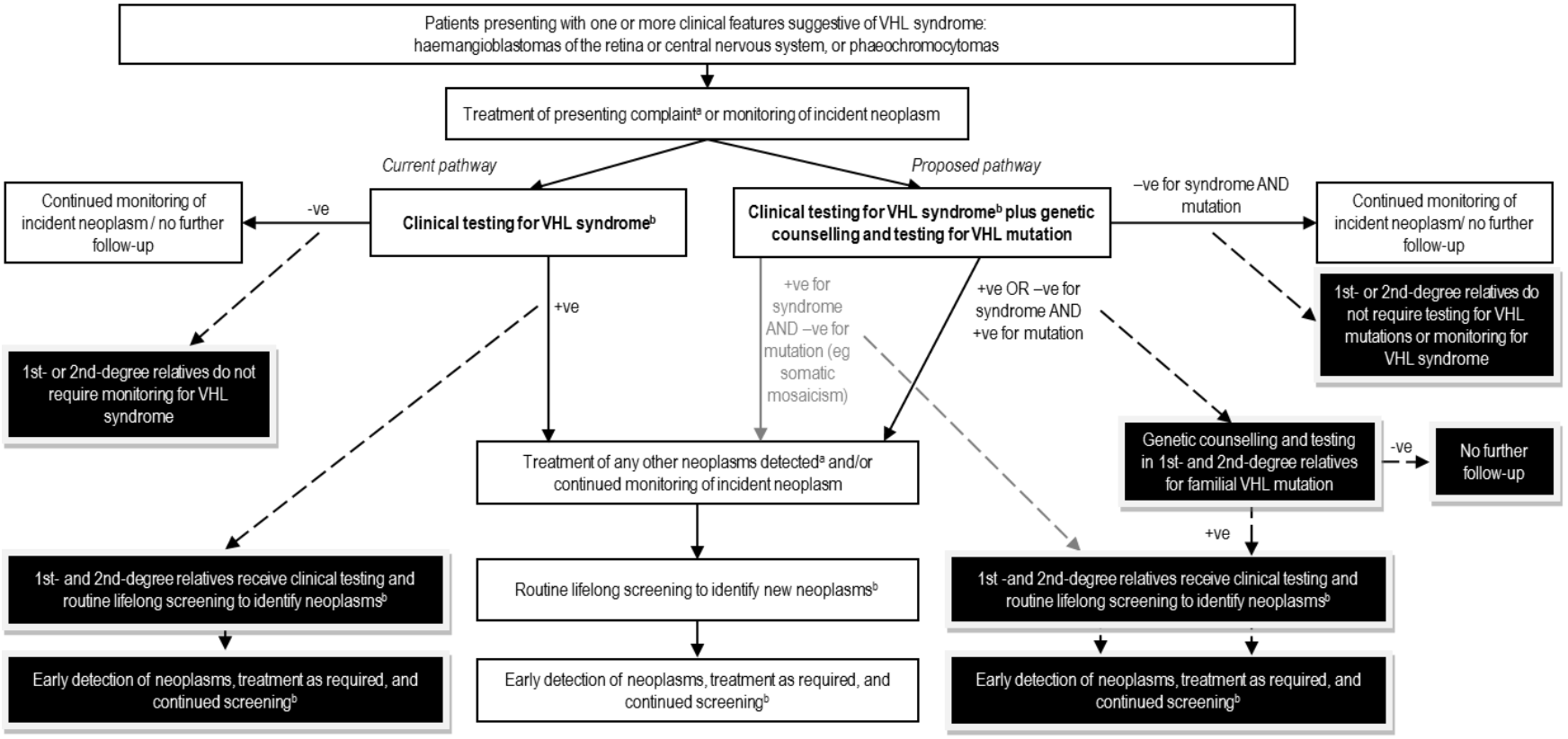
The Protocol Advisory Sub-Committee (PASC) of the MSAC advised that there were unlikely to be any treatment differences in patients diagnosed with VHL-related neoplasms between those who were clinically diagnosed and those who underwent genetic testing. Likewise for family members; although there is likely to be a change in the rate of routine screening (as those who are VHL mutation negative may avoid screening), there is unlikely to be any treatment change. Therefore, a full linked evidence approach was not required. A separate question for linkage 3 was therefore not developed, and a literature search and assessment of likely treatment effectiveness in the population with VHL-related neoplasms were not undertaken.

Clinical place for proposed intervention

A management algorithm is provided below for both the diagnostic and predictive uses of VHL genetic testing (Figure 4). The left side explains the approach to the diagnosis and prediction of VHL syndrome in a setting without genetic testing—assumed to be the current approach for the sake of simplicity, although it is acknowledged that some patients currently receive genetic testing without it being funded by the MBS. The right side of the algorithm shows the proposed approach in which genetic testing is available. The white text boxes and solid lines relate to the diagnosis and treatment of people with clinical features suggestive of having VHL syndrome, while the black boxes and dashes correspond to the management of their close family members. The arrow relevant to patients with somatic mosaicism has been lightened to lessen the emphasis of this pathway, due to its rarity of occurrence.

The main difference between the algorithms is the targeted use of lifelong surveillance in patients who have a definitive diagnosis of VHL syndrome (due to having a VHL mutation), with fewer patients overlooked for surveillance due to a negative misdiagnosis (false negative), and the lack of requirement for surveillance for family members who have not inherited the VHL mutation.

Figure 4 Management algorithm for use of VHL genetic testing in patients who present with clinical features suggestive of VHL syndrome as well as their first- and second-degree relatives



Outcomes

Direct effectiveness :	Primary = mortality, overall/progression-free survival, quality of life, incidence and severity of life-threatening events Secondary = incidence of symptoms, cancer detection rates, tumour stage, age at diagnosis
Predictive accuracy:	Sensitivity and specificity (and therefore rates of false positives and negatives), positive and negative likelihood ratios, positive and negative predictive values (and therefore false alarm and reassurance rates), diagnostic odds ratios, receiver operator characteristic curves, area under the curve, accuracy
Change in management :	Rate and type of referral, frequency and compliance with clinical screening, rate and type of treatment, hospital separations and re-admissions, length of hospital stay
Safety:	Psychological and physical harms from testing and clinical screening

Notes:
1st-degree relatives are parents, offspring and siblings that share 50% of their genes; 2nd-degree relatives are grandparents, grandchildren, uncles, aunties, nephews, nieces and half-siblings that share 25% of their genes
[a] Surgical resection, radiotherapy, laser therapy, anti-VEGF therapy; [b] Clinical testing = computed tomography, magnetic resonance imaging, ultrasound, urine and blood tests, family history, clinical history and other tests as appropriate to identify any signs of disease other than presenting complaint; biopsy and histopathology of any neoplasms; [c]Screening = CT, MRI, ultrasound, urine and blood tests; CNS=central nervous system

The comparator

Diagnosis of VHL syndrome is currently based on clinical criteria. Patients with a family history and a haemangioblastoma (including retinal), phaeochromocytoma, or renal cell carcinoma are diagnosed with the disease. Those with no relevant family history must have two or more haemangioblastomas, or one haemangioblastoma and a visceral tumour (with the exception of epididymal and renal cysts, which are frequent in the general population) to meet the diagnostic criteria (Kim et al 2010; Nordstrom-O'Brien et al 2010).

In first- and second-degree family members of patients with VHL syndrome or a pathogenic VHL mutation, the comparator to VHL genetic testing and annual screening is annual screening alone.

The reference standard

Genetic testing for VHL mutations assists in the prediction of which patients and family members are susceptible to developing VHL-related neoplasms, rather than diagnosing who meet the clinical criteria for VHL syndrome. As such, the reference standard by which the accuracy of genetic testing should be judged is long-term clinical diagnosis, to allow enough time for neoplasms to become clinically evident.

Diagnostic assessment framework

This assessment of genetic testing for mutations in the *VHL* gene is based on the framework outlined in the MSAC *Guidelines for the Assessment of Diagnostic Technologies* (MSAC 2005).

To assess the effectiveness of VHL genetic testing, we need to consider its diagnostic accuracy (in comparison with a reference standard); its impact on the clinical management of people with suspected VHL, or family members of patients with a confirmed VHL mutation; and its ultimate impact on the health outcomes of patients and family members. The first goal of this assessment was therefore to find **direct evidence** of the effectiveness of genetic testing for VHL mutations on patient health outcomes, that is, primary research where one group of people suspected of having VHL would receive genetic testing in addition to clinical testing, treatment and follow-up, and would be compared with another group receiving clinical testing (without genetic testing), treatment and follow-up for suspected VHL. The comparison would occur over a period of time until the impact on health outcomes (eg survival, prevention of symptoms relating to VHL neoplasms) could be evaluated.

In a similar manner, direct evidence assessing the benefit of VHL genetic testing in family members of a patient with a VHL mutation would have compared one group of

family members who were screened annually, without knowledge of their mutation status, against another group of family members who had been genetically tested, knew their mutation status, and subsequently were either screened or did not require screening. Direct evidence in this situation would provide long-term data following the outcomes of screening, any subsequent treatment and health outcomes.

There was no comparative direct evidence available assessing the health impact of genetic testing for VHL mutations in either people suspected of having VHL or family members of someone with a confirmed VHL mutation, so in this assessment a **linked evidence** approach was used.

This means that evidence from studies that report on the following factors would be narratively linked in order to infer the effect of the diagnostic test on patient health outcomes:

- diagnostic test performance (diagnostic accuracy)—sensitivity, specificity, accuracy, diagnostic yield
- whether clinical decision-making—(patient management) changes as a result of the test
- whether patients receiving a change in management benefit in terms of health outcomes

Literature sources and search strategies

Literature sources

The medical literature was systematically searched to identify relevant studies and reviews for the period from 1993 (when the *VHL* gene was first described in the literature) until May 2011 (June 2011 for economic evaluation). Appendix F describes the electronic databases that were used for this search and other sources of evidence that were investigated. Grey literature[6] was included in the search strategy. Unpublished literature, however, was not canvassed as it is difficult to search for it exhaustively and systematically, and trials that are difficult to locate are often smaller and of lower methodological quality (Egger et al 2003). It is, however, possible that these unpublished data could alter the results of the assessment. The search terms used to identify relevant literature in databases for this review are shown in Appendix D.

[6] This is literature that is difficult to find, including published government reports, theses, technical reports and non-peer-reviewed literature.

Selection criteria

The criteria for including studies in this report are presented in the relevant areas of the 'Results' section. The eligibility criteria for research on the safety of VHL genetic testing are presented in Box 2 and Box 3. The criteria for including studies relevant to determining the **direct** effectiveness of VHL genetic testing on health outcomes are presented in Box 4 and Box 5. The criteria for selecting the **linked** evidence components in this assessment of the value of VHL genetic testing as a diagnostic or predictive tool are presented in Box 6, Box 7, Box 8 and Box 9.

All literature also met the following criteria:

- Published within the search period from 1993 – May 2011;
- Non-English language articles were excluded unless they appeared to provide a higher level of evidence than the English language articles identified;
- Conducted on human subjects;
- Provided data or patients that were not duplicated in other articles; and where this occurred, only the most recent and/or comprehensive information was selected;
- Provided data that could be extracted (ie not described graphically); and
- Had study designs that were relevant to the aspect being assessed – namely,
    - Safety: All of the relevant study designs given in the Intervention column of Table 13. If large numbers of case series were identified, all were reviewed but only those that were large case series and/or with long-term follow-up had data extracted.
    - Effectiveness:
        - Direct evidence – All of the relevant study designs listed in the Intervention column of Table 13. However, post-test case series were excluded. If large numbers of pre-test/post-test case series were identified, all were identified and reviewed but only those that were large case series and/or with long-term follow-up had data extracted.
        - Linked evidence –
            - Predictive accuracy: All of the relevant study designs listed in the Diagnostic accuracy column of Table 13.

- Change in management (impact on clinical decision-making): All of the relevant study designs listed in the Intervention column of Table 13. However, post-test case series were excluded. If large numbers of pre-test/post-test case series were identified, all were reviewed but only those that were large case series and/or with long-term follow-up had data extracted.

- Cost-effectiveness: All relevant articles on economic models and trial-based economic evaluations, including the study designs listed in the Intervention column of Table 13 that included cost-effectiveness outcomes (cost, cost per relevant health outcome (eg LYG, QALY, DALY) for VHL genetic testing of patients diagnosed with or suspected of having VHL syndrome and / or their close relatives.

- Ethical issues: All articles identified by the database searches that discussed ethical issues surrounding genetic testing for familial cancer syndromes. The inclusion criteria for these studies were that the articles were in English, were fairly recent and that they discussed ethical issues relevant to genetic testing. All studies designs that investigated the ethical beliefs/behaviour of patients, their families, or medical practitioners with respect to genetic testing for familial cancer syndromes were also included.

Search results

The process of study selection for this systematic literature review went through six phases:

1. All reference citations from all literature sources were collated into an Endnote X3 database.

2. Duplicate references were removed.

3. Studies were excluded, on the basis of the citation information, if it was obvious that they did not meet the pre-specified inclusion criteria. Citations were assessed independently by one reviewer. However, 20% of the database was assessed by a second reviewer, and any discrepancies were discussed in order to reach a consensus and to ensure that eligibility was determined in a robust manner. Studies marked as requiring further evaluation were retrieved for full-text assessment.

4. Studies were included to address the research questions if they met the pre-specified criteria, again independently applied by one reviewer to the full-text articles. Those

articles meeting the criteria formed part of the evidence-base. The remainder provided background information.

5. The reference lists of the included articles were pearled for additional relevant studies. These were retrieved and assessed according to step 4.

6. The evidence-base consisted of articles from steps 4 and 5 that met the inclusion criteria.

Any doubt concerning inclusion at step 4 was resolved by consensus between members of the evaluation team. The results of the process of study selection are provided in Figure 5 and Figure 6.

PRISMA flowcharts

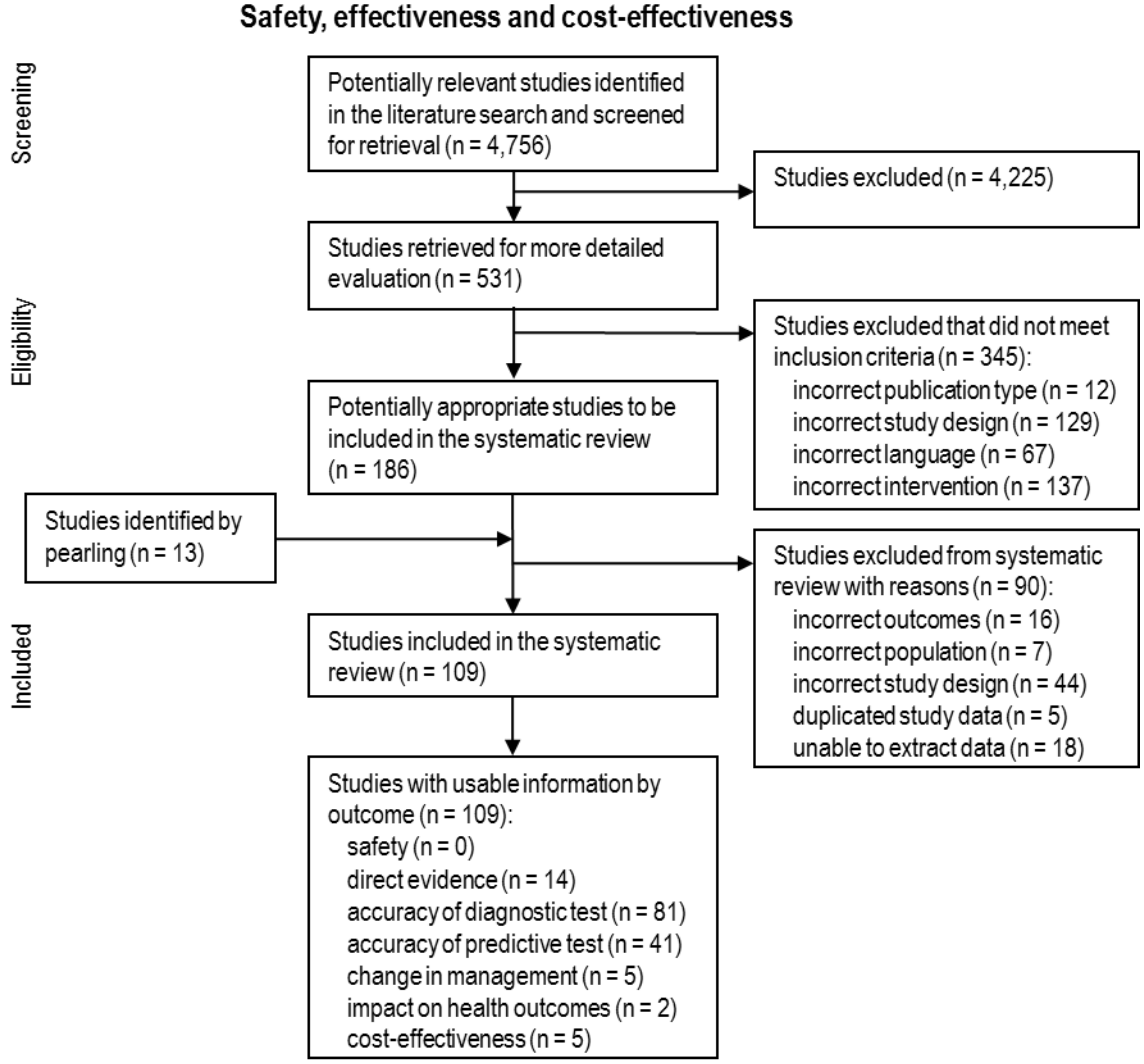


Figure 5 Summary of the process used to identify and select studies for the review of safety, effectiveness and cost-effectiveness outcomes after genetic testing for VHL mutations

Some of the included studies provided usable information for more than one outcome. Adapted from Liberati et al (2009)

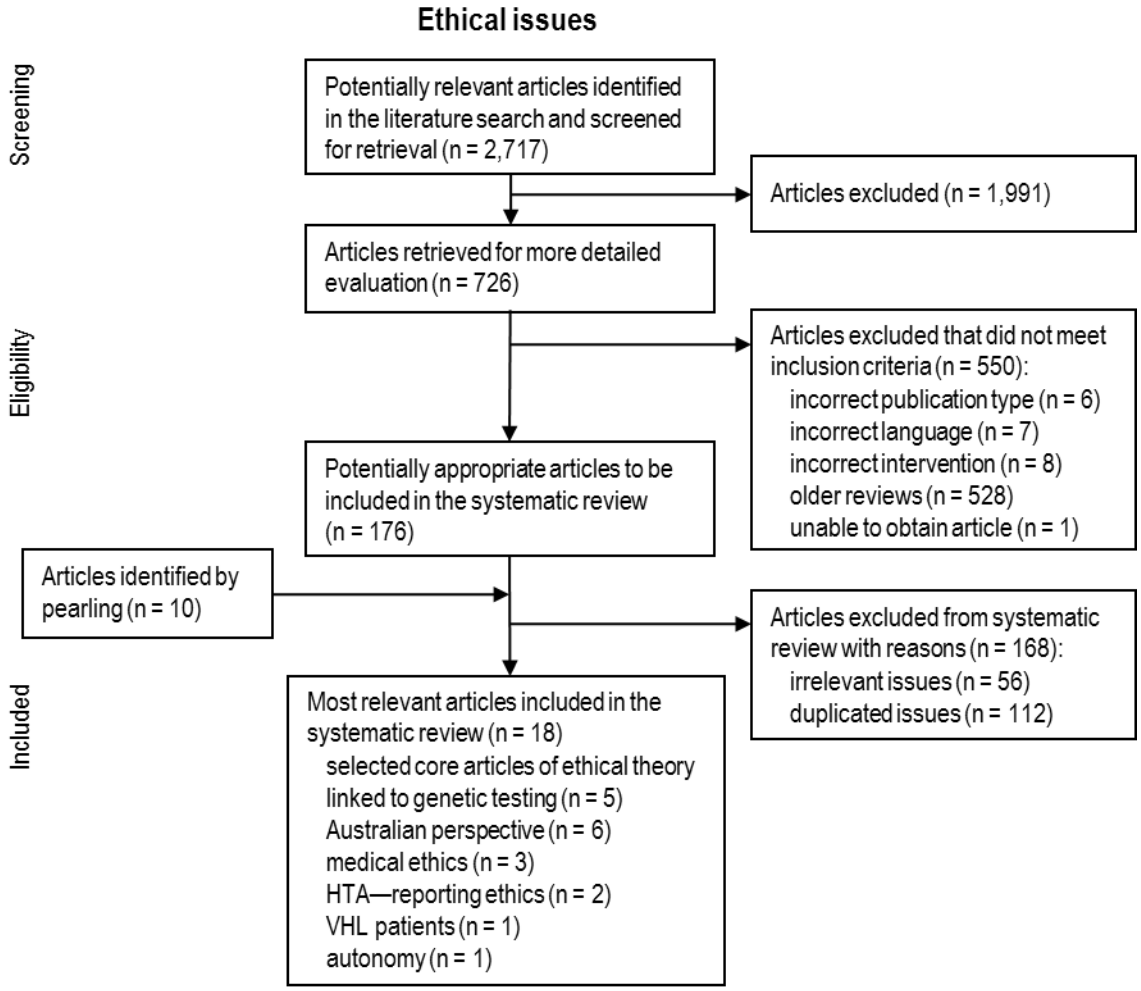


Figure 6 Summary of the process used to identify and select studies for the review of ethical issues surrounding genetic testing for VHL mutations
Adapted from Liberati et al (2009)

Data extraction and analysis

For safety, effectiveness and cost-effectiveness outcomes

A profile of key characteristics including study design and location, level and quality of evidence, population, intervention and outcomes was developed for each study selected for this report (Appendix G).

Studies that potentially may have met the inclusion criteria but contained insufficient or inadequate data are listed according to their reason for exclusion in Appendix H.

For ethical issues

Given that 176 papers represents a superfluous amount of literature on which to base an ethical discussion that forms only a small part of this assessment report, five papers were selected on the criterion of their clear discussion of ethical theory linked to genetic testing. Where possible, sources that presented material from an Australian perspective

and/or dealt with issues related specifically to VHL genetic testing were also used to support the discussion.

Assessing diagnostic accuracy

To assess the diagnostic accuracy of the VHL genetic test, calculations of sensitivity, specificity, negative and positive predictive values of the tests, and 95% confidence intervals were undertaken where possible. Data were extracted using the classic 2 x 2 table, whereby the results of the index diagnostic test were cross-classified against the results of the reference standard (Armitage et al 2002; Deeks 2001), and Bayes' Theorem[7] applied:

		Reference standard All relevant clinical and laboratory information		
		Disease +	Disease –	
Index genetic test	Test +	True positive	False positive	Total test positive
	Test –	False negative	True negative	Total test negative
		Total disease +ve	Total disease –ve	Total tested

Unfortunately, the reference standard defined *a priori* (clinical diagnosis determined from long-term follow-up) was not reported in any studies. In the absence of more informative data, the results of genetic testing were compared with the imperfect reference standard of clinical diagnosis in the short term. It is therefore expected that there would be a proportion of patients in the evidence-base who carried VHL mutations but in whom a clinical diagnosis was not yet able to be made.

In diagnostic yield studies the presence of *VHL* gene mutations was compared in two main populations: i) patients presenting with one or more clinical features suggestive of VHL syndrome (with or without a clinical diagnosis) and ii) first- or second-degree family members of patients with a known VHL genetic mutation.

The sensitivity of the genetic test for VHL mutations was calculated as the proportion of clinically diagnosed patients who were identified as having a VHL mutation:

[7] Bayes Theorem:

Positive predictive value = (prevalence)(sensitivity) / (prevalence)(sensitivity) + (1–prevalence)(1–specificity)

Negative predictive value = (1–prevalence)(specificity) / (prevalence)(1–sensitivity) + (1–prevalence)(specificity)

Sensitivity (true positive rate, %) = number of true positives / total disease positives × 100

The specificity of the genetic test for VHL mutations was calculated as the proportion of patients without a clinical diagnosis of VHL syndrome who did not have a VHL mutation:

Specificity (true negative rate, %) = number of true negatives / total disease negatives × 100

The positive predictive value was calculated as the proportion of patients with a positive clinical diagnosis among those that tested positive for a VHL mutation:

Positive predictive value (PPV, %) = number of true positives / total test positives × 100

The negative predictive value was calculated as the proportion of patients with a negative clinical diagnosis among those that tested negative for a VHL mutation:

Negative predictive value (NPV, %) = number of true negatives / total test negatives × 100

When a 95% confidence interval was not provided in the relevant study, it was calculated using exact binomial methods.

Appraisal of the evidence

Appraisal of the evidence was conducted in three stages:

Stage 1: Appraisal of the applicability and quality of individual studies included in the review.

Stage 2: Appraisal of the precision, size and clinical importance of the primary outcomes used to determine the safety and effectiveness of the intervention.

Stage 3: Integration of this evidence for conclusions about the net clinical benefit of the intervention in the context of Australian clinical practice.

Validity assessment of individual studies

The evidence presented in the selected studies was assessed and classified using the dimensions of evidence defined by the National Health and Medical Research Council (NHMRC 2000).

These dimensions (Table 12) consider important aspects of the evidence supporting a particular intervention, and include three main domains: strength of the evidence, size of

the effect and relevance of the evidence. The first domain is derived directly from the literature identified as informing a particular intervention. Each of the last two requires expert clinical input as part of its determination.

Table 12 Evidence dimensions (NHMRC 2000)

Type of evidence	Definition
Strength of the evidence:	
level	The study design used, as an indicator of the degree to which bias has been eliminated by design.[a]
quality	The methods used by investigators to minimise bias within a study design.
statistical precision	The p-value or, alternatively, the precision of the estimate of the effect. It reflects the degree of certainty about the existence of a true effect.
Size of effect	The distance of the study estimate from the 'null' value and the inclusion of only clinically important effects in the confidence interval.
Relevance of evidence	The usefulness of the evidence in clinical practice, particularly the appropriateness of the outcome measures used.

[a] See Table 13

Strength of the evidence

Three subdomains (level, quality and statistical precision) are collectively a measure of the strength of the evidence.

Level

The 'level of evidence' reflects the effectiveness of a study design to answer a particular research question. This is based on the probability that the design of the study has reduced or eliminated the impact of bias on the results.

The NHMRC evidence hierarchy provides a ranking of various study designs ('levels of evidence') by the type of research question being addressed (Table 13).

Table 13 Designation of levels of evidence according to type of research question (including table notes)

Level	Intervention[a]	Diagnostic accuracy[b]
I[c]	A systematic review of level II studies	A systematic review of level II studies
II	A randomised controlled trial	A study of test accuracy with an independent, blinded comparison with a valid reference standard[d], among consecutive persons with a defined clinical presentation[e]
III-1	A pseudo randomised controlled trial (ie alternate allocation or some other method)	A study of test accuracy with an independent, blinded comparison with a valid reference standard[d], among non-consecutive persons with a defined clinical presentation[e]
III-2	A comparative study with concurrent controls: – non-randomised, experimental trial[f] – cohort study – case-control study – interrupted time series with a control group	A comparison with a reference standard that does not meet the criteria required for level II and III-1 evidence

Level	Intervention[a]	Diagnostic accuracy[b]
III-3	A comparative study without concurrent controls: – historical control study – two or more single-arm studies[f] – interrupted time series without a parallel control group	Diagnostic case-control study[e]
IV	Case series with either post-test or pre-test/post-test outcomes	Study of diagnostic yield (no reference standard)[g]

Source: Merlin et al (2009)

Table notes

[a] Definitions of these study designs are provided in NHMRC (2000), pp. 7–8.

[b] The dimensions of evidence apply only to studies of diagnostic accuracy. To assess the effectiveness of a diagnostic test, there also needs to be a consideration of the impact of the test on patient management and health outcomes (MSAC 2005; Sackett & Haynes 2002).

[c] A systematic review will only be assigned a level of evidence as high as the studies it contains, excepting where those studies are of level II evidence. Systematic reviews of level II evidence provide more data than the individual studies and any meta-analyses will increase the precision of the overall results, reducing the likelihood that the results are affected by chance. Systematic reviews of lower level evidence present results of likely poor internal validity, and thus are rated on the likelihood that the results have been affected by bias rather than whether the systematic review itself is of good quality. Systematic review quality should be assessed separately. A systematic review should consist of at least 2 studies. In systematic reviews that include different study designs, the overall level of evidence should relate to each individual outcome/result, as different studies (and study designs) might contribute to each different outcome.

[d] The validity of the reference standard should be determined in the context of the disease under review. Criteria for determining the validity of the reference standard should be pre-specified. This can include the choice of the reference standard(s) and its/their timing in relation to the index test. The validity of the reference standard can be determined through quality appraisal of the study (Whiting et al 2003).

[e] Well-designed population-based case-control studies (eg population-based screening studies where test accuracy is assessed on all cases, with a random sample of controls) do capture a population with a representative spectrum of disease, and thus fulfil the requirements for a valid assembly of patients. However, in some cases the population assembled is not representative of the use of the test in practice. In diagnostic case-control studies a selected sample of patients already known to have the disease are compared with a separate group of normal/healthy people known to be free of the disease. In this situation patients with borderline or mild expressions of the disease, and conditions mimicking the disease, are excluded, which can lead to exaggeration of both sensitivity and specificity. This is called spectrum bias or spectrum effect because the spectrum of study participants will not be representative of patients seen in practice (Mulherin & Miller 2002).

[f] Comparing single-arm studies, (ie case series from 2 studies). This would also include unadjusted indirect comparisons (ie using A vs B and B vs C to determine A vs C, but with no statistical adjustment for B).

[g] Studies of diagnostic yield provide the yield of diagnosed patients, as determined by an index test, without confirmation of the accuracy of this diagnosis by a reference standard. These may be the only alternatives when there is no reliable reference standard.

Note A: Assessment of comparative harms/safety should occur according to the hierarchy presented for each of the research questions, with the proviso that this assessment occurs within the context of the topic being assessed. Some harms are rare and cannot feasibly be captured within randomised controlled trials; physical harms and psychological harms may need to be addressed by different study designs. Harms from diagnostic testing include the likelihood of false positive and false negative results; harms from screening include the likelihood of false alarm and false reassurance results.

Note B: When a level of evidence is attributed in the text of a document, it should also be framed according to its corresponding research question (eg level II intervention evidence; level IV diagnostic evidence; level III-2 prognostic evidence).

Source: Hierarchies adapted and modified from Bandolier (1999); Lijmer et al (1999); NHMRC (1999); Phillips et al (2001).

Individual studies assessing diagnostic accuracy were graded according to pre-specified quality and applicability criteria (MSAC 2005), as shown in Table 14.

Table 14 Grading system used to rank included studies

Validity criteria	Description	Grading system
Appropriate comparison	Did the study evaluate a direct comparison of the index test strategy with the comparator test strategy?	C1 direct comparison CX other comparison
Applicable population	Did the study evaluate the index test in a population that is representative of the subject characteristics (age and sex) and clinical setting (disease prevalence, disease severity, referral filter and sequence of tests) for the clinical indication of interest?	P1 applicable P2 limited P3 different population
Quality of study	Was the study designed to avoid bias? High quality = no potential for bias based on pre-defined key quality criteria Medium quality = some potential for bias in areas other than those pre-specified as key criteria Poor quality = poor reference standard and/or potential for bias based on key pre-specified criteria	Q1 high quality Q2 medium quality Q3 poor quality poor reference standard or insufficient information

Quality

The appraisal of uncontrolled before-and-after case series was assessed according to a checklist developed by the UK National Health Service (NHS) Centre for Reviews and Dissemination (Khan 2001). The six questions were scored 0–1 and summed to give an estimate of study quality: < 2 = poor quality (Q3); > 2 & ≤ 4 = medium quality (Q2); > 4 = high quality (Q1). Studies of diagnostic accuracy were assessed using the QUADAS quality assessment tool (Whiting et al 2003). The 14 questions were answered yes, no or unclear, and a point was given for each 'yes' answer. The results were tallied to give an estimate of study quality that was consistently applied across all studies: 12–14 = Q1; 10–11 = Q2; ≤ 9 = Q3. The scoring system was not intended to be proscriptive, as it is possible that some items in these checklists could be weighted more highly than others in the subjective assessment of study quality. Rather, the decision rules were simply used to assist with the narrative synthesis of the evidence-base.

Statistical precision

Statistical precision was determined using statistical principles. Small confidence intervals and p-values give an indication as to the probability that the reported effect is real and not attributable to chance (NHMRC 2000). Studies need to be appropriately powered to ensure that a real difference between groups will be detected in the statistical analysis.

Relevance of evidence

The outcomes being measured in this report are clinically relevant, where possible (ie the use of a linked evidence approach can make it difficult to provide clinically relevant

outcomes). Inadequately validated (predictive) surrogate measures of a clinically relevant outcome should be avoided (NHMRC 2000).

Assessment of the body of evidence

Appraisal of the body of evidence was conducted along the lines suggested by the NHMRC in their guidance on clinical practice guideline development (NHMRC 2000). Five components are considered essential by the NHMRC when judging the body of evidence:

- the evidence-base—which includes the number of studies sorted by their methodological quality and relevance to patients
- the consistency of the study results—whether the better quality studies had results of a similar magnitude and in the same direction, that is, either homogenous or heterogeneous findings
- the potential clinical impact—appraisal of the precision, size and clinical importance or relevance of the primary outcomes used to determine the safety and effectiveness of the test
- the generalisability of the evidence to the target Medicare population
- the applicability of the evidence—integration of the evidence for conclusions about the net clinical benefit of the intervention in the context of Australian clinical practice.

A matrix for assessing the body of evidence for each research question, according to the components above, was used for this assessment (Table 15) (NHMRC 2000).

Table 15 Body of evidence assessment matrix

Component	A	B	C	D
	Excellent	**Good**	**Satisfactory**	**Poor**
Evidence-base[a]	Several level I or II studies with low risk of bias	One or two level II studies with low risk of bias, or a SR/multiple level III studies with low risk of bias	Level III studies with low risk of bias, or level I or II studies with moderate risk of bias	Level IV studies, or level I to III studies with high risk of bias
Consistency[b]	All studies consistent	Most studies consistent and inconsistency may be explained	Some inconsistency reflecting genuine uncertainty around clinical question	Evidence is inconsistent
Clinical impact	Very large	Substantial	Moderate	Slight or restricted
Generalisability	Population(s) studied in body of evidence are the same as the target population	Population(s) studied in the body of evidence are similar to the target population	Population(s) studied in the body of evidence differ from target population for guideline, but it is clinically sensible to apply this evidence to target population[c]	Population(s) studied in the body of evidence differ from target population, and it is hard to judge whether it is sensible to generalise to target population
Applicability	Directly applicable to Australian healthcare context	Applicable to Australian healthcare context with few caveats	Probably applicable to Australian healthcare context with some caveats	Not applicable to Australian healthcare context

Adapted from NHMRC (2008)

[a] Level of evidence is determined from the NHMRC evidence hierarchy

[b] If there is only one study, rank this component as 'not applicable'

[c] For example, results in adults that are clinically sensible to apply to children OR psychosocial outcomes for one cancer that may be applicable to patients with another cancer

Expert advice

Members of the MESP and the PASC of the MSAC provided guidance to the Evaluators to ensure that the decision analytic protocol, outlining some of the methodology for this assessment report, included clinically relevant outcomes and comparators. The MESP members had expertise in clinical genetics and oncology.

Results of assessment

Is VHL genetic testing safe?

Genetic testing requires sampling of the patient's blood, generally from veins in the upper limbs. Venesection may rarely be associated with physical harms such as pain, bruising, nerve damage, arterial puncture or infection of the puncture site (Lavery & Ingram 2005; Scales 2008).

This assessment of safety considered any physical harms related to obtaining a sample necessary for genetic testing in the diagnosis of VHL. Relevant studies were assessed by applying the inclusion criteria outlined in Box 2 and Box 3.

Box 2 Inclusion criteria for identification of studies relevant to assessment of the safety of genetic testing for VHL syndrome (index patient)

Research question	
Is VHL genetic testing safe when used as an addition to clinical diagnostic approaches in the diagnosis of patients presenting with symptoms suggestive of VHL syndrome?	
Selection criteria	**Inclusion criteria**
Population	Patients presenting with one or more clinical features suggestive of VHL syndrome
Intervention	VHL genetic testing to diagnose *VHL* gene mutations, **and** clinical diagnosis from family history, clinical history, tests including CT, MRI, ultrasound, hearing test, eye exam, blood tests and other tests as appropriate, to identify any signs of disease other than presenting complaint
Comparator(s)	Clinical diagnosis from family history, clinical history, tests including CT, MRI, ultrasound, hearing test, eye exam, blood tests and other tests as appropriate to identify any signs of disease other than presenting complaint
Outcomes	Psychological and physical harms from genetic testing and clinical screening
Search period	1993 – May 2011
Language	Non-English language articles were excluded unless they appeared to provide a higher level of evidence than the English language articles identified

CT = computed tomography; MRI = magnetic resonance imaging; VHL = von Hippel-Lindau

Box 3 Inclusion criteria for identification of studies relevant to assessment of the safety of genetic testing for VHL mutations (family members)

Research question	
Is VHL genetic testing safe when used as a triage test for lifelong screening of family members of patients who are positive for a VHL mutation?	
Selection criteria	**Inclusion criteria**
Population	Clinically unaffected first- or second-degree family members of patients with clinically diagnosed VHL syndrome and/or a diagnosed VHL genetic abnormality
Intervention	VHL genetic testing to screen for *VHL* gene mutations ± clinical testing (CT, MRI, ultrasound, hearing test, eye exam and blood tests) **and** routine lifelong screening for neoplasms using CT, MRI, ultrasound, hearing test, eye exam and blood tests
Comparator(s)	Clinical testing (CT, MRI, ultrasound, hearing test, eye exam, and blood tests) **and** routine lifelong screening for neoplasms using CT, MRI, ultrasound, hearing test, eye exam and blood tests
Outcomes	Psychological and physical harms from genetic testing and clinical screening
Search period	1993 – May 2011
Language	Non-English language articles were excluded unless they appeared to provide a higher level of evidence than the English language articles identified

CT = computed tomography; MRI = magnetic resonance imaging; VHL = von Hippel-Lindau

No studies were identified that reported safety outcomes related to genetic testing for either the diagnosis of VHL syndrome or for identification of family members with a VHL mutation. Similarly, no case series reported any adverse outcomes associated with the use of genetic testing in the diagnosis of VHL syndrome.

Possible adverse events that can be associated with obtaining samples for use in genetic testing are examined in the 'Discussion' section of this report.

Summary of safety

No studies were identified that could inform an assessment of the safety of genetic testing in the diagnosis of VHL syndrome or for identification of family members with a VHL mutation.

Is VHL genetic testing effective?

Direct evidence

In order to evaluate whether there was a change in patient health outcomes following the use of genetic testing for diagnosing patients suspected of having VHL syndrome or for identifying a VHL mutation in family members, studies were selected on the basis of the inclusion criteria outlined in Box 4 and Box 5.

Box 4 Inclusion criteria for identification of studies relevant to assessment of direct evidence of the effectiveness of genetic testing for VHL syndrome (index patient)

Research question	
Is VHL genetic testing effective when used as an addition to clinical diagnostic approaches in the diagnosis of patients presenting with symptoms suggestive of VHL syndrome?	
Selection criteria	**Inclusion criteria**
Population	Patients presenting with one or more clinical features suggestive of VHL syndrome
Intervention	VHL genetic testing to diagnose *VHL* gene mutations, **and** clinical diagnosis from family history, clinical history, tests including CT, MRI, ultrasound, hearing test, eye exam, blood tests and other tests as appropriate, to identify any signs of disease other than presenting complaint
Comparator(s)	Clinical diagnosis from family history, clinical history, tests including CT, MRI, ultrasound, hearing test, eye exam, blood tests and other tests as appropriate to identify any signs of disease other than presenting complaint
Outcomes	Primary outcomes—mortality/survival, progression-free survival, quality of life, incidence and severity of life-threatening events arising from complications due to haemangioblastomas of the CNS, renal cell carcinomas and other malignant neoplasms associated with VHL syndrome Secondary outcomes—incidence and severity of symptoms (arising from haemangioblastomas of the retina and CNS, endolymphatic sac tumours, phaeochromocytomas, renal cysts and renal cell carcinomas, pancreatic cysts and tumours, and cystoadenomas of the adnexal reproductive organs), age at diagnosis
Search period	1993 – May 2011
Language	Non-English language articles were excluded unless they appeared to provide a higher level of evidence than the English language articles identified

CNS = central nervous system; CT = computed tomography; MRI = magnetic resonance imaging; VHL = von Hippel-Lindau

Box 5 Inclusion criteria for identification of studies relevant to assessment of direct evidence of the effectiveness of genetic testing for VHL mutations (family members)

Research question	
Is VHL genetic testing effective when used as a triage test for lifelong screening of family members of patients who are positive for a VHL mutation?	
Selection criteria	**Inclusion criteria**
Population	Clinically unaffected first- or second-degree family members of patients with clinically diagnosed VHL syndrome and/or a diagnosed VHL genetic abnormality
Intervention	VHL genetic testing to screen for *VHL* gene mutations ± clinical testing (CT, MRI, ultrasound, hearing test, eye exam and blood tests) **and** routine lifelong screening for neoplasms using CT, MRI, ultrasound, hearing test, eye exam and blood tests
Comparator(s)	Clinical testing **and** routine lifelong screening for neoplasms using CT, MRI, ultrasound, hearing test, eye exam and blood tests
Outcomes	Primary outcomes—mortality/survival, progression-free survival, quality of life, incidence and severity of life-threatening events arising from complications due to haemangioblastomas of the CNS, renal cell carcinomas and other malignant neoplasms associated with VHL syndrome Secondary outcomes—incidence of symptoms (arising from haemangioblastomas of the retina and CNS, endolymphatic sac tumours, phaeochromocytomas, renal cysts and renal cell carcinomas, pancreatic cysts and tumours, and cystoadenomas of the adnexal reproductive organs), age at diagnosis
Search period	1993 – May 2011
Language	Non-English language articles were excluded unless they appeared to provide a higher level of evidence than the English language articles identified

CNS = central nervous system; CT = computed tomography; MRI = magnetic resonance imaging; VHL = von Hippel-Lindau

No direct evidence was identified that reported a change in patient health outcomes following genetic testing in addition to usual clinical diagnosis when compared with usual clinical diagnosis alone in patients suspected of having VHL syndrome. Similarly, no direct evidence was identified that assessed the effectiveness of VHL genetic testing when used as a triage test for lifelong screening of family members of patients who are positive for a VHL mutation. However, 14 case series (level IV interventional evidence) with a low–medium risk of bias reported on the likelihood of VHL mutation positive patients developing various VHL-associated neoplasms and their corresponding symptoms (Table 16, Table 17).

Ten of these studies reported on the likelihood of VHL mutation positive patients developing various VHL-associated neoplasms and their mean age of symptom onset. Table 17 summarises the prevalence or incidence rates and age of onset of these neoplasms. The results reported in the studies were mostly consistent; where disparity occurred, it could usually be explained by variability due to small sample size in at least one study. The populations in these studies were the same as the MBS target population (ie patients with a VHL mutation with or without clinical manifestations of disease), making the results generalisable. As these studies were all conducted in the UK, Europe and the United States of America (USA), they are applicable to the Australian healthcare context with few caveats. However, due to the lack of a comparator group in these

studies, no comment can be made about the clinical impact of genetic testing on health outcomes.

The lack of data that directly compared patient health outcomes following clinical diagnosis with and without genetic testing is to be expected, given that the genetic test is considered confirmatory in clinically diagnosed patients, and so does not alter patient management. Adverse health outcomes are avoided by subsequent annual screening for early detection of newly developed neoplasms, and the protocol is identical for all VHL patients irrespective of their VHL mutation status. Six studies reported on health outcomes for patients with both a clinical and a genetic diagnosis of VHL syndrome.

Three studies reported on the likelihood of patients with a VHL mutation suffering from vision loss or blindness due to the presence of, or treatment for, retinal haemangioblastomas (Table 16). The overall probability of a VHL mutation positive patient incurring vision loss was 24–35% (Niemela et al 2000; Webster et al 1999b), but this increased to 55–71% for VHL mutation positive patients who had symptoms at the time of diagnosis (Kreusel et al 2006; Webster et al 1999b). The risk of blindness varied greatly between the 2 studies that reported this outcome; Niemela et al (2000) reported that 18.2% of patients lost sight in an eye, compared with only 5.3% of patients in the study by Kreusel et al (2006). This variability could be explained by the extremely small sample size of the first study (a mutation in the *VHL* gene was confirmed in 6/8 VHL patients), compared with the 43 patients with clinical and genetic diagnosis of VHL syndrome in the study by Kreusel et al (2006).

Neumann et al (1999) reported on the success of adrenal-sparing surgery in treating VHL mutation positive patients with symptomatic phaeochromocytomas. They found that this surgery is usually successful, and that only 1 patient out of 33 became steroid dependent, due to loss of adrenal function over a 6-year follow-up period (Table 16).

Two studies reported on health outcomes in VHL mutation positive patients with renal cell carcinoma (Table 16). Neumann et al (1998) found that the overall 10-year survival rate for VHL patients with renal cell carcinoma was 86%, even though 36% of patients with tumours larger than 7 cm developed metastatic disease. Joly et al (2011) found that 17.7% of VHL patients with renal cell carcinoma required haemodialysis and 8% required renal transplantation. Although the all-cause mortality rate varied between the 2 studies (17.7% in the study by Joly et al (2011) and 33.3% in the study by Neumann et al (1998)), they had similar VHL-associated mortality rates (15.0% and 20.6%, respectively).

Table 16 Health outcomes following genetic testing plus or minus annual screening

Author Location	Study design Quality	Study population Patient group	Outcomes
VHL patients with retinal haemangioblastoma			
(Webster et al 1999b) UK	Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 183 VHL mutation carriers from 81 families recruited from all UK ophthalmic and clinical genetics departments	**Cumulative probability of incurring vision loss by age 50 years** 35% in all gene carriers 55% in patients with haemangioblastomas
(Kreusel et al 2006) Germany	Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 57 consecutive patients at 2 German clinics presenting with capillary retinal angiomatosis resulting from VHL disease n = 43 patients with clinical and genetic diagnosis n = 2 with genetic diagnosis only n = 12 with clinical diagnosis only	**Age-related risk for bilateral retinal angioma** 100% at age 56.4 years **Proportion of eyes with angiomas** 85.8% (97/113) **Proportion of eyes that were enucleated or developed blindness** 5.3% (6/113) **Mean age of onset in VHL patients** 21.0 ± 10.7 years **Mean age of onset for sporadic angiomas** 26.5 ± 13.9 years
(Niemela et al 2000) Finland	Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 11 patients with clinically definite VHL and retinal haemangioblastoma	**Rate of sight loss in an eye of VHL patients** 23.8% (5/21) **Rate of blindness in VHL patients** 18.2% (2/11) **Median age of onset for VHL patients** 27 years (range 11–65)
VHL patients with phaeochromocytoma			
(Neumann et al 1999) Germany	Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 39 patients with phaeochromocytomas that underwent adrenal-sparing surgery n = 21 with VHL mutations n = 13 sporadic cases	**Success of surgery** 94.9% (37/39) **Steroid dependence at end of study** 3% (1/33) **Asymptomatic at end of study** 100% (33/33)
VHL patients with renal cell carcinoma			
(Neumann et al 1998) Germany	Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 63 patients with renal cell carcinoma (RCC) from 30 families with RCC (21/30 families had an identified VHL mutation)	**Overall 10-year survival for VHL patients** 86% **10-year survival for stage adjusted T2 tumours** 94% **Metastatic disease with tumour diameter**: **> 7 cm:** 35.7% (5/14) **< 7 cm:** 0% (0/49) **All cause mortality** 33.3% (21/63) **VHL-associated mortality** 20.6% (13/63) 19.0% (12/63) due to CNS haemangioblastoma **Mean age at diagnosis for VHL patients** 36.3 ± 1.4 (range 20–65)
(Joly et al 2011) France	Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 176 patients who had VHL disease with renal involvement and were VHL mutation positive from a total of 112 families n= 113 who had treatment for	**Severity of kidney disease:** **stage 3 (GFR < 60 ml/min/1.73 m^2)** 12.4% (14/113) **stage 4 (GFR < 30 ml/min/1.73 m^2)** 0.9% (1/113) **end stage (require haemodialysis)**

Author Location	Study design Quality	Study population Patient group	Outcomes
		RCC	8.0% (9/113) **required renal transplantation** 3.5% (4/113) **All-cause mortality rate** 17.7% (20/113) **Mortality due to metastatic RCC** 6.2% (7/113) rate = 0.97/100 patient-years **Mortality due to other VHL complications** 8.8% (10/113) **Mean age at first treatment** 37.6 ± 11.6 years

CNS = central nervous system; GFR = glomerular filtration rate; RCC = renal cell carcinoma; RR = relative risk; VHL = von Hippel-Lindau

Table 17 Prevalence/incidence of VHL-related neoplasms

Author Location	Study design Quality	Study population	Patient group	Prevalence/incidence of VHL-related neoplasm
CNS haemangioblastoma				
(Poulsen et al 2010) Denmark	Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 59 VHL-mutation carriers from 22 unrelated families	n = 54 patients who agreed to participate	**Incidence 1971–2008** 77.8% (42/54) **Incidence of first manifestation** 31.5% (17/54) **Mean age at onset** 31 years (range 8–57)
Retinal haemangioblastoma				
(Webster et al 1999b) UK	Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 183 VHL mutation carriers from 81 families recruited from all UK ophthalmic and clinical genetics departments	n = 183 patients	**Prevalence** 67.8% (124/183)
(Kreusel et al 2006) Germany	Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 57 consecutive patients at 2 clinics presenting with capillary retinal angiomatosis resulting from VHL disease	n = 43 patients with clinical and genetic diagnosis n = 2 with genetic diagnosis only n = 12 with clinical diagnosis only	**Prevalence of ocular lesions in VHL patients** 10% at age 9.3 years 95% at age 37.5 years 100% at age 55.3 years **Mean age at onset** 20.3 ± 10.4 years (range 5.5–55.5)
(Dollfus et al 2002) France	Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 211 patients registered in the French VHL database between 1996 and 1999 who met the diagnostic criteria for VHL	n = 196 patients who agreed to genetic testing n = 149 patients who had a VHL mutation	**Prevalence among patients registered in the French VHL database** 48.8% (103/211) **Prevalence among patients with a known VHL mutation** 49.7% (74/149) **Mean age at onset** 24.4 years (range 6–51)
(Poulsen et al 2010) Denmark	Level IV interventional evidence	N = 59 VHL-mutation carriers from 22 unrelated	n = 54 patients who agreed to participate	**Incidence 1971–2008** 53.7% (29/54) **Incidence of first manifestation**

Author Location	Study design Quality	Study population	Patient group	Prevalence/incidence of VHL-related neoplasm
	Medium quality (NHS CRD = 4/6)	families		27.8% (15/54) **Mean age at onset** 21 years (range 7–62)
Phaeochromocytoma				
(Poulsen et al 2010) Denmark	Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 59 VHL-mutation carriers from 22 unrelated families	n = 54 patients who agreed to participate	**Incidence 1971–2008** 11.1% (6/54) **Incidence of first manifestation** 3.7% (2/54) **Mean age at onset** 20.5 years (range 8–36)
Endolymphatic sac tumour				
(Choo et al 2004) USA	Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 175 individuals, or from families with confirmed VHL disease or who were at risk	n = 129 patients with confirmed VHL and GT positive	**Prevalence in patients with VHL (clinical and GT positive)** 16.3% (21/129)
(Manski et al 1997) USA	Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 374 patients whose brain MRIs were available for review N = 66 consecutive patients from the VHL clinic, without additional screening criteria were studied	n = 121 patients fulfilled the criteria for VHL diagnosis n = 49 patients with proven VHL	**Prevalence in patients with clinical diagnosis of VHL** 10.7% (13/121) **Prevalence in patients with proven VHL (clinical and GT positive)** 6.1% (3/49) **Prevalence in patients with a VHL mutation (± clinical disease)** 4.5% (3/66)
(Poulsen et al 2010) Denmark	Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 59 VHL-mutation carriers from 22 unrelated families	n = 54 patients who agreed to participate	**Incidence 1971–2008** 0% (0/54) **Incidence of first manifestation** 0% (0/54)
Renal cysts				
(Poulsen et al 2010) Denmark	Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 59 VHL-mutation carriers from 22 unrelated families	n = 54 patients who agreed to participate	**Incidence 1971–2008** 46.3% (25/54) **Incidence of first manifestation** 0% (0/54) **Mean age at onset** 32 years (range 12–57)
Renal cell carcinoma				
(Poulsen et al 2010) Denmark	Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 59 VHL-mutation carriers from 22 unrelated families	n = 54 patients who agreed to participate	**Incidence 1971–2008** 18.5% (10/54) **Incidence of first manifestation** 7.4% (4/54) **Mean age at onset** 31.5 years (range 23–55)
Pancreatic cysts				
(Erlic et al 2010) Germany	Level IV interventional evidence	N = 485 registrants from the VHL-registry undergoing	n = 485 patients	**Prevalence of pancreatic cysts** 35.3% (171/485)

Author Location	Study design Quality	Study population	Patient group	Prevalence/incidence of VHL-related neoplasm
	Medium quality (NHS CRD = 4/6)	pancreatic imaging		
(Poulsen et al 2010) Denmark	Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 59 VHL-mutation carriers from 22 unrelated families	n = 54 patients who agreed to participate	**Incidence 1971–2008** 35.2% (19/54) **Incidence of first manifestation** 3.7% (2/54) **Mean age at onset** 29 years (range 12–63)
(Mukhopadhyay et al 2002) UK	Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 25 consecutive patients followed by the Department of Endocrinology at St Bartholomew's Hospital since 1988	n = 17 consecutive patients belonging to 14 families with clinical signs of VHL disease	**Prevalence in patients with clinical diagnosis of VHL** 52.9% (9/17) **Prevalence of pancreatic cysts in patients with VHL (clinical and GT positive)** 55.5% (5/9)
Pancreatic tumours				
(Erlic et al 2010) Germany	Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 485 registrants from the VHL-Registry underwent pancreatic imaging	n = 485 patients	**Prevalence of islet cell tumours of the pancreas** 10.7% (52/485) **Prevalence of malignant Islet cell tumours of the pancreas** 2.7% (13/485)
(Libutti et al 2000) USA	Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 389 patients with VHL disease who were evaluated December 1988 – December 1999	n = 188 VHL mutation positive families	**Prevalence of pancreatic neuro-endocrine tumours (PNETs)** 19.1% (36/188) **Prevalence of metastatic PNETs** 2.1% (4/188)
(Poulsen et al 2010) Denmark	Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 59 VHL-mutation carriers from 22 unrelated families	n = 54 patients who agreed to participate	**Incidence 1971–2008** 9.3% (5/54) **Incidence of first manifestation** 1.9% (1/54) **Mean age at onset** 41 years (range 24–57)
Liver cysts or tumours				
(Poulsen et al 2010) Denmark	Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 59 VHL-mutation carriers from 22 unrelated families	n = 54 patients who agreed to participate	**Incidence 1971–2008** 9.3% (5/54) **Incidence of first manifestation** 0% (0/54) **Mean age at onset** 39 years (range 27–45)
Cysts of the broad ligament				
(Poulsen et al 2010) Denmark	Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 59 VHL-mutation carriers from 22 unrelated families	n = 54 patients who agreed to participate	**Incidence 1971–2008** 1.9% (1/54) **Incidence of first manifestation** 0% (0/54) **Mean age at onset** 23 years

Author Location	Study design Quality	Study population	Patient group	Prevalence/incidence of VHL-related neoplasm
Epididymal cystoadenoma				
(Choyke et al 1997) USA	Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 56 consecutive affected men from the VHL clinic	n = 34 VHL patients with epididymal cystoadenoma and known VHL mutation status	**Prevalence in men with a clinical VHL diagnosis** 53.6% (30/56) **Prevalence in men with VHL disease and a known *VHL* gene mutation** 41.2% (14/34)
(Poulsen et al 2010) Denmark	Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 59 VHL-mutation carriers from 22 unrelated families	n = 54 patients who agreed to participate	**Incidence 1971–2008** 11.1% (6/54) **Incidence of first manifestation** 1.9% (1/54) **Mean age at onset** 21 years (range 10–37)

CNS = central nervous system; GT = genetic test; MRI = magnetic resonance imaging; PNETs = pancreatic neuro-endocrine tumours; VHL = von Hippel-Lindau

Summary of effectiveness

No direct evidence was identified comparing patient health outcomes following genetic testing in addition to usual clinical diagnosis versus usual clinical diagnosis alone in patients suspected of having VHL syndrome, or for assessing the effectiveness of VHL genetic testing when used as a triage test for life-long screening of family members.

Given that health benefits are derived from reduced morbidity and mortality due to annual screening for early detection of newly developed neoplasms, and that the annual screening protocol is identical for all patients clinically diagnosed with VHL syndrome, irrespective of their VHL mutation status, the lack of comparative data was predictable.

It is therefore unclear what impact, if any, the addition of VHL genetic testing to clinical diagnosis has on the health outcomes of patients suspected of VHL syndrome.

Linked evidence

In the absence of comparative direct evidence, a supplementary linked evidence approach was used to assess of the effectiveness of genetic testing to diagnose VHL syndrome in symptomatic patients or to identify family members carrying the VHL mutation. A linked evidence approach normally involves linking evidence of diagnostic accuracy, change in clinical management and treatment effectiveness. However, it was decided *a priori* that no change in clinical management was expected for either patients with VHL syndrome or family members with a VHL mutation. Therefore, treatment effectiveness was not formally assessed.

Is VHL genetic testing accurate in the index case?

For this assessment the diagnostic accuracy of genetic testing for mutations in the *VHL* gene was compared with usual clinical diagnosis in diagnosing patients with suspected VHL syndrome. The studies used in the assessment met the inclusion criteria outlined in Box 6.

Box 6 Inclusion criteria for selecting studies relevant to assess the predictive accuracy of genetic testing for VHL syndrome (index patient)

Research question	
Is genetic testing for mutations in the *VHL* gene, in addition to usual diagnostic assessment, as accurate as, or more accurate than, usual clinical diagnosis in diagnosing patients with suspected VHL syndrome?	
Selection criteria	**Inclusion criteria**
Population	Patients presenting with one or more clinical features suggestive of VHL syndrome
Intervention	VHL genetic testing to diagnose *VHL* gene mutations **and** clinical diagnosis from family history, clinical history, tests including CT, MRI, ultrasound, hearing test, eye exam, blood tests and other tests as appropriate, to identify any signs of disease other than presenting complaint
Comparator(s)	Clinical diagnosis from family history, clinical history, tests including CT, MRI, ultrasound, hearing test, eye exam, blood tests and other tests as appropriate to identify any signs of disease other than presenting complaint
Reference standard	Clinical diagnosis determined from long-term follow-up
Outcomes	Predictive accuracy outcomes: Sensitivity and specificity (and therefore rates of false positives and negatives), positive and negative likelihood ratios, positive and negative predictive values (and therefore false alarm and reassurance rates), diagnostic odds ratios, receiver–operator characteristic curves, area under the curve, accuracy
Search period	1993 – May 2011
Language	Non-English language articles were excluded unless they provided a higher level of evidence than the English language articles identified

CT = computed tomography; MRI = magnetic resonance imaging; VHL = von Hippel-Lindau

Eighty-one studies met the inclusion criteria outlined *a priori,* and reported on the analysis of VHL mutations in the diagnosis of VHL syndrome in patients presenting with one or more VHL-associated neoplasms. The populations in all these studies consisted of patients with symptoms of VHL disease, making the results generalisable to the

population in Australia for whom genetic testing for VHL is expected to occur. Although these studies were conducted all over the world, including Mexico, Brazil, Kuwait, Korea, China and Japan, the majority were conducted in the UK, Europe and the USA, suggesting that the results are applicable to the Australian healthcare context with few caveats.

Fifty-six comparative studies provided data on the diagnostic accuracy (level III-2 diagnostic evidence) of genetic testing compared with a clinical diagnosis for patients that could potentially have VHL syndrome (Table 19). The reference standard outlined *a priori* (ie clinical diagnosis in the long term) was not reported in any of the studies.

As genetic testing is used not only to determine who may have VHL disease currently, but to predict who may meet clinical diagnostic criteria in the future, the comparison against current or short-term clinical diagnosis is flawed. However, in the absence of other data, these studies were included to provide an estimate of the sensitivity and specificity of the genetic test compared with clinical diagnosis. Sensitivity measures the proportion of patients who meet the clinical diagnostic criteria—who are correctly identified as having a genetic mutation—whereas specificity measures the proportion of patients who do *not* have VHL disease based on clinical information—in whom genetic testing correctly finds no pathogenic mutation. Sensitivity and specificity rates are independent of the prevalence of the disease within the population receiving the test (Altman & Bland 1994a; Lalkhen & McCluskey 2008).

Positive and negative predictive values were also determined whenever possible. These measures represent the proportion of patients with a positive or negative genetic test result that were clinically diagnosed correctly. Although these values are useful for determining the clinical value of a test, their dependence on the prevalence of the disease in the study population means that the values observed in one study may not apply to other populations. The more prevalent VHL syndrome is in the population, the greater the likelihood that a positive test indicates a true VHL mutation, and the less likely that a negative result really indicates no VHL mutation (Akobeng 2007; Altman & Bland 1994b).

These 56 comparative studies were grouped according to the study population included, as shown in Table 19. Fifteen studies (3 with a low, and 12 with a medium, risk of bias) compared clinical diagnosis with genetic diagnostic methods to diagnose patients presenting with one or more VHL-associated neoplasms who could potentially have had VHL syndrome. This patient population is representative of the full spectrum of patients expected to undergo genetic testing to diagnose VHL syndrome, particularly incident VHL index cases. Twenty-four studies (7 with a low, 15 with a medium and 2 with a high, risk of bias) investigated the accuracy of genetic testing to detect a VHL mutation

in patients who had already been clinically diagnosed with VHL syndrome. Although this patient group is representative of a large proportion of patients who would be expected to undergo genetic testing, the absence of patients with a negative clinical diagnosis results in a positive predictive value of 100%, and a lack of data for determining test specificity. Results are therefore only applicable to prevalent VHL syndrome patients as (determined by clinical diagnosis) in the targeted MBS population.

The remaining studies included patient groups that were diagnosed with a specific VHL-associated neoplasm (Table 19). In 5 studies (1 with a low, 3 with a medium and 1 with a high, risk of bias) patients were diagnosed with a CNS haemangioblastoma, 33–77% of which were also clinically diagnosed with VHL syndrome. These patients underwent genetic testing to determine the number of apparently sporadic CNS haemangioblastoma cases that were actually due to VHL syndrome. Similar studies were conducted involving patients with retinal haemangioblastoma, where 33.0–88.0% also had VHL syndrome (3 studies; 1 with a low, and 2 with a medium, risk of bias) or phaeochromocytoma with 3–64% also having VHL syndrome (8 studies; 5 with a low, 2 with a medium and 1 with a high, risk of bias). These patient populations are also representative of the type of index cases expected to undergo genetic testing to diagnose VHL syndrome, albeit with a restrictive spectrum of the disease. It is expected that a VHL mutation will be identified in some of the apparently sporadic cases, as these patients may be presenting with their first manifestation of VHL syndrome and do not yet meet the criteria for a clinical diagnosis of VHL syndrome.

The sensitivity of genetic testing was highly variable between studies (3.9–100%; Table 19), which could be due to either the different population subtypes tested or the different genetic testing methodologies used. The data were highly variable for each population subtype and did not appear to suggest any true differences between them, except for phaeochromocytoma patients (discussed below). Conversely, the data did indicate differences according to the testing methodology used.

In broad terms, mutational changes to the DNA sequence occur on two different scales in VHL syndrome, requiring two different detection methods. Small changes, such as missense mutations and small deletions, which lead to a change in the protein sequence or premature termination of protein synthesis are detected by DNA sequencing. In many of these studies the PCR products used for sequencing were pre-screened using methods such as single-strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), denaturing high-performance liquid chromatography (DHPLC) and conformation-sensitive gel electrophoresis (CSGE). These screening methods compare the physical properties of the PCR products obtained from the patient's *VHL* genes with PCR products obtained from normal control *VHL* DNA; only

the PCR products that have different properties to the normal PCR products are sequenced.

There are differences in the ability of these methods to detect single base pair changes between the PCR products. Klein et al (2001) demonstrated that the sensitivity of two commercial DHPLC analytical systems differed (86% and 95%) when analysing the same samples (Table 19). Additionally, Klein et al (2001) used DHPLC to analyse samples from 36 VHL patients who had previously undergone genetic testing using SSCP analysis and in whom no VHL mutation had been detected (Glavac et al 1996). Klein et al (2001) identified mutations in three of these patients, suggesting that DHPLC may pick up different mutations. The current worldwide standard method used by most laboratories offering VHL genetic testing is to directly sequence all three VHL exons without using any pre-screening methods (Gene Tests 1993).

Large deletions or rearrangements involving complete VHL exons, the entire *VHL* gene or an even larger portion of the short arm of chromosome 3 cannot be detected by DNA sequencing if a second normal copy of the *VHL* gene is present. These types of mutations are detected using methods such as universal primer quantitative fluorescent multiplex PCR (UPQFM-PCR), long PCR, multiplex ligation-dependent probe amplification (MLPA), fluorescence in-situ hybridisation (FISH) and Southern blotting. Although the most commonly used method to detect large deletions in these studies was Southern blotting, most genetic laboratories that offer detection of *VHL* gene deletions worldwide use MLPA as their standard technique.

The criteria for a clinical diagnosis of VHL syndrome in these studies generally concurred with the currently accepted clinical criteria in Australia, namely: patients with (i) a family history of VHL disease, plus a CNS or retinal haemangioblastoma, phaeochromocytoma, or renal cell carcinoma, (ii) two or more haemangioblastomas or (iii) one haemangioblastoma and a visceral tumour (with the exception of epididymal and renal cysts, which are frequent in the general population). However, given that a lifetime of follow-up is needed to identify all cases of VHL syndrome, the usual reference standard would be considered an imperfect measure against which the accuracy of the genetic test could be determined. It is generally accepted that a small proportion of patients, especially *de novo* cases where the patient is presenting with a first neoplasm, will not meet the criteria for clinical diagnosis at the time of presentation; however, the genetic test would be expected to detect a VHL mutation, leading to an earlier diagnosis of VHL syndrome.

Interestingly, the 8 studies conducted with phaeochromocytoma patients, with or without a clinical diagnosis of VHL syndrome, showed different sensitivity results to most of the other studies (Table 19). In all these studies, genetic testing included DNA

sequencing (with or without pre-screening) and mutations were detected in all but one patient with a clinical diagnosis of VHL syndrome. This study, in which a VHL mutation could not be detected in a patient with VHL syndrome, pre-screened the PCR products prior to DNA sequencing—a less sensitive method than direct DNA sequencing of all PCR products. Thus, 7 out of 8 studies had sensitivity and negative predictive values of 100%, compared with a sensitivity of 44–91% for DNA sequencing in studies involving other VHL patient groups (Table 19). This difference is most likely due to the high degree of correlation between the risk of developing phaeochromocytoma and the presence of a missense VHL mutation that results in an amino acid substitution in the pVHL (Maher et al 1996; Ong et al 2007; Zbar et al 1996). As missense mutations are detected using DNA sequencing (with or without pre-screening), and large deletions of the *VHL* gene (which cannot be detected by DNA sequencing) are not expected in this patient group, a high sensitivity is expected. Thus, a negative VHL genetic test would effectively rule out a diagnosis of VHL syndrome in these patients. These studies were omitted from the sensitivity comparisons for different genetic testing methodologies described below.

The median sensitivity for studies that used DNA sequencing with and without pre-screening of PCR products was 67% (range 52–88) and 77% (range 44–91), respectively, with false negative rates of 41% and 25%, respectively (Table 18). These values did not alter significantly if studies with a low risk of bias were analysed separately. These results suggest that DNA sequencing of all three PCR products from all three exons of the *VHL* gene (no pre-screening) is more successful at identifying small errors in the *VHL* gene than is sequencing of only those PCR products that have altered physical properties, compared with a control PCR product from a normal *VHL* gene (pre-screening). In fact, a false negative rate of 24.9%, as seen for direct DNA sequencing studies, correlates with the 20–30% of VHL families that have large germ-line deletions of all or part of the *VHL* gene. These deletions are not detectable by DNA sequencing (Ciotti et al 2009). Consequently, the low median sensitivity (17%; range 4–37) found for studies that used methodologies to detect large deletions of the *VHL* gene was predictable.

When DNA sequencing with pre-screening was combined with a deletion detection methodology, the sensitivity improved to 75% (range 14–100). However, when direct DNA sequencing and a deletion detection methodology were both used, the median sensitivity improved even further to 100% (range 70–100) (Table 18). Currently, all three laboratories that offer genetic testing of the *VHL* gene in Australia offer direct DNA sequencing, which is combined with a deletion detection methodology such as MLPA in two of them, suggesting that these two diagnostic laboratories should be able to correctly identify nearly all patients that carry a germ-line VHL mutation. However, these methods were still associated with a false negative rate of 10.2% in the included studies,

suggesting that either some patients may be clinically misdiagnosed with VHL syndrome when they do not have the condition or, more likely, detection of a germ-line mutation is not yet possible for some patients with VHL syndrome.

The median specificity, and positive and negative predictive values, in all studies that involved patients with both a positive and negative clinical diagnosis of VHL syndrome (including the studies involving phaeochromocytoma patients) for the five genetic testing methodology groups are summarised in Table 18. Again, these values did not change significantly when only studies with a low risk of bias were analysed separately. The specificity varied little and was high for all genetic testing methodology groups. The false positive rate also varied little—between 0% and 5%. This suggests that few patients who did *not* meet the criteria for clinical diagnosis of VHL syndrome were found to have an underlying VHL mutation. It is likely that the few patients with 'false positives' actually did have the first manifestations of VHL syndrome but that the disease had not yet progressed sufficiently to obtain a positive clinical diagnosis. The high positive predictive value indicates that a patient with a positive test result has a very high probability of having a true germ-line VHL mutation. The negative predictive value is low for deletion detection methodologies, but this is expected as only a small proportion of patients have large germ-line deletions. It is interesting to note that the group of studies that used direct DNA sequencing plus a deletion detection methodology corresponding to current standard worldwide laboratory VHL genetic testing methods had a median negative predictive value of 100%, indicating that patients with a negative test result are unlikely to have an undetected VHL mutation.

Table 18 Median and range of diagnostic accuracy data from studies with a low–medium risk of bias for different genetic testing methodologies

Genetic testing methodology	Sensitivity[a]	Specificity[b]	PPV[b]	NPV[b]
Pre-screened DNA sequencing	66.9% (51.8–87.5) FN = 40.5% [37.5, 43.6] k = 8 (2 Q1)	95.0% (88.9–100) FP = 3.4% [1.1, 9.0] k = 5 (3 Q1)	97.8% (85.7–100) k = 5 (3 Q1)	72.2% 30.3–100) k = 5 (3 Q1)
Direct DNA sequencing	76.9% (44.4–91.4) FN = 24.9% [21.5, 28.6] k = 13 (5 Q1)	100% (57.1–100) FP = 5.2% [3.3, 8.1] k = 8 (2 Q1)	100% (36.0–100) k = 8 (2 Q1)	80.9% (14.3–100) k = 8 (2 Q1)
Deletion detection (DD) methods	17.4% (3.9–36.6) FN = 85.7% [83.6, 87.5] k = 18 (5 Q1)	100% (100–100) FP = 0% [0, 10.0] k = 5 (0 Q1)	100% (100–100) k = 5 (0 Q1)	17.1% (4.8–52.4) k = 5 (0 Q1)
Pre-screened DNA sequencing plus DD	74.6% (14.3–100) FN = 27.4% [25.2, 29.8] k = 15 (6 Q1)	94.9% (50.0–100) FP = 5.1% [3.9, 6.5] k = 9 (4 Q1)	97.1% (54.2–100) k = 9 (4 Q1)	80.0% (12.5–100) k = 9 (4 Q1)
Direct DNA sequencing (no pre-screening) plus DD	100% (70.0–100) FN = 10.2% [7.8, 13.0] k = 17 (7 Q1)	100% (50.0–100) FP = 4.2% [1.6, 10.1] k = 8 (1 Q1)	100% (77.8–100) k = 9 (1 Q1)	100% (33.3–100) k = 8 (1 Q1)

[a] Median and range measured using all studies except those involving phaeochromocytoma patients; [b] Median and range measured using all studies including those involving phaeochromocytoma patients, except those involving only patients with a clinical diagnosis of VHL syndrome; the median values for all groups did not vary significantly if only studies with a low risk of bias were included in the analysis; low-quality studies with a high risk of bias were also excluded from the calculations; the 95% CI for false positives and false negatives are within square brackets

CI = confidence interval; FN = false negatives; FP = false positives; k = number of studies; NPV = negative predictive value; PPV = positive predictive value; Q1 = high-quality study with a low risk of bias

Table 19 Diagnostic accuracy of genetic testing for *VHL* gene mutations in the diagnosis of VHL syndrome

Study Location	Study quality[a]	Population and comparator	Genetic test technique	Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
Patients suspected of having VHL disease							
(Klein et al 2001) Germany	Level III-2 diagnostic evidence CX P2 Q1	N = 79 unrelated VHL patients n = 68 VHL cases n = 11 sporadic disease cases (n = 36 with unknown VHL mutation status n = 43 with VHL mutations) ***Reference standard*** Clinical diagnosis	DHPLC and DNA sequencing	**All patients** 66.2% [54.3, 76.3] FN = 33.8% (23/68)	90.9% [62.3, 98.4] FP = 9.1% (1/11)	97.8% [88.7, 99.6]	30.3% [17.4, 47.3]
				Patients with known mutation			
			DHPLC (V-H system)	86.0% [71.4, 94.2] FN = 14.0% (6/43)		100% [88.3% 100]	
			(WNAFA system)	94.7% [80.9, 99.1] FN = 2/38		100% [88.0, 100]	
(Rasmussen et al 2006) Mexico	Level III-2 diagnostic evidence CX P1 Q1	N = 23 patients with suspected VHL syndrome n = 14 VHL cases n = 4 possible VHL cases n = 5 sporadic disease cases ***Reference standard*** Clinical diagnosis	DNA sequencing	85.7% [56.2, 97.5] FN = 14.3% (2/14)	100% [62.9, 100] FP = 0% (0/9)	100% [69.9, 100]	81.8% [47.8, 96.8]
(Hoebeeck et al 2005) Belgium	Level III-2 diagnostic evidence CX P1 Q1	N = 17 patients with suspected VHL syndrome n = 16 VHL cases n = 1 sporadic disease cases ***Reference standard*** Clinical diagnosis	DNA sequencing, real-time Q-PCR, Southern blotting	100% [75.9, 100] FN = 0% (0/16)	100% [5.5, 100] FP = 0% (0/1)	100% [75.9, 100]	100% [5.5, 100]
(Hes et al 2007) Netherlands	Level III-2 diagnostic evidence CX P1 Q2	N = 146 probands n = 38 classic VHL cases n = 17 non-classic VHL cases n = 91 sporadic disease cases ***Reference standard*** Clinical diagnosis	DNA sequencing, Southern blotting, MLPA	**All VHL patients** 72.7% [59.8, 82.7] FN = 27.3% (15/55)	96.7% [90.8, 98.9] FP = 3.3% (3/91)	93.0% [81.4, 97.6]	85.4% [77.4, 91.0]
				Classic VHL patients 94.7% [82.7, 98.5] FN = 5.3% (2/38)	96.7% [90.8, 98.9] FP = 3.3% (3/91)	92.3% [79.7, 97.3]	97.8% [92.3, 99.4]
(Olschwang	Level III-2	N = 110 patients	DGGE and DNA	71.7% [0.62, 0.80]	88.9% [0.67, 0.97]	97.1% [0.90, 0.99]	38.1% [0.25, 0.53]

Study Location	Study quality[a]	Population and comparator	Genetic test technique	Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
et al 1998) France	diagnostic evidence CX P1 Q2	n = 92 unrelated VHL patients n = 18 patients with sporadic haemangioblastoma ***Reference standard*** Clinical diagnosis	sequencing, Southern blotting	FN = 28.3% (26/92)	FP = 11% (2/18)		
			DGGE and DNA sequencing	66.3% [0.56, 0.75] FN = 33.7% (31/92)	88.9% [0.67, 0.97] FP = 11.1% (2/18)	96.8% [0.89, 0.99]	34.0% [0.22, 0.48]
			Southern blotting	5.4% [2.0, 12.8] FN = 94.6% (87/92)	100% [78.1, 100] FP = 0% (0/18)	100% [46.3, 100]	17.1% [10.7, 26.0]
(Ciotti et al 2009) Italy	Level III-2 diagnostic evidence CX P1 Q2	N = 43 patients n = 27 classic VHL cases n = 3 non-classic VHL cases n = 13 VHL-associated disease ***Reference standard*** Clinical diagnosis	Real-time Q-PCR	90.0% [72.3, 97.4] FN = 10.0% (3/30)	100% [71.7, 100] FP = 0% (0/13)	100% [84.5, 100]	81.3% [53.7, 95.0]
(Hattori et al 2006) Japan	Level III-2 diagnostic evidence CX P1 Q2	N = 31 patients n = 27 from 19 families diagnosed with VHL syndrome n = 4 unrelated patients with single VHL manifestations ***Reference standard*** Clinical diagnosis	Real-time Q-PCR	100% [75.9, 100] FN = 14.8% (4/27)	100% [5.5, 100] FP = 0% (0/4)		100% [5.5, 100]
(Zhang et al 2008) China	Level III-2 diagnostic evidence CX P1 Q2	N = 27 index patients suspected of having VHL disease from unrelated families n = 23 with a family history n = 3 with *de novo* disease n = 1 who did not fulfil the current clinical VHL diagnostic criteria ***Reference standard*** Clinical diagnosis	DNA sequencing, UPQFM-PCR	100% [84.0, 100] FN = 0% (0/26)	100% [5.5, 100] FP = 0% (0/1)	100% [84.0, 100]	100% [5.5, 100]
			DNA sequencing	76.9% [55.9, 90.2] FN = 23.1% (6/26)	100% [5.5, 100] FP = 0% (0/1)	100% [80.0, 100]	14.3% [0.8, 58.0]
			UPQFM-PCR	23.1% [9.8, 44.1] FN = 76.9% (20/26)	100% [5.5, 100] FP = 0% (0/1)	100% [51.7, 100]	4.8% [0.2, 25.9]
(Cho et al 2009) Korea	Level III-2 diagnostic evidence CX	N = 26 patients with suspected VHL syndrome n = 15 VHL cases n = 11 sporadic disease cases ***Reference standard***	DNA sequencing, MLPA	100% [74.6, 100] FN = 0% (0/15)	100% [67.9, 100] FP = 0% (0/11)	100% [74.6, 100]	100% [67.8, 100]
			DNA sequencing	66.7% [38.7, 87.0] FN = 33.3% (5/15)	100% [67.9, 100] FP = 0% (0/11)	100% [65.5, 100]	68.8% [41.5, 87.9]

Study Location	Study quality[a]	Population and comparator	Genetic test technique	Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
	P1 Q2	Clinical diagnosis	MLPA	33.3% [13.0, 61.3] FN = 66.7% (10/15)	100% [67.9, 100] FP = 0% (0/11)	100% [46.3, 100]	52.4% [30.3, 73.6]
(Magnani et al 2001) Italy	Level III-2 diagnostic evidence CX P1 Q2	N = 18 Italian patients from the San Raffaele Clinica and Policlinico Hospital n = 9 diagnosed with VHL (n = 4 patients with family history of VHL) n = 9 patients with VHL-associated disease ***Reference standard*** Clinical diagnosis	DGGE and DNA sequencing, Southern blotting	100% [62.9, 100] FN = 0% (0/9)	77.8% [40.2, 96.1] FP = 22.2% (2/9)	81.8% [47.8, 96.8]	100% [56.1, 100]
(Rasmussen et al 2010) Mexico	Level III-2 diagnostic evidence CX P1 Q2	N = 17 patients from 17 suspected VHL families n = 10 patients diagnosed with VHL n = 7 patients with possible VHL ***Reference standard*** Clinical diagnosis	DNA sequencing	90.0% [54.1, 99.5] FN = 10.0% (1/10)	57.1% [20.2, 88.2] FP = 42.9% (3/7)	75.0% [42.8, 93.3]	80.0% [29.9, 98.9]
(Martin et al 1998b) Australia	Level III-2 diagnostic evidence CX P1 Q2	N = 16 patients from Australia and New Zealand with clinical manifestations suggestive of VHL disease n = 14 with suspected VHL n = 2 with phaeochromocytoma ***Reference standard*** Clinical diagnosis	SSCP and DNA sequencing, Southern blotting	50.0% [24.0, 76.0] FN = 50.0% (7/14)	50.0% [2.7, 97.3] FP = 50.0% (1/2)	87.5% [46.7, 99.3]	12.5% [0.7, 53.3]
(Kang et al 2005) Korea	Level III-2 diagnostic evidence CX P1 Q2	N = 15 patients n = 11 diagnosed with VHL syndrome from 7 families n = 2 cases from 1 family with phaeochromocytoma n = 2 sporadic phaeochromocytoma patients	DNA sequencing, long PCR	90.9% [57.1, 99.5] FN = 9.1% (1/11)	50.0% [9.2, 90.8] FP = 50.0% (2/4)	83.3% [50.9, 97.1]	66.7% [12.5, 98.2]

Study Location	Study quality[a]	Population and comparator	Genetic test technique	Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
		Reference standard Clinical diagnosis					
(Gomy et al 2010) Brazil	Level III-2 diagnostic evidence CX P1 Q2	N = 10 families n = 9 VHL families n = 1 with sporadic disease ***Reference standard*** Clinical diagnosis	DNA sequencing, MLPA	77.8% [40.2, 96.1] FN = 22.2% (2/9)	100% [5.5, 100] FP = 0% (0/1)	100% [56.1, 100]	33.3% [1.8, 87.5]
			DNA sequencing	44.4% [15.3, 77.3] FN = 55.6% (5/9)	100% [5.5, 100] FP = 0% (0/1)	100% [39.6, 100]	16.7% [0.9, 63.5]
			MLPA	33.3% [9.0, 69.1] FN = 66.7% (6/9)	100% [5.5, 100] FP = 0% (0/1)	100% [31.0, 100]	14.3% [0.8, 58.0]
(Siu et al 2011) China	Level III-2 diagnostic evidence CX P1 Q2	N = 9 probands from unrelated families n = 7 VHL patients n = 2 patients with bilateral phaeochromocytoma ***Reference standard*** Clinical diagnosis	DNA sequencing, MLPA	100% [56.1, 100] FN = 0% (0/7)	FP = 100% (2/2)	77.8% [40.2, 96.1]	
Patients clinically diagnosed with VHL syndrome							
(Zbar et al 1996) USA	Level III-2 diagnostic evidence CX P1 Q1	N = 469 unrelated VHL families that were evaluated at one of the 8 participating laboratories ***Reference standard*** Clinical diagnosis	SSCP or DGGE and DNA sequencing, Southern blotting	64.0% [59.4, 68.3] FN = 36.0% (169/469)		100% [98.4, 100]	
			SSCP or DGGE and DNA sequencing	51.8% [47.2, 56.4] FN = 48.2% (226/469)		100% [98.1, 100]	
			Southern blotting	13.7% [10.6, 17.5] FN = 86.3% (358/415)		100% [92.1, 100]	
(Maher et al 1996) UK	Level III-2 diagnostic evidence CX P1 Q1	N = 138 unrelated patients clinically diagnosed with VHL disease ***Reference standard*** Clinical diagnosis	Southern blotting, DNA sequencing	79.0% [71.1, 85.3] FN = 21.0% (29/138)		100% [95.8, 100]	
			Southern blotting, SSCP and DNA sequencing	73.2% [64.9, 80.2] FN = 26.8% (37/138)		100% [95.4, 100]	
			DNA sequencing of Southern blotting and SSCP negatives	28.6% [14.0, 48.9] FN = 71.4% (20/28)		100% [59.8, 100]	

Study Location	Study quality[a]	Population and comparator	Genetic test technique	Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
			Southern blotting	18.8% [12.9, 26.6] FN = 81.2% (112/138)		100% [84.0, 100]	
(Stolle et al 1998) USA	Level III-2 diagnostic evidence CX P1 Q1	N = 93 patients from consecutive VHL families ***Reference standard*** Clinical diagnosis	DNA sequencing and Southern Blotting	100% [95.1, 100] FN = 0% (0/93)		100% [95.1, 100]	
			DNA sequencing	63.4% [52.8, 73.0] FN = 36.6% (34/93)		100% [92.4, 100]	
			Southern Blotting	36.6% [30.0, 47.2] FN = 63.4% (59/93)		100% [87.4, 100]	
(Libutti et al 2000) USA	Level III-2 diagnostic evidence CX P1 Q1	N = 44 patients that were clinically diagnosed with VHL and pancreatic neuro-endocrine tumours ***Reference standard*** Clinical diagnosis	DNA sequencing, Southern blotting	100% [90.0, 100] FN = 0% (0/44)		100% [90.0, 100]	
			DNA sequencing	86.4% [72.0, 94.3] FN = 13.6% (6/44)		100% [88.6, 100]	
			Southern blotting	13.6% [5.7, 28.0] FN = 86.4% (38/44)		100% [51.7, 100]	
(Ruiz-Llorente et al 2004) Spain	Level III-2 diagnostic evidence CX P1 Q1	N = 35 unrelated patients suspected of having VHL disease, 24 of which had a familial history ***Reference standard*** Clinical diagnosis	DNA sequencing, Southern blotting	91.4% [75.8, 97.8] FN = 8.6% (3/35)		100% [86.7, 100]	
			DNA sequencing	68.6% [50.6, 82.6] FN = 31.4% (11/35)		100% [82.8, 100]	
			Southern blotting	22.9% [11.0, 40.6] FN = 77.1% (27/35)		100% [59.8, 100]	
(Rocha et al 2003) Brazil	Level III-2 diagnostic evidence CX P1 Q1	N = 20 patients diagnosed with VHL ***Reference standard*** Clinical diagnosis	DNA sequencing, Southern blotting	100% [80.0, 100] FN = 0% (0/20)		100% [80.0, 100]	
			DNA sequencing	80.0% [55.7, 93.4] FN = 20.0% (4/20)		100% [75.9, 100]	
			Southern blotting	20.0% [6.6, 44.3] FN = 80.0% (16/20)		100% [39.6, 100]	
(Gergics et al 2009) Hungary	Level III-2 diagnostic evidence CX	N = 11 patients diagnosed with VHL ***Reference standard*** Clinical diagnosis	DNA sequencing, real-time PCR, MLPA	100% [76.9, 100] FN = 0% (0/11)		100% [76.9, 100]	

Study Location	Study quality[a]	Population and comparator	Genetic test technique	Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
	P1 Q1						
(Manski et al 1997) USA	Level III-2 diagnostic evidence CX P1 Q1	N = 10 patients diagnosed with VHL and endolymphatic sac tumours ***Reference standard*** Clinical diagnosis	SSCP and DNA sequencing, Southern blotting	70.0% [35.4, 91.9] FN = 30.0% (3/10)		100% [56.1, 100]	
(Franke et al 2009) Germany	Level III-2 diagnostic evidence CX P1 Q2	N = 308 unrelated familial or sporadic VHL index cases from the Freiburg VHL registry ***Reference standard*** Clinical diagnosis	DNA sequencing	82.5% [77.7, 86.5] FN = 21.3% (54/254)		100% [98.1, 100]	
			MLPA	100% [91.7, 100] FN = 0% (0/54)		100% [91.7, 100]	
(Dollfus et al 2002) France	Level III-2 diagnostic evidence CX P1 Q2	N = 196 VHL patients who agreed to genetic testing n = 103 patients with ocular manifestations (OM) n = 108 patients without OM ***Reference standard*** Clinical diagnosis	SSCP and DNA sequencing, Southern blotting	**All patients** 76.0% [69.3, 81.7] FN = 24.0% (47/196)		100% [96.9, 100]	
				Patients with OM 81.3% [71.5, 88.4] FN = 18,7% (17/91)		100% [93.9, 100]	
				Patients without OM 71.4% [61.7, 81.7] FN = 28.6% (30/105)		100% [69.3, 100]	
			SSCP and DNA sequencing	69.9% [62.9, 76.1] FN = 30.1% (59/196)		100% [96.6, 100]	
			Southern blotting	6.1% [3.3, 10.7] FN = 93.9% (184/196)		100% [69.9, 100]	
(Chen et al 1995) USA	Level III-2 diagnostic evidence	N = 114 affected members of apparently unrelated VHL families from the USA, Canada, Puerto	SSCP and DNA sequencing, Southern blotting	74.6% [65.4, 82.0] FN = 25.4% (29/114)		100% [94.6, 100]	

Study Location	Study quality[a]	Population and comparator	Genetic test technique	Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
	CX P1 Q2	Rico and Hawaii ***Reference standard*** Clinical diagnosis	SSCP and DNA sequencing	58.8% [49.2, 67.8] FN = 41.2% (47/114)		100% [93.2, 100]	
			Southern blotting	5.8% [9.9, 24.1] FN = 84.2% (96/114)		100% [78.1, 100]	
(Yoshida et al 2000) Japan	Level III-2 diagnostic evidence CX P1 Q2	N = 77 unrelated patients diagnosed with VHL syndrome ***Reference standard*** Clinical diagnosis	SSCP and DNA sequencing, Southern blotting	71.4%% [59.8, 80.9] FN = 28.6% (22/77)		100% [91.9, 100]	
			SSCP and DNA sequencing	67.5%% [55.8, 77.5] FN = 32.5% (25/77)		100% [91.4, 100]	
			Southern blotting	3.9%% [1.0, 11.7] FN = 96.1% (74/77)		100% [31.0, 100]	
(Glavac et al 1996) Slovenia	Level III-2 diagnostic evidence CX P1 Q2	N = 65 affected members of 65 families diagnosed with VHL syndrome ***Reference standard*** Clinical diagnosis	SSCP and DNA sequencing, Southern blotting	81.5% [69.6, 89.7] FN = 18.5% (12/65)		100% [91.6, 100]	
			MLPA	10.8% [4.8, 21.5] FN = 89.2% (58/65)		100% [56.1, 100]	
(Corcos et al 2008) France	Level III-2 diagnostic evidence CX P1 Q2	N = 35 patients diagnosed with VHL syndrome and a pancreatic endocrine tumour from 29 different VHL families ***Reference standard*** Clinical diagnosis	DNA sequencing, Southern blotting	100% [87.7, 100] FN = 0% (0/35)		100% [87.7, 100]	
			DNA sequencing	91.4% [75.8, 97.8] FN = 8.6% (3/35)		100% [86.7, 100]	
			Southern blotting	8.6% [2.2, 24.2] FN = 91.4% (32/35)		100% [31.0, 100]	
(Cybulski et al 2002) Poland	Level III-2 diagnostic evidence CX P1 Q2	N = 34 patients diagnosed with VHL syndrome from different VHL families ***Reference standard*** Clinical diagnosis	DNA sequencing, long PCR, UPQFM-PCR	88.2% [71.6, 96.2] FN = 11.8% (4/34)		100% [85.9, 100]	
			DNA sequencing	52.9% [35.4, 69.6] FN = 47.1% (16/34)		100% [78.1, 100]	
			Long PCR	14.7% [5.5, 31.8] FN = 85.3% (29/34)		100% [46.3, 100]	

Study Location	Study quality[a]	Population and comparator	Genetic test technique	Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
			UPQFM-PCR	20.6% [9.3, 38.4] FN = 79.4% (27/34)		100% [56.1, 100]	
(Pack et al 1999) USA	Level III-2 diagnostic evidence CX P1 Q2	N = 31 patients with clinical and genetic diagnosis for VHL disease n = 30 with known *VHL* gene deletions n = 1 with a point mutation ***Reference standard*** Clinical diagnosis	FISH	93.5% [77.2, 98.9] FN = 6.5% (2/31)		100% [85.4, 100]	
(Marcos et al 2002) USA	Level III-2 diagnostic evidence CX P1 Q2	N = 25 patients who were clinically diagnosed with VHL and pancreatic neuro-endocrine tumours ***Reference standard*** Clinical diagnosis	Not reported	100% [83.4, 100] FN = 0% (0/25)		100% [83.4, 100]	
(Mukhopadhyay et al 2002) UK	Level III-2 diagnostic evidence CX P1 Q2	N = 16 patients belonging to 14 families with clinical signs of VHL disease n = 10 with pancreatic cysts or tumours ***Reference standard*** Clinical diagnosis	Genetic testing (method not reported)	**VHL patients** 56.3% [30.6, 79.2] FN = 43.8% (7/16) **VHL patients with pancreatic lesions** 55.6% [22.7, 84.7] FN = 25.0% (4/16)		100% [62.9, 100] 100% [46.3, 100]	
(Cybulski et al 1999) Poland	Level III-2 diagnostic evidence CX P2 Q2	N = 16 patients diagnosed with VHL syndrome n = 5 with large gene deletions n = 11 unrelated patients with no sequence mutations ***Reference standard*** Clinical diagnosis	Long PCR	**All patients** 56.3% [30.6, 79.2] FN = 43.8% (7/16) **Patients with unknown status** 36.4% [12.4, 68.4] FN = 63.6% (7/11)		100% [62.9, 100] 100% [39.6, 100]	
(Kim et al 2009b)	Level III-2 diagnostic	N = 12 patients who were diagnosed with VHL	Not reported	58.3% [28.6, 83.5] FN = 41.7% (5/12)		100% [56.1, 100]	

Study Location	Study quality[a]	Population and comparator	Genetic test technique	Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
Korea	evidence CX P1 Q2	***Reference standard*** Clinical diagnosis					
(Wong et al 2008) USA	Level III-2 diagnostic evidence CX P1 Q2	N = 11 patients with atypical ocular lesions and VHL disease who had genetic testing n = 10 with clinical VHL n = 1 family history of VHL ***Reference standard*** Clinical diagnosis	Not reported	100% [67.8, 100] FN = 0% (0/11)		100% [67.8, 100]	
(Li et al 1998) Sweden	Level III-2 diagnostic evidence CX P1 Q2	N = 10 probands from unrelated VHL families ***Reference standard*** Clinical diagnosis	DNA sequencing, Southern blotting	70% [35.4, 91.9] FN = 30.0% (3/10)		100% [56.1, 100]	
(Kanno et al 1996) Japan	Level III-2 diagnostic evidence CX P1 Q2	N = 8 individuals that were clinically diagnosed with VHL syndrome ***Reference standard*** Clinical diagnosis	SSCP and DNA sequencing	87.5% [46.7, 99.3] FN = 12.5% (1/8)		100% [56.1, 100]	
(Shuin et al 1999) Japan	Level III-2 diagnostic evidence CX P1 Q3	N = 69 unrelated VHL patients ***Reference standard*** Clinical diagnosis	SSCP and DNA sequencing, Southern blotting	49.3% [37.1, 61.5] FN = 50.7% (35/69)		100% [87.4, 100]	
			SSCP and DNA sequencing	44.9% [33.1, 57.3] FN = 55.1% (38/69)		100% [86.3, 100]	
			Southern blotting	4.3% [1.1, 13.0] FN = 95.7% (66/69)		100% [31.0, 100]	
(Weil et al	Level III-2 diagnostic	N = 12 patients that were clinically diagnosed with VHL and	SSCP and DNA sequencing, Southern	100% [67.9, 100] FN = 0% (0/11)		100% [67.9, 100]	

Study Location	Study quality[a]	Population and comparator	Genetic test technique	Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
2003) USA	evidence CX P1 Q3	brainstem haemangioblastomas ***Reference standard*** Clinical diagnosis	blotting SSCP and DNA sequencing Southern blotting	 45.5% [18.1, 75.4] FN = 54.5% (6/11) 54.4% [24.6, 81.9] FN = 45.5% (5/11)	 	 100% [46.3, 100] 100% [51.7, 100]	
CNS haemangioblastoma							
(Glasker et al 1999) Germany	Level III-2 diagnostic evidence CX P1 Q1	N = 141 patients with symptomatic CNS haemangioblastomas n = 94 diagnosed with VHL ***Reference standard*** Clinical diagnosis	Southern blotting, SSCP and DNA sequencing	86.2% [77.2, 92.1] FN = 13.8% (13/94)	100% [90.6, 100] FP = 0% (0/47)	100% [94.4, 100]	78.3% [65.5, 87.5]
(Glasker et al 2001) Germany	Level III-2 diagnostic evidence CX P1 Q2	N = 31 patients with CNS haemangioblastoma n = 18 patients with VHL disease n = 13 patients with sporadic tumours ***Reference standard*** Clinical diagnosis	SSCP and DNA sequencing, Southern blotting SSCP and DNA sequencing Southern blotting	94.4% [70.6, 99.7] FN = 5.6% (1/18) 72.2% [46.4, 89.3] FN = 27.8% (5/18) 22.2% [7.4, 48.1] FN = 77.8% (14/18)	100% [71.7, 100] FP = 0% (0/13) 100% [71.7, 100] FP = 0% (0/13) 100% [71.7, 100] FP = 0% (0/13)	100% [77.1, 100] 100% [71.7, 100] 100% [39.6, 100]	92.9% [64.2, 99.6] 72.2% [46.4, 89.3] 48.1% [29.2, 67.6]
(Ronning et al 2010) Norway	Level III-2 diagnostic evidence CX P1 Q2	N = 31 patients who were clinically diagnosed with haemangioblastoma n = 7 with clinical VHL ***Reference standard*** Clinical diagnosis	DHPLC, DNA sequencing, MLPA	14.3% [0.8, 58.0] FN = 85.7% (6/7)	100% [82.8, 100] FP = 0% (0/24)	100% [5.5, 100]	80.0% [60.9, 91.6]
(Fisher et al 2002) USA	Level III-2 diagnostic evidence CX P1 Q2	N = 6 children with cerebellar haemangioblastoma n = 2 diagnosed with VHL n = 4 sporadic cases ***Reference standard*** Clinical diagnosis	DNA sequencing, Southern blotting	100% [19.8, 100] FN = 0% (0/2)	100% [39.6, 100] FP = 0% (0/4)	100% [19.8, 100]	100% [39.6, 100]

Study Location	Study quality[a]	Population and comparator	Genetic test technique	Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
(Gläsker et al 2005) Germany	Level III-2 diagnostic evidence CX P1 Q3	N = 6 patients with spinal nerve haemangioblastoma n = 4 patients with VHL disease n = 2 patients with sporadic tumours ***Reference standard*** Clinical diagnosis	Not reported	75.0% [21.9, 98.7] FN = 25.0% (1/4)	100% [19.8, 100] FP = 0% (0/2)	100% [31.0, 100]	66.7% [12.5, 98.2]
Retinal haemangioblastoma							
(Kreusel et al 2000) Germany	Level III-2 diagnostic evidence CX P1 Q1	N = 37 non-related patients presenting with capillary retinal angioma n = 29 diagnosed with VHL n = 8 with sporadic retinal angioma ***Reference standard*** Clinical diagnosis	SSCP and DNA sequencing, Southern blotting	93.1% [75.8, 98.8] FN = 6.9% (2/29)	75.0% [35.6, 95.5] FP = 25.0% (2/8)	93.1% [75.8, 98.8]	75.0% [35.6, 95.5]
(Niemela et al 2000) Finland	Level III-2 diagnostic evidence CX P1 Q2	N = 29 patients with retinal haemangioblastoma who agreed to a genetic test n = 8 with clinically definite VHL n = 21 patients with haemangioblastomas but not VHL disease ***Reference standard*** Clinical diagnosis	DNA sequencing	75.0% [35.6, 95.5] FN = 25.0% (2/8)	100% [80.8, 100] FP = 0% (0/21)	100% [51.7, 100]	91.3% [70.5, 98.5]
(Kreusel et al 2007) Germany	Level III-2 diagnostic evidence CX P1 Q2	N = 11 patients with a solitary juxtapapillary capillary retinal angioma n = 6 clinically diagnosed with VHL ***Reference standard*** Clinical diagnosis	Not reported	83.3% [36.5, 99.1] FN = 16.7% (1/6)	80.0% [29.9, 98.9] FP = 20.0% (1/5)	83.3% [36.5, 99.1]	80.0% [29.9, 98.9]

Study Location	Study quality[a]	Population and comparator	Genetic test technique	Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
Phaeochromocytoma							
(Erlic et al 2009) Germany	Level III-2 diagnostic evidence CX P1 Q1	N = 1,149 index cases presenting with symptomatic phaeochromocytoma n = 65 diagnosed with VHL n = 989 sporadic phaeochromocytoma cases ***Reference standard*** Clinical diagnosis	MLPA, PCR-based mutation scanning	100% [93.0, 100] FN = 0% (0/65)	94.9% [93.4, 96.1] FP =5.1% (55/1084)	54.2% [44.9, 63.2]	100% [99.5, 100]
(Meyer-Rochow et al 2009) Australia	Level III-2 diagnostic evidence CX P1 Q1	N = 74 patients with phaeochromocytoma n = 6 VHL patients n = 18 patients with other familial syndromic disease n = 50 with no family history of disease ***Reference standard*** Clinical diagnosis	DHPLC analysis, DNA sequencing	100% [51.7, 100] FN = 0% (0/6)	98.5% [91.0, 99.9] FP = 1.5% (1/68)	85.7% [42.0, 99.2]	100% [93.2, 100]
(Bender et al 2000) Germany	Level III-2 diagnostic evidence CX P1 Q1	N = 38 patients with phaeochromocytoma n = 21 diagnosed with VHL n = 17 sporadic cases ***Reference standard*** Clinical diagnosis	Southern blotting, SSCP and DNA sequencing	100% [80.8, 100] FN = 0% (0/21)	100% [77.1, 100] FP = 0% (0/17)	100% [80.8, 100]	100% [77.1, 100]
(Cotesta et al 2009) Italy	Level III-2 diagnostic evidence CX P1 Q1	N = 30 phaeochromocytoma patients n = 4 patients with VHL n = 17 with other syndromes n = 9 sporadic cases ***Reference standard*** Clinical diagnosis	DNA sequencing	100% [39.6, 100] FN = 0% (0/4)	96.2% [78.4, 99.8] FP = 3.8% (1/26)	80.0% [29.9, 98.9]	100% [83.4, 100]
(De Krijger et al 2006)	Level III-2 diagnostic	N = 10 paediatric phaeochromocytoma patients	DGGE, SSCP and DNA sequencing	50.0% [26.7, 97.3] FN = 50.0% (1/2)	100% [59.8, 100] FP = 0% (0/8	100% [5.5, 100]	88.9% [50.7, 99.4]

Study Location	Study quality[a]	Population and comparator	Genetic test technique	Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
Netherlands	evidence CX P1 Q1	n = 2 diagnosed with VHL ***Reference standard*** Clinical diagnosis					
(Amar et al 2005) France	Level III-2 diagnostic evidence CX P1 Q2	N = 314 patients with a phaeochromocytoma or a functional paraganglioma n = 9 patients with VHL n = 47 patients with other familial syndromes n = 258 sporadic cases ***Reference standard*** Clinical diagnosis	DNA sequencing	100% [62.9, 100] FN = 0% (0/9)	94.8% [91.5, 96.9] FP = 5.2% (16/305)	36.0% [18.7, 57.4]	100% [98.4, 100]
(Hering et al 2006) Germany	Level III-2 diagnostic evidence CX P1 Q2	N = 14 children with phaeochromocytoma n = 9 with VHL syndrome ***Reference standard*** Clinical diagnosis	DNA sequencing, MLPA	100% [62.9, 100] FN = 0% (0/9)	100% [46.3, 100] FP = 0% (0/5)	100% [62.9, 100]	100% [46.3, 100]
(Patocs et al 2004) Hungary	Level III-2 diagnostic evidence CX P2 Q3	N = 41 patients with phaeochromocytoma n = 2 with VHL syndrome ***Reference standard*** Clinical diagnosis	DNA sequencing	100% [19.8, 100] FN = 0% (0/2)	100% [88.8, 100] FP = 0% (0/39)	100% [19.8, 100]	100% [88.8, 100]

[a] A description of study quality characteristics is provided in Table 13 and Table 14

CI = confidence intervals; DGGE = denaturing gradient gel electrophoresis; DHPLC = denaturing high-performance liquid chromatography; DNA = deoxyribonucleic acid; FISH = fluorescence in-situ hybridisation; FN = false negative; FP = false positive; MLPA = multiplex ligation-dependent probe amplification; NPV = negative predictive value; OM = ocular manifestations; PCR = polymerase chain reaction; PPV = positive predictive value; Q-PCR = quantitative PCR; Sn = sensitivity; Sp = specificity; SSCP = single-strand conformational polymorphism; UPQFM-PCR = universal primer quantitative fluorescent multiplex PCR; V-H = Varian-Helix DHPLC system; VHL = von Hippel-Lindau; WNAFA = Wave Nucleic Acid Fragment Analysis DHPLC system

Twenty-three case series reported on the diagnostic yield (level IV diagnostic evidence) of genetic testing for VHL mutations when used to diagnose patients presenting with clinical signs of disease (Table 20 to Table 22). The study populations used in these studies were separated into eight distinct groups, involving either an unknown number of patients with a clinical diagnosis of VHL syndrome or patients without a positive clinical diagnosis (patients with a clinical diagnosis of VHL were excluded). The genetic testing methods varied between the studies, but all studies included DNA sequencing with or without pre-screening of the PCR products. Southern blotting or MLPA were used to detect large deletions in some of these studies. However, the impact of any differences between genetic testing methods on the diagnostic yield could not be determined in most cases due to the small number of studies addressing each testing methodology.

One study included both symptomatic and asymptomatic patients belonging to families with VHL syndrome (Webster et al 1999b).This study could not be used to determine diagnostic accuracy as the number of genetic test positive patients with and without VHL syndrome could not be determined. Overall, 84% (154/183) of patients had VHL syndrome and 88.0% (161/183) had a VHL mutation (Table 20).

Table 20 Diagnostic yield of genetic testing for *VHL* gene mutations in the diagnosis of VHL syndrome in patients ± VHL syndrome

Study Location	Study quality[a]	Population	Clinical diagnostic criteria used	Genetic test[b]	Diagnostic yield
(Webster et al 1999b) UK	Level IV diagnostic evidence CX P1 Q3	N = 183 VHL mutation carriers from 81 families n = 154 with VHL syndrome n = 29 asymptomatic gene carriers	Interview and medical records review determined each patient's ocular and family history	Southern blotting, SSCP and DNA sequencing	**All patients** 161/183 (88.0%) **All families** 69/81 (85.2%)

[a] A description of study quality characteristics is provided in Table 13 and Table 14; [b] Detection limit of genetic test was not reported
DNA = deoxyribonucleic acid; SSCP = single-strand conformational polymorphism; VHL = von Hippel-Lindau

Sixteen studies, divided into three groups, provided diagnostic yield data for VHL genetic testing of patients diagnosed with phaeochromocytoma (Table 21). The first group of 3 studies involved phaeochromocytoma patients with or without VHL and other syndromic diseases such as multiple endocrine neoplasia type 2 (MEN 2). Overall, 10.2% (79/777) of phaeochromocytoma patients with or without VHL syndrome had a VHL mutation that was identified by genetic testing. Thus, in a group of randomly selected patients presenting with phaeochromocytoma, approximately 10% of them would be expected to have a germ-line VHL mutation.

Four studies provided diagnostic yield data for VHL genetic testing of patients with familial phaeochromocytoma, but no other symptoms for syndromic diseases such as

VHL or MEN 2. Overall, VHL mutations were detected in 45.8% (11/24) of patients with familial phaeochromocytoma. The prevalence of detected mutations did not vary greatly if the studies were divided according to the DNA sequencing method used (with or without a method to detect large deletions). Studies that used direct DNA sequencing of all PCR products had a diagnostic yield of 46% (range 25–80%), compared with 46% (range 38–67%) also for studies that used pre-screening of the PCR products prior to DNA sequencing. This suggests that approximately half of all patients who present with phaeochromocytoma and have a family history of only this type of neoplasm carry a VHL mutation corresponding to type 2C VHL syndrome, which is characterised by phaeochromocytoma in the absence of other clinical manifestations.

The third group of 12 studies reported the diagnostic yield for VHL genetic testing of patients with sporadic phaeochromocytoma. Patients with either clinical signs of, or a family history of, syndromic diseases such as VHL and MEN 2 were excluded. Studies were divided according to the DNA sequencing method used for genetic testing (with or without a method to detect large deletions), and the 7 studies that used pre-screening of the PCR products prior to sequencing had a slightly higher diagnostic yield of 9% (range 3–13%), compared with 4% (range 1–50%) for the 5 studies that used direct DNA sequencing. Overall, 6.5% (61/940) of patients with apparently sporadic phaeochromocytoma had a mutation in the *VHL* gene that was identified by genetic testing. Surprisingly, this yield (with a range of 1–50%) of sporadic phaeochromocytomas is similar to the 10% yield obtained in the 3 studies described above that involved all patients presenting with phaeochromocytomas. This suggests that the proportion of patients with syndromic phaeochromocytomas who have a VHL mutation is similar to the proportion of patients clinically diagnosed with sporadic phaeochromocytoma who have a VHL mutation.

Table 21 Diagnostic yield of genetic testing for *VHL* gene mutations in the diagnosis of VHL syndrome in patients presenting with phaeochromocytoma

Study Location	Study quality[a]	Population	Clinical diagnostic criteria used	Genetic test[b]	Diagnostic yield
Phaeochromocytoma ± VHL syndrome					
(Mannelli et al 2009) Italy	Level IV diagnostic evidence CX P2 Q3	N = 501 consecutive patients (adults and children) with phaeochromocytoma and/or paraganglioma n = 32 with VHL syndrome	Based on levels of catecholamines and confirmed by scintigraphy and/or surgical histology	DNA sequencing, MLPA	48/501 (9.6%)
(Cascon et al 2009) Spain	Level IV diagnostic evidence CX P2 Q3	N = 237 consecutive patients with phaeochromocytoma or paraganglioma n = 45 patients with hereditary syndrome	Criteria not reported	Complete genetic characterisation	**All patients** 10/237 (4.2%) **Hereditary syndromes** 3/45 (6.7%)
(Neumann et al 1999) Germany	Level IV diagnostic evidence CX P2 Q3	N = 39 patients with phaeochromocytoma that underwent adrenal-sparing surgery n = 26 diagnosed with familial syndromes (eg VHL or MEN2)	Presence of retinal angiomas or haemangiomas in the patient or a first-degree relative	SSCP, DNA sequencing, Southern blotting and MLPA	21/39 (53.8%)
Familial phaeochromocytoma					
(Woodward et al 1997) UK	Level IV diagnostic evidence CX P2 Q3	N = 8 with familial phaeochromocytoma	Criteria not reported	SSCP and DNA sequencing	3/8 (37.5%)
(Pigny et al 2009) France	Level IV diagnostic evidence CX P1 Q3	N = 8 patients with phaeochromocytoma and a positive familial history of adrenal tumour	Review of medical records and family history by inquiry	DNA sequencing, MLPA	2/8 (25.0%)
(Tong et al 2006) China	Level IV diagnostic evidence CX P2 Q3	N = 5 unrelated families with non-syndromic familial phaeochromocytoma	Screening for clinical manifestations of VHL syndrome	DNA sequencing	4/5 (80.0%)
(Crossey et al 1995) UK	Level IV diagnostic evidence CX P2 Q3	N = 3 families with phaeochromocytoma in more than 1 relative and with no other signs of VHL disease	Ophthalmological and radiological screening for VHL disease	SSCP and DNA sequencing, Southern blotting	2/3 (66.7%)

Study Location	Study quality[a]	Population	Clinical diagnostic criteria used	Genetic test[b]	Diagnostic yield
Phaeochromocytoma (sporadic)					
(Neumann et al 2002) Germany	Level IV diagnostic evidence CX P2 Q3	N = 271 patients with non-syndromic phaeochromocytoma that were consecutively registered in the population registries of Freiburg, Germany, and Warsaw, Poland	Clinical evaluation (personal and family history and physical examination) and review of medical records	SSCP and DNA sequencing	30/271 (11.1%)
(Cascon et al 2009) Spain	Level IV diagnostic evidence CX P2 Q3	N = 192 patients with sporadic disease	Criteria not reported	Complete genetic characterisation	7/192 (3.6%)
(Pigny et al 2009) France	Level IV diagnostic evidence CX P1 Q3	N = 100 patients with apparently sporadic phaeochromocytoma	Review of medical records and family history by inquiry	DNA sequencing, MLPA	1/100 (1.0%)
(Gimenez-Roqueplo et al 2003) France	Level IV diagnostic evidence CX P2 Q3	N = 84 patients with apparently sporadic phaeochromocytoma (no family history or clinical signs of familial or syndromic disease)	Thorough clinical examination to search for signs of syndromic disease	DNA sequencing	2/84 (2.4%)
(Van der Harst et al 1998) Netherlands	Level IV diagnostic evidence CX P2 Q3	N = 68 patients with sporadic phaeochromocytoma	Criteria not reported	SSCP and DNA sequencing	7/68 (10.3%)
(Brauch et al 1997) Germany	Level IV diagnostic evidence CX P2 Q3	N = 62 patients who had undergone surgery, 1995–96, for sporadic phaeochromocytoma	Based upon history, physical examination, and family history	SSCP and DNA sequencing	2/62 (3.2%) [95%CI 1–11%]
(Krawczyk et al 2010) Poland	Level IV diagnostic evidence CX P2 Q3	N = 53 patients with phaeochromocytoma	Clinical manifestations, biochemical abnormalities, radiological or scintigraphic imaging, and histopathology	SSCP and DNA sequencing	3/53 (5.7%)
(Castellano et al 2006) Italy	Level IV diagnostic evidence CX P2	N = 45 patients with phaeochromocytoma or paragangliomas n = 35 with phaeochromocytoma n = 7 with PGL	Clinical, biochemical (catecholamines, chromogranin A) and imaging data (CT and MRI)	DNA sequencing	**All patients** 4/45 (8.9%) **Phaeochromo-cytoma patients** 3/35 (8.6%)

Study Location	Study quality[a]	Population	Clinical diagnostic criteria used	Genetic test[b]	Diagnostic yield
	Q3	n = 3 with head and neck PGL			**PGL cases** 1/7 (14.3%)
(Bar et al 1997) Israel	Level IV diagnostic evidence CX P2 Q3	N = 27 patients with sporadic phaeochromocytomas (no personal or familial history of syndromic disease)	Detailed physical exam including fundoscopy, CT and/or MRI	SSCP and DNA sequencing	1/27 (3.7%)
(Waldmann et al 2009) Germany	Level IV diagnostic evidence CX P2 Q3	N = 26 patients with sporadic phaeochromocytoma (no family history of disease)	24-hour urine catecholamine levels, CT or MRI of abdomen	DHPLC and DNA sequencing	1/26 (3.8%)
(Woodward et al 1997) UK	Level IV diagnostic evidence CX P2 Q3	N = 8 patients with phaeochromocytoma and no clinical evidence or family history of MEN 2, VHL, or NF1	Criteria not reported	SSCP and DNA sequencing	1/8 (12.5%)
(Bender et al 1997) Germany	Level IV diagnostic evidence CX P2 Q3	N = 4 patients with thoracic phaeochromocytoma and no other signs of VHL disease	Criteria not reported	DNA sequencing	2/4 (50%)

[a] A description of study quality characteristics is provided in Table 13 and Table 14; [b] Detection limit of genetic test was not reported

CT = computed tomography; DHPLC = denaturing high-performance liquid chromatography; DNA = deoxyribonucleic acid; MEN 2 = multiple endocrine neoplasia type 2; MLPA = multiplex ligation-dependent probe amplification, MRI = magnetic resonance imaging; NF1 = neurofibromatosis type 1; PGL = paraganglioma; SSCP = single-strand conformational polymorphism

Two studies each reported the diagnostic yield for VHL genetic testing of patients with sporadic CNS and retinal haemangioblastomas; 1 study used patients with sporadic pancreatic neuro-endocrine tumours and the final study was of patients with sporadic renal cell carcinomas (Table 22). The overall yield of VHL mutations in patients diagnosed with sporadic CNS or retinal haemangioblastomas, pancreatic neuro-endocrine tumours and renal cell carcinomas was 5.1% (5/98), 0% (0/27), 1.0% (1/101) and 1.6% (3/187), respectively. As retinal haemangioblastomas are a common first manifestation of VHL disease (according to Poulsen et al (2010) in 27% of patients), the lack of VHL mutations identified in patients with sporadic retinal haemangioblastomas was probably due to the small size of the 2 studies.

Table 22 Diagnostic yield of genetic testing for *VHL* gene mutations in the diagnosis of VHL syndrome in patients with haemangioblastomas, pancreatic tumours or renal cell carcinomas

Study Location	Study quality[a]	Population	Clinical diagnostic criteria used	Genetic test[b]	Diagnostic yield
CNS haemangioblastoma					
(Hes et al 2000a) Netherlands	Level IV diagnostic evidence CX P2 Q3	N = 84 haemangioblastoma patients with a single haemangioblastoma N = 4 patients with multiple haemangioblastomas but no other evidence of VHL disease	Ophthalmological examination and abdominal sonography or MRI	DNA sequencing, Southern blotting	**Single HB** 3/84 (3.6%) **Single HB patients aged < 50 yrs** 3/69 (4.3%) **Multiple HB** 2/4 (50.0%)
(Catapano et al 2005) Italy	Level IV diagnostic evidence CX P2 Q3	N = 14 patients with CNS haemangioblastoma who were operated on 1993 – 2002	History, MRI, test for urinary catecholamines, ophthalmological examination, upper abdominal ultrasound	DHPLC and DNA sequencing	2/14 (14.3%)
Retinal haemangioblastoma					
(Webster et al 1999a) UK	Level IV diagnostic evidence CX P2 Q3	N = 17 patients had a VHL-like ocular angioma in the absence of any other VHL complications in the patients or their relatives	Interview and review of medical records, and ophthalmic examination	Southern blotting, SSCP	0/17 (0%)
(Singh et al 2002) USA	Level IV diagnostic evidence CX P2 Q3	N = 10 patients with solitary retinal capillary haemangioma and no other signs of VHL disease	Family history of VHL disease, systemic features of VHL disease, or more than one haemangioma	CSGE and DNA sequencing, Southern blotting,	0/10 (0%)
Pancreatic tumours (sporadic)					
(Erlic et al 2010) Germany	Level IV diagnostic evidence CX P1 Q3	N = 101 unrelated patients in the German NET-Registry that had pancreatic islet cell tumours	Based on histo-pathological confirmation	DHPLC and DNA sequencing, MLPA	1/101 (1.0%)
Renal cell carcinoma (sporadic)					
(Neumann et al 1998) Germany	Level IV diagnostic evidence CX P1 Q3	N = 189 unselected sporadic RCC patients from a register of all patients surgically treated for RCC	Ultrasonography CT and MRI imaging, ophthalmoscopy 24-hour urine catecholamine assay	SSCP and DNA sequencing, Southern blotting	3/187 (1.6%)

[a] A description of study quality characteristics is provided in Table 13 and Table 14; [b] Detection limit of genetic test was not reported

CNS = central nervous system; CT = computed tomography; CSGE = conformation sensitive gel electrophoresis; DHPLC = denaturing high-performance liquid chromatography; DNA = deoxyribonucleic acid; HB =

haemangioblastoma; MLPA = multiplex ligation-dependent probe amplification, MRI = magnetic resonance imaging; PCR = polymerase chain reaction; RCC = renal cell carcinoma; SSCP = single-strand conformational polymorphism

Summary of the diagnostic accuracy of genetic testing in the diagnosis of VHL syndrome

The current standard VHL genetic testing methods of direct DNA sequencing of PCR products from all three exons of the *VHL* gene, plus a method to detect large deletions of the *VHL* gene such as MLPA, appear to be the most accurate of the modalities available. This dual testing methodology is highly accurate, with median 100% sensitivity, specificity, positive predictive and negative predictive values. However, despite this accuracy, a false negative rate of 10.2% and a false positive rate of 4.2% were observed.

The false negative rate of 10.2% suggests that detection of a germ-line mutation is not yet possible for some patients with VHL syndrome. Thus, VHL genetic testing should not be used as a standalone test for the diagnosis of VHL syndrome in patients presenting with VHL-related neoplasms, but as a confirmatory test after clinical diagnosis in the index case.

The false positive rate of 4.2% was expected, as there will always be a few patients who do *not* meet the criteria for clinical diagnosis of VHL syndrome but have an underlying VHL mutation. In these patients the disease may not yet have progressed sufficiently to obtain a positive clinical diagnosis.

Genetic diagnosis of a VHL mutation was more accurate in patients with phaeochromocytoma than in any other patient group (100% sensitivity in 7/8 studies). This was due to missense VHL mutations (detected by DNA sequencing) being the most common cause of phaeochromocytoma in VHL syndrome. Patients with phaeochromocytoma (syndromic or sporadic) had an overall 7% probability of having an underlying germ-line VHL mutation, whereas patients with familial phaeochromocytoma had a 50% probability of having a VHL mutation that is indicative of type 2C VHL syndrome.

Is VHL genetic testing accurate in first- or second-degree family members?

Studies were selected to determine the accuracy of testing for *VHL* gene mutations, compared with annual screening, for predicting VHL syndrome in relatives of patients with a known VHL mutation if they met the criteria outlined in Box 7.

Box 7 Inclusion criteria for identification of studies relevant to assessment of the predictive accuracy of genetic testing for VHL mutations (family members)

Research question	
Is genetic testing for mutations in the *VHL* gene, plus annual screening, as accurate as, or more accurate than, annual screening for diagnosing relatives of patients with a known VHL mutation?	
Selection criteria	**Inclusion criteria**
Population	Clinically unaffected first- or second-degree family members of patients with clinically diagnosed VHL syndrome and/or a diagnosed VHL genetic abnormality
Intervention	VHL genetic testing to screen for *VHL* gene mutations **±** clinical testing (CT, MRI, ultrasound, hearing test, eye exam and blood tests) **and** routine lifelong screening for neoplasms using CT, MRI, ultrasound, hearing test, eye exam and blood tests
Comparator(s)	Clinical testing (CT, MRI, ultrasound, hearing test, eye exam and blood tests) **and** routine lifelong screening for neoplasms using CT, MRI, ultrasound, hearing test, eye exam and blood tests
Reference standard	Clinical diagnosis determined from lifelong follow-up
Outcomes	Predictive accuracy outcomes: Sensitivity and specificity (and therefore rates of false positives and negatives), positive and negative likelihood ratios, positive and negative predictive values, diagnostic odds ratios, receiver–operator characteristic curves, area under the curve, accuracy
Search period	1993 – May 2011
Language	Non-English language articles were excluded unless they provided a higher level of evidence than the English language articles identified

CT = computed tomography; MRI = magnetic resonance imaging; VHL = von Hippel-Lindau

Forty-one studies met the inclusion criteria outlined *a priori*, and reported on the analysis of VHL mutations in the pre-symptomatic genetic testing of close relatives of index patients (or probands) that carry a known VHL mutation. Fifteen studies provided comparative data (level III-2 diagnostic evidence) reporting on the predictive accuracy of genetic testing compared with clinical diagnosis. Four studies provided data on first-degree relatives, only 1 study on second-degree relatives, and 12 studies on a combination of first- and second-degree relatives (Table 24). Twenty-six studies reported the diagnostic yield (level IV diagnostic evidence) of pre-symptomatic genetic testing of close relatives; 12 reported on first-degree relatives, 2 on second-degree relatives and 17 on a combination of first- and second-degree relatives (Table 25). The populations in all these studies consisted of close relatives of patients with a known germ-line VHL mutation, making the results generalisable to the population eligible for VHL genetic testing under the proposed MBS listing. Although these studies were conducted all over the world, including Mexico, Brazil, Kuwait, Korea, China and Japan,

the majority were conducted in the UK, Europe and the USA, suggesting that the results are applicable to the Australian healthcare context with few caveats.

The different genetic testing methodologies should have less impact on the accuracy or yield measures for pre-symptomatic screening of family members than with index cases because a germ-line VHL mutation has already been identified in the proband. When repeated, this method should be able to identify all relatives presenting with the same mutation. In comparison, the annual screening protocols used can only identify relatives that are presenting with early signs of disease. Thus, routine clinical screening provides an imperfect reference standard against which the accuracy of pre-symptomatic genetic testing would be measured. The screening procedures used in these studies were similar to the Australian VHL screening protocol described in the background section of this report.

The VHL genetic test was compared with clinical screening of relatives in two different ways. In 10 studies relatives with and without symptoms of VHL syndrome (ie they had been screened) were tested to determine their VHL mutation status. In 5 studies asymptomatic relatives underwent VHL genetic testing, then only those who carried the VHL mutation were clinically screened for early signs of VHL disease. These studies are subject to verification bias, as only relatives with the VHL mutation were screened, and so no estimates of test specificity could be determined.

The median (and range) accuracy data from the studies with a low–medium risk of bias for first-degree and combined first- and second-degree relatives of a known VHL mutation carrier are shown in Table 23. All included studies involved relatives of patients with a known germ-line VHL mutation, and had a sensitivity of 100% with no false negatives (Table 24). This is to be anticipated, as all relatives that show early clinical symptoms of VHL syndrome would be expected to have inherited the familial germ-line VHL mutation. Conversely, 3 studies involving first-degree relatives with and without VHL-related symptoms (with a medium risk of bias), and 5 studies involving first- and second-degree relatives with and without VHL-related symptoms (3 with a low, and 2 with a medium, risk of bias), had a median specificity of 78% (range 50.0–100) and 85.0% (range 42.9–100) and a false positive rate of 23.5% and 16.9%, respectively. A high level of false positives was anticipated, as VHL genetic testing was expected to identify relatives who have inherited the familial germ-line VHL mutation before the manifestation of clinical signs of disease. The true false positive rate would only be known with long-term clinical follow-up.

The specificity and false positive rate appear to be dependent on the age of the relatives being tested. The older the relatives, the more likely it is that some clinical signs of disease would have been detected by clinical screening. Thus, as the average age of the

relatives increases, so does the specificity, and the false positive rate decreases. The difference in the timeframe required for a clinical versus a genetic diagnosis was also reflected in the positive predictive values. The median positive predictive value for studies involving first-degree relatives was 69% (range 33–100) and for studies involving both first- and second-degree relatives was 48% (range 20–100), suggesting that a positive genetic test result does not always correlate with a positive clinical test result. The higher positive predictive value for first-degree relatives compared with first- and second-degree relatives is probably due to the increased number of VHL mutation carriers among first-degree relatives (50% probability of inheriting the germ-line VHL mutation) compared with second-degree relatives (25% probability of inheriting the VHL mutation).

All studies involving VHL genetic testing of first- and/or second-degree relatives of a known VHL mutation carrier had a negative predictive value of 100%. This was predictable, as a relative with a negative VHL genetic test result will also be expected to have a negative clinical diagnosis for VHL syndrome.

Table 23 Median and range of diagnostic accuracy data from studies with a low–medium risk of bias for relatives of a known VHL mutation carrier

Relatives	Sensitivity	Specificity	PPV	NPV
First degree relatives	100% (100–100) FN = 0% [0, 11.4] k = 4 (1 Q1)	83.3% (50–100) FP = 23.5% [7.8, 50.2] k = 3 (0 Q1)	69.4% (33.3–100) k = 4 (1 Q1)	100% (100–100) k = 3 (0 Q1)
First and second degree relatives	100% (100–100) FN = 0% [0, 8.0] k = 10 (5 Q1)	85.0% (42.9–100) FP = 16.9% [10.9, 25.2] k = 5 (3 Q1)	47.8% (20.0–100) k = 10 (5 Q1)	100% (100–100) k = 5 (3 Q1)

[a] Median (range) for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) measured using all studies except those with a high risk of bias; The 95% CI for false positives and false negatives are within the square brackets

CI = confidence interval; FN = false negatives; FP = false positives; k = number of studies; Q1 = high quality study with a low risk of bias

Table 24 Diagnostic accuracy of genetic testing for *VHL* gene mutations in relatives of patients with a known mutation

Study	Study quality[a]	Population and comparator	Genetic test technique	Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
First-degree relatives							
(Magnani et al 2001) Italy	Level III-2 diagnostic evidence CX P1 Q2	N = 21 first-degree relatives of 5 index cases n = 9 with clinical signs of disease n = 2 with no clinical signs of disease ***Reference standard*** Clinical screening	DGGE analysis, DNA sequencing and Southern blotting	100% [62.9, 100] FN = 0% (0/9)	83.3% [50.9, 97.1] FP = 16.7% (2/12)	81.8% [47.8, 96.8]	100% [65.5, 100]
(Ritter et al 1996) Germany	Level III-2 diagnostic evidence CX P1 Q2	N = 7 first-degree family members with familial phaeochromocytomas n = 6 with clinical signs of phaeochromocytomas n = 1 with no clinical signs of disease ***Reference standard*** Clinical screening	SSCP and DNA sequencing	100% [51.7, 100] FN = 0% (0/6)	100% [5.5, 100] FP = 0% (0/1)	100% [51.7, 100]	100% [5.5, 100]
(Martin et al 1998a) Australia	Level III-2 diagnostic evidence CX P1 Q2	N = 5 first-degree relatives from a family with familial phaeochromocytoma n = 1 with VHL disease n = 4 with no signs of disease ***Reference standard*** Clinical screening	SSCP and DNA sequencing	100% [54.6, 100] FN = 0% (0/1)	50.0% [9.2, 90.8] FP = 50.0% (2/4)	33.3% [17.7, 87.5]	100% [19.8, 100]
First-degree relatives with VHL mutations							
(Bender et al 2001) Germany	Level III-2 diagnostic evidence CX P1 Q1	N = 51 first-degree asymptomatic relatives who were **diagnosed as VHL c.505 T/C mutation carriers** ***Reference standard*** Clinical screening	PCR with modified primers to create restriction-site polymorphisms	100% [85.4, 100] FN = 0% (0/22)	FP = 100% (29/29)	56.9% [42.3, 70.4] **Penetrance at:** 35 years = 48% 70 years = 88%	

Study	Study quality[a]	Population and comparator	Genetic test technique	Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
First- and second-degree relatives							
(Rasmussen et al 2010) Mexico	Level III-2 diagnostic evidence CX P1 Q1	N = 92 first- and second-degree relatives n = 85 asymptomatic n = 7 symptomatic ***Reference standard*** Clinical screening	DNA sequencing	**All relatives** 100% [69.9, 100] FN = 05 (0/12) **Asymptomatic relatives** 100% [46.3, 100] FN = 0% (0/5)	85.0% [74.9, 91.7] FP = 15.0% (12/80) 85.0% [74.9, 91.7] FP = 15.0% (12/80)	50.0% [29.6, 70.4] 29.4% [11.4, 56.0]	100% [93.3, 100] 100% [93.3, 100]
(Patocs et al 2008) Hungary	Level III-2 diagnostic evidence CX P1 Q1	N = 22 kindred from a large VHL type 2 family spanning five generations n = 6 with clinical disease n = 16 with no disease ***Reference standard*** Clinical screening	DNA sequencing	100% [51.7, 100] FN = 0% (0/6)	87.5% [60.4, 97.8] FP = 12.5% (2/16)	75.0% [35.6, 95.5]	100% [73.2, 100]
(Atuk et al 1998) USA	Level III-2 diagnostic evidence CX P1 Q1	N = 13 members of a VHL family followed at the University of Virginia since 1964 n = 6 affected members n = 7 unaffected members ***Reference standard*** Clinical screening	DNA sequencing	100% [51.7, 100] FN = 0% (0/6)	42.9% [11.8, 79.8] FP = 57.1% (4/7)	60.0% [27.4, 86,3]	100% [31.0, 100]
(Hes et al 2000b) Netherlands	Level III-2 diagnostic evidence CX P1 Q1	N = 11 patients from 1 VHL family n = 3 with clinical disease n = 8 with no disease ***Reference standard*** Clinical screening	Southern blotting	100% [31.0, 100] FN = 0% (0/3)	100% [59.8, 100] FP = 0% (0/8)	100% [31.0, 100]	100% [59.8, 100]

Study	Study quality[a]	Population and comparator	Genetic test technique	Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
(Huang et al 2004) China	Level III-2 diagnostic evidence CX P1 Q1	N = 6 kindred from a large family with VHL disease n = 2 with clinical disease n = 4 with no disease ***Reference standard*** Clinical screening	DNA sequencing	100% [19.8, 100] FN = 0% (0/2)	FP = 100% (4/4)	33.3% [6.0, 75.9]	
(Martin et al 1998a) Australia	Level III-2 diagnostic evidence CX P1 Q2	N = 9 relatives (5 first- and 4 second-degree relatives) from a family with familial phaeochromocytoma n = 2 with VHL disease (1 first-, 1 second-degree) n = 7 with no signs of disease ***Reference standard*** Clinical screening	SSCP and DNA sequencing	**All relatives** 100% [19.8, 100] FN = 0% (0/2) **2° relatives** 100% [5.5, 100] FN = 0% (0/1)	71.4% [30.3, 94.9] FP = 28.6% (2/7) 100% [31.0, 100] FP = 0% (0/3)	50.0% [9.2, 90.8] 100% [5.5, 100]	100% [46.3, 100] 100% [31.0, 100]
(Sovinz et al 2010) Austria	Level III-2 diagnostic evidence CX P1 Q3	N = 5 (3 first- and 2 second-degree) relatives of an index case n = 1 with clinical disease n = 4 with no disease ***Reference standard*** Clinical screening	Mutation analysis, method not reported	100% [5.5, 100] FN = 0% (0/1)	100% [39.6, 100] FP = 0% (0/4)	100% [5.5, 100]	100% [39.6, 100]
(Shah et al 2000) USA	Level III-2 diagnostic evidence CX P1 Q3	N = 5 family members (4 first- and 1 second-degree) of an index patient with RCC n = 1 with clinical disease n = 4 with no disease ***Reference standard*** Clinical screening	DNA sequencing	100% [5.5, 100] FN = 0% (0/1)	75.0% [21.9, 98.7] FP = 25.0% (1/4)	50.0% [2.7, 97.3]	100% [31.0, 100]

Study	Study quality[a]	Population and comparator	Genetic test technique	Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
First- and second-degree relatives with VHL mutations							
(Rasmussen et al 2006) Mexico	Level III-2 diagnostic evidence CX P2 Q1	N = 14 asymptomatic relatives **with VHL mutations** ***Reference standard*** Clinical screening	DNA sequencing	100% [51.7, 100] FN = 0% (0/6)	FP = 100% (8/8)	42.9% [18.8, 70.4]	
(Akcaglar et al 2008) Turkey	Level III-2 diagnostic evidence CX P1 Q2	N = 22 relatives **with VHL mutations** n = 10 with clinical disease n = 12 with no disease ***Reference standard*** Clinical screening	Moorehead karyotyping method	100% [65.5, 100] FN = 0% (0/10)	- FP = 100% (12/12)	45.5% [25.1, 67.3]	-
(Glavac et al 1996) Slovenia	Level III-2 diagnostic evidence CX P1 Q2	N = 15 asymptomatic relatives **with VHL mutations** ***Reference standard*** Clinical screening	DNA sequencing, SSCP analysis, Southern Blotting and MLPA	100% [51.7, 100] FN = 0% (0/6)	FP = 100% (9/9)	40.0% [17.5, 67.1]	
(Gross et al 1996) Israel	Level III-2 diagnostic evidence CX P1 Q2	N = 5 asymptomatic relatives **with VHL mutations** ***Reference standard*** Clinical screening	SSCP and DNA sequencing	100% [5.5, 100] FN = 0% (0/1)	FP = 100% (4/4)	20.0% [1.1, 70.1]	

[a] A description of study quality characteristics is provided in Table 13 and Table 14

CI = confidence intervals; DGGE = denaturing gradient gel electrophoresis; DNA = deoxyribonucleic acid; FN = false negatives; FP = false positives; MLPA = multiplex ligation-dependent probe amplification, NPV = negative predictive value; PCR = polymerase chain reaction; PPV = positive predictive value; Sn = sensitivity; Sp = specificity; SSCP = single-strand conformational polymorphism; VHL = von Hippel-Lindau

The 12 studies (6 with a low, and 6 with a medium, risk of bias) that reported on the likelihood of first-degree relatives inheriting a germ-line VHL mutation had an overall diagnostic yield of 36.0% (41/114) (Table 25), which is lower than the 50% of first-degree relatives predicted to inherit the VHL mutation. This may be because other symptomatic members of the family had been tested previously, or older family members may have died due to complications arising from VHL-related neoplasms without being diagnosed with VHL syndrome. This effectively reduces the proportion of first-degree relatives carrying the VHL mutation. The 2 studies with a medium risk of bias that reported data for second-degree relatives involved very small study populations, and none of the nine relatives were found to carry an inherited VHL germ-line mutation. The 17 studies (10 with a low, and 7 with a medium, risk of bias) that involved a combination of first- and second-degree relatives had an overall yield of 38.1% (203/533) (Table 25). The similar yields for studies involving either first-degree or both first- and second-degree relatives are probably due to the larger representation of siblings, parents and children (first-degree relatives) compared with grandparents, aunts, uncles, nieces, nephews and cousins (second-degree relatives) in the 17 studies that included both first- and second-degree relatives. These results suggest that approximately 4 out of 10 relatives that undergo VHL genetic testing would be identified as carriers of the familial VHL mutation.

However, these studies included both asymptomatic relatives, with no obvious clinical signs of disease (early signs of developing neoplasms may be detectable when screened by CT or MRI) and relatives with symptoms indicative of VHL syndrome. When only asymptomatic relatives are considered, 26.8% (19/71) of first-degree relatives and 22.4% (93/415) of first- and second-degree relatives inherited the familial VHL mutation. Thus, only 2 first-degree relatives and 3 first- or second-degree relatives out of 10, without any symptoms indicative of VHL syndrome, would be identified as VHL mutation carriers.

Table 25 Diagnostic yield of genetic testing for specific *VHL* gene mutations in relatives of patients with a known mutation

Study	Study quality[a]	Population	Genetic test[b]	Diagnostic yield
First-degree relatives				
(Kreusel et al 2000) Germany	Level IV diagnostic evidence CX P1 Q3	N = 22 first-degree relatives (20 parents and 2 siblings) of VHL patients with a known VHL mutation	SSCP and DNA sequencing, Southern blotting	32% (7/22)
(Gross et al 1996) Israel	Level IV diagnostic evidence CX P1 Q3	N = 19 first-degree relatives from a Jewish VHL family of Kurdish origin n = 4 symptomatic n = 15 asymptomatic	SSCP and DNA sequencing	**All relatives** 9/19 (47.4%) **Asymptomatic relatives** 5/15 (33.3%)
(Kanno et al 1996) Japan	Level IV diagnostic evidence CX P1 Q3	N = 17 first-degree asymptomatic relatives from 1 of 5 Japanese VHL families	SSCP	**Asymptomatic relatives** 3/17 (18.8%)
(Siu et al 2011) China	Level IV diagnostic evidence CX P1 Q3	N = 10 first-degree relatives from 4 families n = 2 symptomatic n = 8 asymptomatic	DNA sequencing, MLPA	**First-degree relatives** 5/10 (50.0%) **Asymptomatic First-degree relatives** 3/8 (37.5%)
(Stanojevic et al 2007) Serbia	Level IV diagnostic evidence CX P1 Q3	N = 7 asymptomatic first-degree relatives from 3 families of hospitalised VHL patients	SSCP and DNA sequencing	**Asymptomatic relatives** 2/7 = 28.6%
(Cruz et al 2007) Brazil	Level IV diagnostic evidence CX P1 Q3	N = 7 first-degree asymptomatic relatives of 2 siblings with phaeochromocytoma who have a *VHL* gene mutation	DNA sequencing	**Asymptomatic relatives** 2/7 (28.6%)
(Priesemann et al 2006) UK	Level IV diagnostic evidence CX P1 Q3	N = 7 first-degree relatives (children) of 3 probands (2 were siblings) who had a clinical and genetic diagnosis of VHL syndrome	Not reported	5/7 (71.4%)
(Brauch et al 1997) Germany	Level IV diagnostic evidence CX P1 Q3	N = 7 first-degree asymptomatic relatives of 2 index cases with phaeochromocytoma and known VHL mutations	SSCP and DNA sequencing	**Asymptomatic relatives** 2/7 (28.6%)
(Bender et al 1997) Germany	Level IV diagnostic evidence CX P1 Q3	N = 5 first-degree relatives of 2 index cases with thoracic phaeochromocytoma and known VHL mutations	DNA sequencing	3/5 (60%)

Study	Study quality[a]	Population	Genetic test[b]	Diagnostic yield
(Garcia et al 1997) Spain	Level IV diagnostic evidence CX P1 Q3	N = 6 first-degree asymptomatic members of a family with suspected VHL syndrome	Restriction-site polymorphism	**Asymptomatic relatives** 1/6 (16.7%)
(Wu et al 2000) Japan	Level IV diagnostic evidence CX P1 Q3	N = 4 first-degree asymptomatic relatives of a proband with clinical diagnosis of VHL	Restriction-site polymorphism	**Asymptomatic relatives** 1/4 (25.0%)
(Kang et al 2005) Korea	Level IV diagnostic evidence CX P1 Q3	N = 3 first-degree relatives of 1 VHL patient	DNA sequencing	1/3 (33.3%)
Second-degree relatives				
(Stanojevic et al 2007) Serbia	Level IV diagnostic evidence CX P1 Q3	N = 6 asymptomatic second-degree relatives from 3 families of hospitalised VHL patients	SSCP and DNA sequencing	**Asymptomatic relatives** 0/6 (0%)
(Garcia et al 1997) Spain	Level IV diagnostic evidence CX P1 Q3	N = 3 asymptomatic second-degree relatives from a family with suspected VHL syndrome	Restriction-site polymorphism	**Asymptomatic relatives** 0/3 (0%)
First- and second-degree relatives				
(Ruiz-Llorente et al 2004) Spain	Level IV diagnostic evidence CX P1 Q3	N = 103 relatives from 20 families n = 25 who presented with some clinical symptoms	DNA sequencing and Southern blotting	**All relatives** 41/103 (39.8%) **Asymptomatic relatives** 16/78 (20.5%)
(Rasmussen et al 2006) Mexico	Level IV diagnostic evidence CX P1 Q3	N = 70 asymptomatic relatives from 7 families with VHL disease	DNA sequencing	**Asymptomatic relatives** 14/70 (20.0%)
(Glavac et al 1996) Slovenia	Level IV diagnostic evidence CX P1 Q3	N = 50 asymptomatic family members from 8 large VHL families	DNA sequencing, SSCP analysis, Southern blotting and MLPA	**Asymptomatic relatives** 15/50 (30.0%)
(Huang et al 2007) Taiwan	Level IV diagnostic evidence CX P1 Q3	N = 38 relatives from 3 unrelated families n = 5 clinically diagnosed with VHL syndrome	DNA sequencing, Southern blotting	**All relatives** 13/38 (34.2%) **Asymptomatic relatives** 7/32 (21.9%)
(Gergics et al 2009)	Level IV diagnostic evidence	N = 36 relatives n= 28 family members of VHL	DNA sequencing,	**All relatives** 15/36 (41.7%)

Study	Study quality[a]	Population	Genetic test[b]	Diagnostic yield
Hungary	CX P1 Q3	patients n = 8 relatives of 3 VHL mutation +ve phaeochromocytoma patients	real-time PCR and MLPA	**Asymptomatic relatives** 5/19 (26.3%)
(AlFadhli et al 2008) Kuwait	Level IV diagnostic evidence CX P1 Q3	N = 33 members of a VHL family n = 13 clinically diagnosed with VHL syndrome	SSCP	**All relatives** 13/33 (39.4%) **Asymptomatic relatives** 0/20 (0%)
(Akcaglar et al 2008) Turkey	Level IV diagnostic evidence CX P1 Q3	N = 31 kindred of 4 index patients with VHL and RCC	Moorehead karyotyping method to detect deletion of the short arm of chromosome 3	**All relatives** 22/31 (71.0%) **Asymptomatic relatives** 12/21 (57.1%)
(Green 1996) Canada	Level IV diagnostic evidence CX P1 Q3	N = 28 members of a large VHL family from Bonavista Bay with no clinical signs of VHL syndrome	Restriction-site polymorphism	**Asymptomatic relatives** 4/28 (14.3%)
(Huang et al 2004) China	Level IV diagnostic evidence CX P1 Q3	N = 27 kindred from a large family with VHL disease n = 9 clinically diagnosed with VHL syndrome	DNA sequencing	**All relatives** 15/27 (55.6%) **Asymptomatic relatives** 6/18 (33.3%)
(Kanno et al 1996) Japan	Level IV diagnostic evidence CX P1 Q3	N = 25 individuals belonging to 1 of 5 Japanese VHL families n = 8 clinically diagnosed with VHL syndrome	SSCP	**All relatives** 10/25 (40.0%) **Asymptomatic relatives** 3/17 (17.6%)
(Cybulski et al 1999) Poland	Level IV diagnostic evidence CX P1 Q3	N = 24 relatives of 9 VHL patients with deletions identified by means of long PCR	Long PCR	**All relatives** 15/24 (62.5%) **Asymptomatic relatives** 3/12 (25.0%)
(Stanojevic et al 2007) Serbia	Level IV diagnostic evidence CX P1 Q3	N = 18 first- and second-degree relatives from 3 families of hospitalised VHL patients n = 5 symptomatic n = 13 asymptomatic	SSCP and DNA sequencing	**All relatives** 7/18 = 38.9% **Asymptomatic relatives** 2/13 = 15.4%
(Garcia et al 1997) Spain	Level IV diagnostic evidence CX P1 Q3	N = 15 members of a family with suspected VHL syndrome n = 5 clinically diagnosed with VHL syndrome	Restriction-site polymorphism	**All relatives** 6/15 (40%) **Asymptomatic relatives** 1/10 (10%)
(Chen et al 1996) USA	Level IV diagnostic evidence CX P1 Q3	N = 12 members of a large Pennsylvanian VHL type 2A phaeochromocytoma family n = 5 clinically diagnosed with VHL syndrome	SSCP and DNA sequencing	**All relatives** 5/12 (41.7%) **Asymptomatic relatives** 0/7 (0%)

Study	Study quality[a]	Population	Genetic test[b]	Diagnostic yield
(AlFadhli et al 2004) Kuwait	Level IV diagnostic evidence CX P1 Q3	N = 9 asymptomatic relatives of a proband with clinical diagnosis of VHL	SSCP	**Asymptomatic relatives** 3/9 (33.3%)
(Tong et al 2009) China	Level IV diagnostic evidence CX P1 Q3	N = 8 family members n = 3 patients with phaeochromocytomas n = 5 asymptomatic relatives	DNA sequencing	**All relatives** 3/8 (37.5%) **Asymptomatic relatives** 0/5 (0%)
(Pack et al 1999) USA	Level IV diagnostic evidence CX P2 Q3	N = 6 asymptomatic relatives of 4 VHL patients	FISH	**Asymptomatic relatives** 2/6 (33.3%)

[a] A description of study quality characteristics is provided in Table 13 and Table 14; [b] Detection limit of genetic test was not reported

DNA = deoxyribonucleic acid; PCR = polymerase chain reaction; RCC = renal cell carcinoma; DNA = deoxyribonucleic acid; FISH = fluorescence in-situ hybridisation; MLPA = multiplex ligation-dependent probe amplification, PCR = polymerase chain reaction; SSCP = single-strand conformational polymorphism; VHL = von Hippel-Lindau

Summary of the diagnostic accuracy of genetic testing in the detection of an inherited VHL mutation in first- and/or second-degree relatives of index cases with a known germ-line VHL mutation

Once an index case has a pathogenic VHL mutation identified, their close relatives need only be tested for that specific mutation, using a testing methodology known to be able to detect that type of mutation. Therefore, contrary to testing of the index case, the diagnostic accuracy of genetic testing within family members did not vary to any great extent by specific genetic testing methodology. Every included study reporting accuracy data for relatives of someone with a known VHL mutation reported a sensitivity of 100%. This indicates that, as expected, patients who met the clinical diagnostic criteria for VHL syndrome carried the familial VHL mutation. The median specificity of 83.3–85.0% and the false positive rates of 16.9–23.5% reflect the difference in the timeframe required for a positive clinical diagnosis compared with a positive genetic test. Younger relatives are more likely to receive a positive genetic test before any clinical signs of disease can be detected by clinical screening.

The 100% negative predictive value indicates that a negative genetic test result is likely to reflect the true disease status of the patient. However, the median positive predictive value of 69.4% for first-degree relatives and 47.8% for first- and second-degree relatives reflects both the potential lag between a genetic and clinical diagnosis and the greater prevalence of VHL mutation carriers among first-degree relatives compared with second-degree relatives. That is, it reflects the imperfect nature of the reference standard at predicting which relatives would likely develop clinical symptoms over time, rather than reflecting poorly on the accuracy of the genetic test.

Approximately 4 out of 10 of all first- and second-degree relatives, and 2–3 out of 10 asymptomatic first- and second-degree relatives, who undergo VHL genetic testing were identified as carriers of the familial VHL mutation.

Does VHL genetic testing change patient management?

Studies meeting the inclusion criteria outlined in Box 8 and Box 9 were used to determine if testing for mutations in the *VHL* gene plus usual diagnostic screening and assessment changed patient management, compared with usual screening and assessment alone, either in patients suspected of having VHL syndrome or in their family members.

Box 8 Inclusion criteria for identification of studies relevant to assessment of a change in patient management as a result of genetic testing for VHL syndrome (index patient)

Research question	
Does genetic testing for mutations in the *VHL* gene, in addition to usual diagnostic assessment, change patient management compared with usual clinical diagnosis in patients with suspected VHL syndrome?	
Selection criteria	**Inclusion criteria**
Population	Patients presenting with one or more clinical features suggestive of VHL syndrome
Intervention	VHL genetic testing to diagnose *VHL* gene mutations, **and** clinical diagnosis from family history, clinical history, tests including CT, MRI, ultrasound, hearing test, eye exam, blood tests and other tests as appropriate, to identify any signs of disease other than presenting complaint
Comparator(s)	Clinical diagnosis from family history, clinical history, tests including CT, MRI, ultrasound, hearing test, eye exam, blood tests and other tests as appropriate to identify any signs of disease other than presenting complaint
Outcomes	Rate and type of referral, frequency and compliance with clinical screening, rate and type of treatment, type of referral, hospital separations and readmissions, hospital length of stay
Search period	1993 – May 2011
Language	Non-English language articles were excluded unless they provided a higher level of evidence than the English language articles identified

CT = computed tomography; MRI = magnetic resonance imaging; VHL = von Hippel-Lindau

Box 9 Inclusion criteria for identification of studies relevant to assessment of a change in patient management as a result of genetic testing for VHL syndrome (family members)

Research question	
Does genetic testing for mutations in the *VHL* gene, in addition to usual diagnostic assessment, change patient management compared with usual clinical diagnosis for relatives of patients with a known VHL mutation?	
Selection criteria	**Inclusion criteria**
Population	Clinically unaffected first- or second-degree family members of patients with clinically diagnosed VHL syndrome and/or a diagnosed VHL genetic abnormality
Intervention	VHL genetic testing to screen for *VHL* gene mutations **±** clinical testing (CT, MRI, ultrasound, hearing test, eye exam, and blood tests) **and** routine lifelong screening for neoplasms using CT, MRI, ultrasound, hearing test, eye exam and blood tests
Comparator(s)	Clinical testing (CT, MRI, ultrasound, hearing test, eye exam, and blood tests) **and** routine lifelong screening for neoplasms using CT, MRI, ultrasound, hearing test, eye exam and blood tests
Outcomes	Rate and type of referral, frequency and compliance with clinical screening, rate and type of treatment, type of referral, hospital separations and readmissions, hospital length of stay
Search period	1993 – May 2011
Language	Non-English language articles were excluded unless they provided a higher level of evidence than the English language articles identified

CT = computed tomography; MRI = magnetic resonance imaging; VHL = von Hippel-Lindau

Minimal evidence was identified regarding a change in patient management following diagnosis of VHL syndrome using genetic testing in combination with clinical diagnosis. Only 5 studies (level IV interventional evidence) were included that provided any evidence associated with the clinical management of patients with a germ-line VHL mutation, but none provided a direct comparison between patients with a known VHL mutation and those that had not been tested (Table 26). Nevertheless, as these studies all included patients with VHL syndrome and their families, the results are generalisable for the purposes of this assessment. Additionally, as the studies were conducted in the USA, Canada, UK and Europe, the results are applicable to the Australian healthcare context with few caveats. However, due to the lack of an appropriate comparator group in these studies, no comment can be made about the clinical impact of genetic testing on patient management.

A good-quality narrative systematic review (of level IV interventional evidence) reported on genotype–phenotype correlations in patients with VHL syndrome and their relatives (Ho et al 2003). Knowledge of a specific germ-line VHL mutation in a patient with a clinical diagnosis of VHL syndrome is not expected to alter patient management significantly. However, it may provide some information about the types of neoplasms that are likely to develop in a particular patient, and thus affect the type of screening offered. Patients with VHL type 1 syndrome are more likely to have germ-line VHL mutations that inactivates pVHL, for example large deletions and nonsense mutations that truncate the protein. They are also more likely to develop renal cell carcinoma and CNS haemangioblastomas without phaeochromocytoma. Patients with VHL type 2 syndrome are more likely both to develop phaeochromocytoma in addition to the other VHL-associated neoplasms, and to have germ-line missense mutations predicted to produce altered full-length pVHLs. Thus, management of patients with type 1 or type 2 VHL syndrome could be tailored to ensure early detection of the neoplasms most likely to occur.

Although no difference in patient management is expected for patients presenting with the same VHL-associated neoplasms based on VHL mutation status, early detection of neoplasms is expected to affect long-term outcomes. Thus, identifying VHL mutations in patients presenting with early stages of *de novo* VHL syndrome, that is, with their first neoplasm and no family history, enables early commencement of routine screening to monitor disease progression. Currently, as these patients would not meet the criteria for a positive clinical diagnosis of VHL syndrome, the clinicians may consider them to have sporadic disease and may not offer routine screening until they present with a second neoplasm. The availability of VHL genetic testing may change this.

Conversely, the VHL genetic test is expected to change patient management for asymptomatic relatives when used as a triage test for lifelong screening. Relatives with a

negative genetic test result would not require lifelong screening, saving potential anguish and unnecessary use of healthcare resources. Lifelong screening programs designed to detect and then treat any new neoplasms early, thus preventing serious morbidity and/or mortality outcomes, can then be targeted towards relatives that have inherited the VHL mutation and are therefore likely to develop VHL-associated neoplasms.

One level IV study of moderate quality investigated the proportion of VHL patients with and without retinal disease who agreed to genetic testing (Dollfus et al 2002). The authors found that 88.0% (91/103) of patients with retinal manifestations and 97.0% (105/108) of patients without retinal manifestations agreed to genetic testing. This rate is quite high when compared with the number of at-risk relatives of patients diagnosed with VHL syndrome and a known VHL mutation, who agreed to genetic testing. Rasmussen et al (2010) and Evans et al (1997) reported in 2 good-quality level IV studies that 58.5% (92/157) and 65.8% (48/73), respectively, of at-risk relatives agreed to genetic testing. Evans et al (1997) found that relatives aged over 20 years (94.9%; 37/39) were more likely to undergo genetic testing than children aged less than 5 years (0%; 0/6). This suggests that parents are reluctant to have very young children genetically tested. Their reluctance diminished with increasing age of the child, agreeing to genetic testing of 33.3% (6/18) of children aged 5–9 years and 50.0% (5/10) of children aged over 10 years. Gender had little influence on this decision, as 82.6% (19/23) of males and 90.9% (20/22) of females aged over 16 years agreed to genetic testing.

Rasmussen et al (2010) conducted a study in Mexico City that investigated the likelihood that symptomatic and asymptomatic patients with a VHL mutation would continue annual screening after 5 years. The authors found that only 38.9% (14/36) of patients with a VHL mutation continued screening after 5 years. Symptomatic patients (57.9%; 11/19) were significantly more likely to continue screening after 5 years than asymptomatic patients (17.6%; 3/17; OR = 5 [95% CI 1.2, 20.3]; p = 0.02), which the authors suggested was due to complacency. Patients who have symptoms or have had a neoplasm detected are more aware of the personal risks involved in discontinuation of screening than patients who have not developed any detectable neoplasms. Whether this compliance with annual screening, irrespective of symptomatic status, is higher with the knowledge of having a VHL mutation than without cannot be determined from the available evidence.

Table 26 Effectiveness of genetic testing at influencing management of patients with VHL syndrome and asymptomatic relatives with a *VHL* gene mutation

Author Location	Study design Quality	Study population	Outcome
(Ho et al 2003) Canada	Systematic review of level IV interventional evidence Good quality (SIGN 2008)	Patients with VHL syndrome plus a VHL mutation, and their families	**Genotype–phenotype correlations:** **VHL type 1:** Germ-line mutations predicted to inactivate the VHL protein are associated with renal cell carcinoma and CNS haemangioblastomas without phaeochromocytoma **VHL type 2:** Germ-line mutations predicted to produce full-length VHL proteins are associated with phaeochromocytoma in addition to the other manifestations of VHL **Large deletions and mutations:** Predicted to cause a truncated protein are associated with a lower risk of phaeochromocytoma **Missense at codon 167:** Associated with a high risk of phaeochromocytoma (53% and 82% at 30 and 50 years, respectively).
(Evans et al 1997) UK	Level IV interventional evidence Good quality (NHS CRD = 5/6)	N = 73 at-risk members of VHL families	**% relatives that agreed to genetic testing** **Overall** 65.8% (48/73) **Aged < 5 years** 0% (0/6) **Aged 5–9 years** 33.3% (6/18) **Aged 10–20 years** 50.0% (5/10) **Aged > 20 years** 94.9% (37/39) **Males aged ≥ 16 years** 82.6% (19/23) **Females aged ≥ 16 years** 90.9% (20/22)
(Rasmussen et al 2010) Mexico	Level IV interventional evidence Good quality (NHS CRD = 4.5/6)	N = 157 first- and second-degree relatives of 12 +ve probands N = 36 patients that receive annual screening n = 12 VHL patients n = 24 GT positive relatives	**% relatives that agreed to genetic testing** 58.5% (92/157) **% patients with a VHL mutation that continued screening after 5 years:** 38.9% (14/36) **% symptomatic patients that continued screening after 5 years:** 57.9% (11/19) **% asymptomatic patients that continued screening after 5 years:** 17.6% (3/17) **Likelihood of symptomatic versus asymptomatic patients to continue screening program** OR = 5 [95% CI 1.2, 20.3]; p = 0.02
(Neumann et al 1999) Germany	Level IV interventional evidence Good quality (NHS CRD = 4.5/6)	N = 39 patients with phaeochromocytomas who underwent adrenal-sparing surgery n = 21 patients with VHL mutations n = 13 sporadic cases	**Length of hospital stay** mean = 13 days
(Dollfus et al 2002) France	Level IV interventional evidence Moderate quality (NHS CRD = 4/6)	N = 103 patients with VHL retinal manifestations N = 108 patients without VHL retinal manifestations	**% patients that agreed to genetic testing:** **with retinal manifestations:** 88% (91/103) **without retinal manifestations:** 97% (105/108)

CNS = central nervous system; GT = genetic test; OR = odds ratio; VHL = von Hippel-Lindau

Summary of change in patient management

Minimal evidence was identified regarding patient management following diagnosis of VHL syndrome using genetic testing in combination with clinical diagnosis. No study provided a direct comparison between patients with a known VHL mutation and those that had not been tested. Therefore, due to the lack of an appropriate comparator in these studies, no conclusions can be made about the *change* in patient management (ie the clinical impact) from genetic testing.

Knowledge of a specific germ-line VHL mutation in a patient with a clinical diagnosis of VHL syndrome may provide some information about the VHL syndrome type, which then determines the types of neoplasms that are likely to develop in a particular patient. Thus, management of patients with a known VHL mutation could be tailored to ensure early detection of the neoplasms most likely to occur.

Although no difference in patient management is expected for patients presenting with the same VHL-associated neoplasms, based on the method of diagnosis, the VHL genetic test is expected to change patient management for asymptomatic relatives when used as a triage test for lifelong screening. Relatives with a negative genetic test result would not require lifelong screening, saving potential anguish and unnecessary use of healthcare resources. Lifelong screening programs can then be targeted towards relatives who have inherited the VHL mutation and are likely to develop VHL-associated neoplasms.

While 88.0–97.0% of clinically diagnosed VHL patients agreed to genetic testing in the evidence-base, only 58.5–65.8% of at-risk relatives agreed. Additionally, relatives aged over 20 years were more likely to undergo genetic testing than children aged less than 5 years, suggesting that parents are reluctant to have very young children genetically tested. This reluctance appears to diminish with increasing age of the child.

Interestingly, only 38.9% of patients with a VHL mutation continued screening after 5 years. Symptomatic patients were much more likely to continue than asymptomatic patients. Patients who have symptoms or have a neoplasm detected early are more aware of the personal risks involved than patients who have not developed any detectable neoplasms, and thus may be complacent. Whether compliance with annual screening is higher with knowledge of a VHL mutation than without could not be determined from the available evidence.

Does change in management alter patient health outcomes?

Adverse health outcomes are avoided by subsequent annual screening for early detection of newly developed neoplasms. As the screening protocol is identical for all VHL patients, irrespective of their VHL mutation status, a systematic literature search for linkage 3 evidence (investigating the impact of a change in patient management on health outcomes) was not undertaken after advice from PASC. However, 2 studies were identified that provided some data on the health outcomes for patients with both a clinical and a genetic diagnosis of VHL syndrome presenting with neoplasms detected by annual screening compared with detection due to symptomatic manifestations (Kreusel et al 2006; Rasmussen et al 2010).

Kreusel et al (2006) found that, whereas 71.4% of eyes treated for symptomatic retinal haemangioblastomas had adverse visual outcomes, normal vision was maintained after treatment in 97.0% of asymptomatic eyes (Table 27). Rasmussen et al (2010) followed a group of 36 patients with a VHL mutation (24 initially had no clinical signs of disease) for a period of 5 years and compared the mortality rate of the 14 patients who continued annual screening with the 22 patients who did not. They found that the mortality rate for the group that discontinued screening was slightly higher than those that were screened (9.1% versus 7.1%), although this difference was not statistically significant ($p>0.05$). The authors also found that each patient who continued screening developed an average of 2.3 new neoplasms over the 5-year period (Table 27).

These results support the premise that there are significant health benefits associated with annual screening to detect newly developed neoplasms. The treatment of neoplasms before they become symptomatic is associated with less morbidity and mortality.

Table 27 Health outcomes after annual screening compared with no screening

Author Location	Study design Quality	Study population/ Patient group	Outcomes
(Rasmussen et al 2010) Mexico	Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 36 patients who received annual screening n = 12 VHL patients n = 24 GT positive relatives n = 14 patients who participated in screening for 5 years n = 22 patients who stopped participating in screening during first 5 years	**5-year mortality rate of participants** 7.1% (1/14) **5-year mortality rate of non-participants** 9.1% (2/22) **Relative risk of mortality for participants compared with non-participants** RR = 0.85 [0.16, 4.43] **Number of new neoplasms** 32/14 patients mean = 2.3/patient / 5 years
(Kreusel et al 2006) Germany	Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 57 consecutive patients presenting with capillary retinal angiomatosis resulting from VHL disease at 2 clinics n = 43 patients with clinical and genetic diagnosis n = 2 with genetic diagnosis only n = 12 with clinical diagnosis only	**Age-related risk for bilateral retinal angioma** 100% at age 56.4 years **Proportion of eyes with angiomas** 85.8% (97/113) **Proportion of eyes that were enucleated or developed blindness** 5.3% (6/113) **Risk of adverse visual outcomes** 71.4% for symptomatic eyes 3.0% for asymptomatic eyes

GT = genetic test; VHL = von Hippel-Lindau

Summary of change in management effects on health outcomes

The data obtained highlighted that health benefits are derived from reduced morbidity and mortality due to annual screening for early detection of newly developed neoplasms.

Other relevant considerations

In addition to the relative safety and effectiveness of genetic testing, there are other issues outside the scope of the research questions assessed by the systematic review that may impact on the assessment of the technology, as well as any decision to publicly fund VHL testing.

Counselling services

Genetic counselling services are funded by the state/territory governments, and thus there may be difficulties getting access to a service. Genetic counsellors are not reimbursed for their services under the MBS, whereas the more costly service provided by a clinical geneticist is reimbursed (MBS item number 132). Hence, there may be an incentive to use these medical specialists for genetic counselling over genetic counsellors, despite the higher cost associated with the service.

Australian VHL registry

Currently, there is no Australian registry either for patients with VHL syndrome or their relatives who carry a VHL mutation. Hence, it is unknown how many people and/or families in Australia are affected by this disease. It is also unknown how many patients with a positive clinical diagnosis and their at-risk relatives have been genetically tested.

An Australian VHL registry (managed in a similar way to a cancer registry) could provide important data for the management of patients with VHL, while maintaining the individual's privacy and confidentiality.

Quality assurance and molecular methodologies

The Australian Genetic Testing Survey (2006) reported that three laboratories accredited by the National Association of Testing Authorities (NATA) conducted genetic testing to identify mutations in the *VHL* gene (Royal College of Pathologists of Australasia 2008).

Previously, there had not been a quality assurance program (QAP) to monitor the performance of laboratories providing VHL genetic testing (RCPA Quality Assurance Programs Pty Ltd 2009). The RCPA and the Human Genetics Society of Australasia (HGSA) reached a landmark agreement to jointly offer an enhanced Molecular Genetics QAP in 2010, and seek International Laboratory Accreditation Cooperation (ILAC) G13:2000 accreditation at the earliest opportunity. As a consequence, the RCPA/HGSA Molecular Genetics QAP Committee will be able to monitor the performance within and between the three laboratories that offer VHL genetic testing, using in-house methodologies to ensure that their performance is optimal. Should other laboratories

seek to offer VHL testing in the future, it would be reasonable to suggest that they participate in this program.

Additional applications for VHL genetic testing

Somatic VHL genetic testing

The proposed MBS items do not allow for reimbursement for somatic VHL genetic testing as they have been limited to the 'detection of germ-line mutations of the *VHL* gene'.

However, there are instances where somatic VHL genetic testing may be beneficial. Therefore, the use of VHL genetic tests for the detection of somatic VHL mutations may need to be addressed by MSAC in the future.

CNS haemangioblastomas

Currently, patients presenting with isolated CNS haemangioblastomas are routinely tested for both germ-line VHL mutations in the peripheral blood and somatic VHL mutations in the tumour itself (expert advice of MESP clinical expert). As CNS haemangioblastomas are a common first manifestation of VHL disease (in 31.5% of patients; Poulsen et al 2010), this procedure provides a greater degree of certainty that the VHL genetic test results are accurate. For example, if a VHL mutation is found in tissue samples from the tumour but not in the normal tissue surrounding the tumour or in the peripheral blood, it confirms that the patient does not have a germ-line VHL mutation and has a sporadic haemangioblastoma.

Somatic genetic mosaicism

Some patients will have somatic genetic mosaicism, and there is accumulating evidence to suggest that it is more prevalent than previously believed. In these patients a VHL mutation will be present in particular embryonic cell lineages (eg the CNS) but not in others (eg the peripheral blood). Thus, a VHL mutation will not be detected using standard genetic testing protocols. It would be useful for managing these patients to be able to determine if one or more organs is affected with a VHL mutation, so that annual screening can be tailored to checking those organs only and avoid unnecessary screening of unaffected organs.

Prenatal and pre-implantation VHL genetic testing

A worldwide birth incidence for VHL disease is often quoted at 1 in 36,000 (Barontini & Dahia 2010; Lonser et al 2003; Maher et al 1991). However, one recent study conducted in the UK found the birth incidence to be 1 in 42,987 (Evans et al 2010). The decreased birth incidence found in this study may reflect an increased understanding of the inheritance of genetic diseases and the availability of improved reproductive technology. In fact, one study involving adults aged 20–40 years with a known VHL mutation found

that a positive test result may lead to altered reproductive intentions. Approximately 25% of individuals did not want to have children and another 50% planned to use prenatal diagnosis and then termination of an affected pregnancy (Levy & Richard 2000).

Prenatal diagnostic tests use procedures such as chorionic villus sampling (at 11–13 weeks) and amniocentesis (at 15–19 weeks) to procure samples for genetic testing. These procedures carry a small risk of miscarriage (less than 1 in 100) and are only useful if the parents are willing to abort an affected foetus (Barlow-Stewart & Saleh 2007b). This has the potential to have long-term psychological and/or religious consequences for the parents and their extended family, depending on their beliefs (McGivern 1995). This decision may be especially difficult with a disease such as VHL syndrome. Although VHL syndrome is highly penetrant, there is vast clinical unpredictability in its manifestations, with no clear phenotype–genotype correlation to severity of the disease.

Another difficulty is that prenatal predictive VHL genetic testing would not be reimbursed under Medicare as the foetus is not considered an 'eligible person' for health insurance. The *Health Insurance Act 1973* states (section 3) that an 'eligible person' means an Australian resident or an eligible overseas representative. Currently, Australian law still considers birth to be the moment when the foetus obtains the full legal rights of a citizen. The possibility of MBS funding covering prenatal genetic testing is being explored by the Genetics Working Party, which is being established under the new Pathology Funding Agreement.

An alternative for parents not willing to abort an affected foetus is pre-implantation genetic diagnosis, which is performed prior to implantation and before pregnancy occurs. First, an embryo is created using assisted reproductive technologies such as in vitro fertilisation, and then one or two cells are removed from the embryo at the eight-cell stage (after 3 days) or at blastocyst stage (after 5 days) for genetic testing. Only those embryos that did not have the specific genetic condition would be transplanted into the uterus (Barlow-Stewart & Saleh 2007a).

In Australia pre-implantation genetic diagnosis is currently only offered in the private health setting. The Victorian Assisted Reproductive Treatment Authority lists the *VHL* gene on its website as one of the single gene disorders that were tested for use of pre-implantation genetic diagnosis in 2010 (Victorian Assisted Reproductive Treatment Authority 2010).

Emergence of targeted therapies

The elucidation of how pVHL functions in tumour suppression has increased our understanding of how cancer develops. This has led to the development of therapies that target proteins that function in HIF-dependent pathways, as shown in Figure 7. Disrupting the function of these proteins interferes with tumour progression, and these targeted therapies have been quite successful in treating renal cell carcinomas. For example, loss of pVHL leads to activation of the HIF-dependent pathways, increasing the levels of VEGF and PDGF, which stimulate angiogenesis. The use of angiogenic inhibitors (sorafenib, sunitib, pazopanib and axitinib) or VEGF antibodies (bevacizumab) can slow down the rate of angiogenesis, thus inhibiting growth of the tumour. Hence, they are now part of the standard treatment course for renal cell carcinomas (Barontini & Dahia 2010; Heng et al 2010; Singer et al 2011).

Mammalian target of rapamycin (mTOR) regulates HIF transcription and translation, and mTOR inhibitors (temsirolimus and everolimus), which act to lower the amount of HIF protein present in tumour cells, have recently shown promise in the treatment of patients with renal cell carcinoma (Barontini & Dahia 2010; Heng et al 2010). Future treatments may also include using a histone deacetylase inhibitor (sodiumbutyrate), which inhibits HIF activation and may be beneficial in restoring cell adhesions that are disrupted by VHL inactivation (Barontini & Dahia 2010; Singer et al 2011).

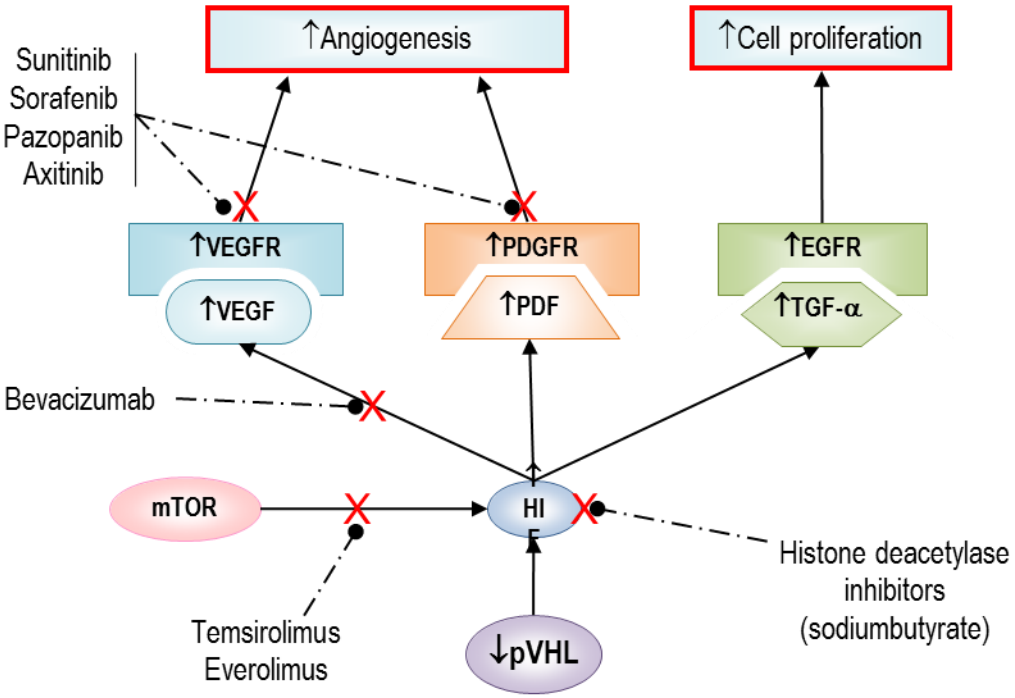


Figure 7 Potential drug targets for pVHL HIF-regulated functions.

EGFR = epidermal growth factor receptor; HIF = hypoxia-inducible factor; mTOR = mammalian target of rapamycin; PDGF = platelet derived growth factor; PDGFR = platelet-derived growth factor receptor; pVHL = von Hippel-Lindau protein; TGF-α = transforming growth factor α; VEGF = vascular epidermal growth factor; VEGFR = vascular epidermal growth factor receptor

Several of these targeted therapies have been used to treat other VHL-related neoplasms with limited success (Kaelin Jr 2005). In particular, the mTOR inhibitor everolimus has been used to treat malignant phaeochromocytoma (Druce et al 2009), the anti-VEGF antibody bevacizumab to treat CNS haemangioblastomas (Gilheeney et al 2007) and the VEGF inhibitor pegaptanib to treat retinal haemangioblastomas (Dahr et al 2007). However, the use of targeted therapies to treat these tumours is still experimental and not used in routine clinical practice.

As our understanding of the differences in activation of the various HIF-dependent and HIF-independent pathways between type 1 and type 2 VHL disease increases (presumably due to the differences in pVHL function resulting from different *VHL* gene mutations), therapies can be targeted to counteract the neoplastic effects of specific pathways, to hopefully impede tumour growth and offer new treatment options to patients with known VHL mutations.

Ethical considerations

Introduction

The ethical issues raised by genetic testing differ from those usually discussed with respect to health technologies. Genetic tests for germ-line VHL mutations raise these issues, but they also raise additional issues of their own. These will be discussed, where possible, in the assessment that follows.

The aim of this assessment is to synthesise available evidence in order to inform a public funding decision. In the case of ethical issues, such synthesis equates to reviewing relevant literature and assessing the balance of the arguments. The assessment is descriptive insofar as it reviews available literature, but it is also normative insofar as it seeks to identify ethical ideals for framing policy on how medical professionals should universally conduct themselves.

Methods of evidence synthesis

Five core papers were selected from the 176 articles identified as potentially relevant in the literature search using the following criteria: clear discussion and linking of ethical theory to genetic testing (Giarelli 2001; Kinder 1998; Korf 1999; Offit & Thom 2007; Winslow et al 2005). These constituted the main body of evidence. Where possible, they were supported by additional atricles that presented (i) material from an Australian perspective and (ii) issues relating specifically to VHL genetic testing. Some of these additional articles were identified by the literature search, while others came from outside the formal search. These included key texts in medical ethics (Beauchamp & Childress 2001; Munson 2000; Rogers & Braunack-Mayer 2004), web resources (ALRC 2003; HGSA 2008) and a number of papers that did not consider ethical issues.

Genetic exceptionalism

Genetic exceptionalism is the idea that genetic information is special and distinct from other forms of information. Several features of genetic testing support this idea:

1. Genetic testing provides information that is private and personal but also relevant to more than just the tested individual. Test results have implications not only for the tested individual but also for family members, who may or may not wish to be informed of their revealed risk status for a given disease.

2. Many tests predict for disease development that may occur many years in the future. Because of this, the psychological ramifications can be very different from those of tests within the context of symptomatic disease. For instance, patients may perceive diseases with genetic origins as predicted to emerge from 'within themselves', while diseases without such origins are thought to associate with factors that are external to themselves and hence beyond 'their control'.

3. Most patients have only a limited knowledge of genetics. Because of this, an informed consent process requires adequate counselling on an extensive array of issues. These include both standard considerations and those particular to individual and/or family circumstances (Kinder 1998).

Genetic exceptionalism is not universally accepted. Some contend that the clinical integration of genetic risk assessment for common malignancies such as colon and breast cancer has negated the need for treating genetic information as special. Particularly within the field of clinical oncology, some believe that genetic and non-genetic predictive testing feature more similarities than differences. This belief has emerged, at least in part, due to the increasing use of predictive testing over the past 10–15 years.[8] The belief entails that medical interventions are now reliant on genetic information in order to offer the best possible clinical care (Offit & Thom 2007).

Despite these counterarguments to genetic exceptionalism, there is no broad acceptance that genetic information is the same as other kinds of information, principally family history. Family history can provide valuable information for diagnosing and predicting the risk of disease. As with genetic testing, it also raises ethical issues concerning the preservation of autonomous choice, privacy and confidentiality (Suthers 2008). However,

[8] A prominent example is pharmacogenetic testing for drug toxicity.

this assessment adopts the conservative view of genetic exceptionalism—that genetic information is indeed unique and particularly vulnerable to misuse.[9]

Ethical framework

The approach adopted by the current assessment is principlism, philosophically developed elsewhere (Beauchamp & Childress 2001). This approach was adopted because it is predominant within the field of biomedical ethics (Beauchamp & Childress 2001; Munson 2000; Rogers & Braunack-Mayer 2004). Furthermore, of the 18 papers included in this assessment, none used an alternative approach. Recently, it has been recommended that health technology assessments should incorporate comprehensive ethical analysis (Duthie & Bond 2011); however, a philosophical defence of principlism has not been possible within the confines of this assessment. This does not unduly undermine the assessment's capacity to report on the main ethical issues as identified by the literature search. Nor does it preclude a reasonable understanding of the main issues identified.

The 'four principles' approach

Principlism outlines four main principles—autonomy, non-maleficence, beneficence and justice, which are used to assess the ethical issues associated with genetic testing, as briefly described below.

Autonomy

Autonomy refers to self-rule. Individual autonomy refers to the governing of oneself and the directing of one's own life, free from coercive interference on the part of others and from limitations that might prevent one from making meaningful choices. A respect for individuals' autonomy entails that they have a right to self-determination or to act freely in accordance with a self-chosen plan. Such respect underpins the process of informed consent in medical care and research, and provides the basis for privacy of medical records. Most theories of autonomy agree that two conditions are required for autonomous choice—liberty, meaning independence from controlling influences, and agency, the capacity for intentional action (Beauchamp & Childress 2001; Winslow et al 2005).

Non-maleficence

Non-maleficence refers to not inflicting harm or injury to others and is associated with the dictum *Primum non nocere*: 'Above all (first) do no harm'. The principle also finds

[9] For further arguments, see the Australian Law Reform Commission President's preliminary account of the 2003 joint inquiry into the protection of human genetic information (Weisbrot 2003).

expression in the modern Hippocratic oath: 'I will use treatment to help the sick according to my ability and judgment, but I will never use it to injure or wrong them'. In clinical practice the principle of non-maleficence is often combined with, and sometimes balanced against, the principle of beneficence, a version of which is expressed in the first half of the above Hippocratic oath (Beauchamp & Childress 2001; Giarelli 2001). For instance, even the best diagnostic tests and treatments can carry certain risks of harm, and it is practically impossible for medical professionals to act without ever causing harm. Indeed, causing some harms may be warranted in the light of greater potential benefits. Hence, the avoidance of unwarranted or unnecessary harm, even if unintentional, is paramount to the non-maleficent conduct of health professionals. Inextricably linked to the concept of non-maleficence is the obligation to exercise 'due care', which is not always explicitly defined but rather implied in many professional codes of clinical practice. Aspects of non-maleficent practice that are implied in the clinician's duty of care are neither more nor less important than those explicitly defined (Munson 2000).

Beneficence

The principle of beneficence asserts that it is not enough to respect the autonomy of patients and to avoid causing them harm; in addition, clinicians and providers of health services should act in ways that actively promote the welfare of patients (Kinder 1998). Just as there are standards of due care that explicitly and implicitly define appropriate conduct in the protection of patients from harm, so too are there explicit and implicit standards of beneficence. For example, an obvious expectation in medical care is the physician's duty to help patients by providing appropriate treatment. More implicit is the wider societal expectation that physicians should make reasonable sacrifices for the sake of their patients. In the absence of a reasonable cause to act otherwise, a physician's neglect of a patient requiring medical intervention understandably warrants the disapproval of that patient and of the physician's colleagues, placing the ethical conduct of the physician in serious question even before potential legal ramifications are considered.

Practical constraints must be applied in acting beneficently. There are countless ways to promote the welfare of a patient, but the majority of people will distinguish between expectations that are reasonable and those that are not. In this way, whether or not clinicians fulfil their duty of beneficence relies on judgment and is constrained by various practical considerations. It is also constrained by the duty to act in accordance with other, sometimes conflicting, ethical principles (Munson 2000). Ethical dilemmas arise precisely when one is torn between acting in accordance with two or more ethical principles that commend different courses of action.

Justice

Justice refers to treating equals equally. In medical ethics the principle of justice finds expression in the belief that everyone deserves equal access to advances in medicine, and in the importance of fairness in the treatment of patients, particularly in the distribution of scarce resources. Different theories of justice focus on conditions of entitlement, fair and equal treatment, and concerns that the distribution of social goods such as healthcare occurs on the basis of relevant factors, for example degree of need, capacity to benefit and/or particular rights. Distributive justice concerns how resources are distributed, to whom and for what reasons. Difficult choices are sometimes made between, for instance, greatly benefiting the few (those with rare diseases) and benefiting to a lesser degree the many (Giarelli 2001; Winslow et al 2005).

The main ethical issues raised by genetic testing

The main ethical issues associated with genetic testing and their most relevant ethical principles are listed in Table 28.

Table 28 Main ethical issues and their most relevant principle

Issue	Most relevant principle(s)
Informed consent	Autonomy, non-maleficence, beneficence
Privacy and confidentiality	Autonomy
Balancing risks and benefits	Non-maleficence, beneficence
Potential for discrimination	Justice
Access	Justice
Direct-to-consumer genetic testing	Non-maleficence, beneficence

Questions relevant to ethical inquiry when assessing a health technology have been listed previously (Hofmann 2005) and have provided valuable guidance. However, the questions proposed by Hofmann have not been used as a 'checklist' on a question-by-question basis as individual concepts could not be logically separated. The emergent themes are most comprehensibly captured when discussed in a collective manner.

Informed consent

Many people do not have a good understanding of genetics, and seeking informed consent for genetic testing poses particular challenges for clinicians and counsellors. Emphasis is placed on the need for an explicit agreement between the health provider and the patient. The basic elements of informed consent, adapted from guidelines of the American Society of Clinical Oncology (Kinder 1998), are listed in Table 29. In line with the principle of respect for autonomy, clinicians and counsellors should stress that testing for a genetic mutation is completely voluntary and optional. The competence of the individual to be tested should be assessed, and information must be provided in a format that the patient can understand, with particular emphasis on the likely accuracy

of the diagnosis and the fact that test results will not always provide definitive information about whether the development of disease will ensue. The limits of other methods for predictive testing, if applicable, need to be discussed.

Table 29 Informed consent for genetic testing

Autonomy provisions • Information on the specific test being performed • Implications of a positive and negative test result • Possibility that the test will be inconclusive or not informative • Options for risk estimation without genetic testing • Risk of passing mutation to children • Options to withdraw from study (in the case of genetic tests conducted for research)
Beneficence provisions • Options for medical surveillance, risk reduction and screening following testing
Non-maleficence provisions • Technical accuracy of the test • Risks of psychological distress • Risk of insurance or employer discrimination
Paternity provisions • Procedures if relatedness (ie paternity/maternity) is not as expected • Procedures governing notification of family
Privacy-professional responsibilities • Confidentiality issues • Fees involved in testing, counselling and follow-up care
Special considerations • Ownership and research uses of DNA remaining after testing • Reproductive uses of genetic information

Source: adapted from Offit & Thom (2007)

In the context of VHL syndrome, information regarding the natural history of the types of tumour to which individuals with a VHL mutation are predisposed should form an integral part of the pre-test counselling. Pre-symptomatic individuals who are seeking testing subsequent to genetic diagnosis of a family member should be informed of the likelihood of tumour development (mean age of tumour onset is 26 years) (Maher et al 1990). They should also be given information on non-genetic periodic screening and its limitations and benefits. As to limitations, they should be informed of the potential for false negative and false positive results attached to the following screening methods: magnetic resonance imaging (MRI) to detect haemangioblastoma; computed tomography (CT), MRI and ultrasound to diagnose renal cell carcinoma; CT for the diagnosis of pancreatic tumours; the measurement of plasma metanephrine levels to rule in or out the possibility of phaeochromocytoma; MRI and CT to detect endolymphatic sac tumours; and palpation, CT and ultrasound to diagnose cystoadenomas of the adnexal reproductive organs (Barontini & Dahia 2010; Lonser et al

2003). One considerable benefit of screening is an increased life expectancy to levels approaching those of the general population (Nordstrom-O'Brien et al 2010).

These considerations of consent based on counselling procedures that provide comprehensive information on the nature and likelihood of disease occurrence and development may be complicated by the fact that genetic disorders, including VHL syndrome, display incomplete penetrance, as emphasised in a discussion of ethical issues involved in genetic testing for renal disease (Korf 1999). As a consequence of incomplete penetrance, not all carriers of genetic mutations express the associated disorder at a given age. In the past, estimates of penetrance have often come from studies of families observed to have high penetrance—those with several affected members who are ideal subjects for research investigating gene linkage or identification. Data from these studies may overestimate the penetrance in the population; therefore, there is a possibility that counselling based on available penetrance estimates leads to exaggerated perceptions of risk among those considering genetic testing. This may in turn cause undue anxiety and even lead to unnecessary modes of surveillance. For this reason, the genetic counsellor needs to ensure that individuals and their families (where applicable) receive adequate information regarding the effectiveness and quality of all relevant screening methods, and that this information is understood (Korf 1999).

Special concerns have arisen with regard to contexts in which the intended recipients of genetic tests are unable to give informed consent, specifically children and embryos (Offit et al 2004). In Australia the genetic testing of children for clinical purposes is not regulated by legislation. However, the World Health Organization, the Nuffield Council on Bioethics and the American Society of Human Genetics have developed guidelines on the genetic testing of children (ALRC 2003), and the Human Genetics Society of Australasia (HGSA) has published a position statement, *Pre-symptomatic and predictive testing in children and young people* (HGSA 2008). In essence, these guidelines and statements affirm that the predictive genetic testing of minors should only be conducted when there is an availability of treatment options that directly benefit the child. Testing for adult-onset diseases for which no known treatment or preventive measures exist should not be performed on children, but rather deferred until adulthood or at least until the minor can adequately understand the implications of testing and make their own informed decision. The HGSA position statement specifically acknowledges that there is a lack of research on the issue of informed consent for the genetic testing of children; therefore, it expects that any further guidelines will be of benefit only if more research emerges in this area. Furthermore, the HGSA does not provide direction on the matter of neonatal or prenatal testing.

The issue of prenatal testing introduces particular ethical considerations insofar as definitions of personhood are contentious. Ethical guidelines for Australian practice in the

area of genetic testing at the embryonic stage of human development appear to be lacking; however, various medical associations in the USA and Europe share similar positions to Australia. The main message is that, while prenatal testing is usually considered acceptable in instances of increased risk of foetal genetic disorders, embryo selection to avoid genetic disease is not appropriate in all circumstances. It depends on the gestational period at which selection would occur, and other factors including the disease's severity, probability of occurrence and age of onset (Offit & Thom 2007).

Testing for VHL mutations in children and embryos should be considered relative to the best available guidelines. Although such guidelines indicate that it should be avoided where possible, VHL syndrome certainly has the potential to manifest in childhood. This means that the issue of informed consent cannot safely be deferred until the child is of age. It is important that parents with children at risk of having a VHL mutation are well informed about the nature of the disease, screening procedures that may be avoided if mutations are ruled out by the testing of family members, and disease treatment regimes, with an unbiased presentation of the risks and benefits. The same information is also necessary for mutation carriers who are contemplating prenatal testing. Out of continuing respect for autonomy, these individuals should also be informed of the risk of conceiving affected offspring.

Privacy and confidentiality

The principle of autonomy affirms the right to voluntary genetic testing, entailing access to the best available evidence of risks and benefits. Furthermore, it affirms the individual's right to privacy. While a patient may choose to reveal information, genetic test results must usually be kept confidential by medical personnel. In the case of inherited genetic conditions, keeping the results of a genetic test confidential protects an individual's right to privacy. However, it also limits the ability of family members to make informed choices with respect to their own health. Given that inheritable genetic disorders are both an individual and a family matter, ethical dilemmas can arise when a clinician is torn between maintaining the confidentiality of a patient's test results and informing family members of their own corollary predisposition to disease (Giarelli 2001).

Although healthcare professionals recognise the need to maintain confidentiality in most clinical scenarios, some circumstances exist in which disclosure may be permissible, even required. Especially from a legal perspective, these are typically scenarios in which a threat to a third party is considered 'imminent' and 'serious'. Judging which specific clinical situations warrant a breach of confidentiality remains one of the most difficult ethical issues raised by genetic testing. For this reason, groups such as the US Institute of Medicine's Committee on Assessing Genetic Risks have proposed the following criteria that must be met before a clinician contemplates any disclosure of genetic information:

1. All attempts to bring about voluntary disclosure must be exhausted.

2. The seriousness of the harms posed by the genetic mutation must be imminent and certain.

3. Effective means of preventive or therapeutic intervention must be available.

If the harms risked by maintaining confidentiality are small and unlikely, and no reasonable medical intervention can be provided or accessed, a breach in confidentiality is considered wrong. By contrast, if the harms risked by maintaining confidentiality are substantial and certain, and there is effective treatment that can be readily accessed, a breach in confidentiality is considered ethically justified (Winslow et al 2005).

Some authors have identified that the duty to inform family members of genetic risk—so that they can adopt early monitoring and prophylactic treatment if required—may be justified on the grounds that the clinician regards the entire family as the patient and, in this sense, revealing genetic information among family members does not represent a breach of confidentiality (Rogers & Braunack-Mayer 2004). However, as per considerations of informed consent, counselling is required before the disclosure of test results. Counselling helps the initial test recipient understand and deal with information, but also consider and state how much information they are prepared to share. Counselling the initial test recipient on the benefits of sharing information with close relatives and in turn providing counselling to those relatives, whether they be directly affected or not, requires considerable skill and sensitivity.

While best practice is represented by striving to avoid breaking confidentiality, the Australian Government has enabled genetic counsellors to legally do so in serious cases through its 2006 amendment to the *Privacy Act 1988*. This amendment allows disclosure of genetic information without consent to relatives provided such disclosure is 'necessary to lessen or prevent a serious threat to the life, health or safety whether or not the threat is imminent' (Suthers et al 2011). In 2009 the NHMRC developed guidelines that provide the formal mechanism for implementation of the new provisions of the Privacy Act, which practitioners must comply with (National Health and Medical Research Council 2009).

Patients who undergo genetic testing are likely to have concerns, not only about sharing test results with family members, but also about who will have access to their test results, how the information will be used and for what purposes. Patients may be particularly concerned about the potential for health and life insurance companies, employers and financial institutions to use genetic information in order to discriminate against them. Confidentiality and privacy are of particular importance in these respects

(Beauchamp & Childress 2001). The perceived risk of this type of discrimination is discussed further directly below.

Weighing risks and benefits

In general, risks are to be minimised and benefits maximised. Risks associated with genetic testing are generally psychological and social, but by no means should risks to physical health be neglected. For example, tests that erroneously present an individual as a non-carrier of some genetic mutation may result in substantial physical harms to that individual, especially in the case of pre-symptomatic testing. Pre-symptomatic individuals will be afforded a false sense of security about their risk and will almost certainly miss the opportunity for screening procedures that offer the potential for early detection and intervention against disease.

On the other hand, the ramifications of a **false negative** test in a symptomatic individual seeking to confirm a clinical diagnosis will be very different. In this case it is likely that the patient will experience some level of anxiety about symptoms that are not supported by a genetic diagnosis.

True negative results are considered harmful mainly in the context of common diseases for which at-risk populations are large (eg colon and breast cancers). An individual may see a true negative result as reason to ignore screening recommendations for which there is an evidentiary basis. Logically, true negative results could also be of concern when considering rarer conditions whose genetic basis is only partly understood (eg those disorders for which only a portion of the responsible genes have been found). However, no literature was found expressing this concern. Paradoxically, negative test results may also lead to experiences of 'survivor guilt'.

When it comes to the psychologically harmful effects of a positive genetic test result, the risk must be weighed against the potential benefit of information that can lead to targeted surveillance, preventive measures and/or more specific and effective treatment (Offit & Thom 2007).

Testing for VHL mutations in children and embryos should be considered in line with the best available guidelines. Although such guidelines indicate that it should be avoided where possible, the development of symptoms of VHL syndrome is possible throughout childhood, suggesting that there may well be cause for the exercise of parental choice in the decision. While many of the tumours, on average, do not appear until the third and fourth decades of life, this can obscure the fact that a substantial proportion of children with VHL mutations will experience tumour development and associated neurological deficits. Haemangioblastoma is relatively common during the second decade of life and may occur before the age of 10 years (Barontini & Dahia 2010). Prior to the genetic testing of children suspected of VHL syndrome, it is important that parents are well

informed about disease development and progression, screening procedures that may be avoided if relatives are tested (and mutations ruled out) and treatment regimes, with an unbiased presentation of the risks and benefits. One major concern is that the child and family may be informed of the carrier state years in advance of any clinical signs of disease. This carries the possibility of substantial psychological harms for the child and family. The potential impact of the result on the child's development, together with the emotional reactions of the affected and unaffected parent and of any siblings, all require consideration (Levy & Richard 2000). The same information is also necessary for mutation carriers who are contemplating prenatal testing. Obviously, these individuals should also be informed of the risk of conceiving affected offspring.

The potential for genetic discrimination was touched on above in discussion of patient privacy and confidentiality. Respecting a patient's wish to keep genetic information private is good in itself. It also minimises the risk, however small to begin with, that disclosed information will lead to the patient being discriminated against and to attendant psychological, social and economic harms. Fear of genetic discrimination, particularly from insurance companies, represents one of the most active areas of debate in the ethics literature on genetic testing. Such fear is felt by many people invited to undergo genetic testing (Offit & Thom 2007; Winslow et al 2005).

Some commentators have argued that the underwriting of health insurance premiums on the basis of genetic test results should not be an issue for Australian patients. Community rating dictates that all people pay the same rates for the same level of health cover, regardless of their health status and family history (Delatycki 2008). Life insurance, on the other hand, is not afforded the same level of protection against genetic discrimination. This may be defensible if health care is considered a basic or fundamental good or right, whereas death benefits are considered a commodity. The reduction of life insurance to a commodity, and thus very different from health insurance, is contestable.

Either way, if genetic information is to be disclosed and used in underwriting, it must be used justly and fairly. If a person who has a known positive test for a genetic condition pays a higher premium than another, untested, person with an identical mutation, the underwriting practice is unjust, as it does not treat equally those with equal health risks. The potential for genetic discrimination raises an important issue. Societal acceptance of genetic disorders cannot be regulated by law, and those individuals known to suffer from conditions of a hereditary nature may be subject to stigma due to shortened life expectancy, of the kind that surrounds much disability. Statutory provisions provide protection against discrimination at the level of the workplace and insurance industries, but societal discrimination can only be ameliorated through education of the public (Winslow et al 2005).

Access issues

Individuals who undergo genetic testing deserve justice in resource allocation. But how would this work? Access to treatment should be provided in the event that results provide evidence of mutation. How cost-effective this treatment must be before being publicly funded is a matter for debate. Suspected VHL syndrome patients from both rural and remote areas and metropolitan centres would have a blood sample taken by their general practitioner, which would then be sent away for analysis. Given the rarity of the condition, only three Australian pathology laboratories offer VHL genetic testing. It is expected that referral overseas would not be a common occurrence given the relatively low demand for this service. With current funding for VHL genetic testing being provided either by the state/territory governments, where testing may be limited by budgetary constraints, or at a personal cost to the patient, it is probable that not all patients with VHL syndrome or at-risk relatives are being tested, raising questions of justice. Listing VHL genetic testing on the MBS should increase access to the test for all individuals who require it.

It should be noted that the quality of genetic testing varies significantly (Offit & Thom 2007) and that VHL genetic testing is not an exception. The test available from the Cancer Genetics Diagnostic Laboratory, Royal North Shore Hospital, NSW, cannot detect large deletions, and therefore may miss some VHL diagnoses. On the other hand, the Molecular Pathology Division of the Institute of Medical and Veterinary Science in Adelaide, SA, and the service offered in WA undertake procedures that enable detection of virtually all VHL cases.

Direct-to-consumer testing

The potential for psychological harm caused by genetic testing in the context of poor or absent counselling cannot be overstated. Nor can the potential for psychological and physical harm caused by inappropriate clinical decisions based on an inaccurate performance or interpretation of genetic tests. In light of these concerns, direct-to-consumer (DTC) genetic testing has emerged as an issue of ethical significance. A key example of DTC genetic testing is that for breast cancer in the USA. The advertising of these tests has generated huge increases in demand while simultaneously resulting in substantial decreases in the referral of high-risk individuals. This suggests that advertisements for DTC genetic tests may downplay the limitations of genetic testing and increase the number of individuals who, having been tested, do not adequately appreciate the meaning of their results. Furthermore, much unnecessary and harmful anxiety and complacency may be generated, depending on the nature of test results, and mistakes in health management are likely to occur (Offit & Thom 2007).

Summary

Genetic testing raises the following range of ethical issues and, in some cases, dilemmas:

- On balance, it appears ethically acceptable provided that testing is both preceded and followed by adequate counselling on, among other things, the limitations and significance of test results, including possible ramifications for family members and the possible courses of effective treatment should a test result be positive.
- Diseases without effective treatment options should not be tested for.
- Counselling is necessary in order to ensure informed consent and minimise risks of harm, both psychological and, in the longer term, physical.
- Test results should remain confidential, although the patient should be counselled on the benefit of sharing information with family members who may benefit.
- As always, confidentiality should be broken only if risks to others are serious, imminent, certain and avoidable, and attempts at encouraging voluntary disclosure have been exhausted.
- Testing should be available, and not overly financially burdensome, to all who might benefit from it.
- If genetic tests were to underwrite health or life insurance premiums, they would have to do so fairly. It is far from clear how that would work, nor what role voluntary disclosure would play.
- Direct-to-consumer genetic testing appears to carry substantial risks.

Conclusion

With respect to VHL testing, the above ethical analysis would suggest that the test should only be offered on the MBS if it is performed in conjunction with genetic counselling from accredited counsellors with familiarity both in the interpretation of VHL test results and in the management implications for the index case and family members.

What are the economic considerations?

In its assessment of a new service, the MSAC is required to consider not only the comparative effectiveness and safety of the service but also the comparative cost and cost-effectiveness of the service. Thus, an economic evaluation based on the clinical evidence of adding/substituting the service under MSAC consideration to/for the main comparator(s) in the population, and in the setting for which subsidy is required, is presented. In addition, an analysis that examines the financial impact to the Australian healthcare system of subsidising the proposed new service is presented.

The purpose of an economic evaluation is to inform the MSAC as to the additional costs and additional gains (health or other socially relevant outcomes) of the proposed service over the comparator when used in the Australian healthcare system. This is to ensure that society's ultimately scarce resources are allocated to those activities from which it will get the most value; that is, it seeks to enhance economic efficiency.

The costing exercise conducted is not intended for fee scheduling purposes, and is not necessarily a recommendation for funding the service at these levels.

Existing literature

The literature from the databases listed in Appendix F was searched to identify studies that met the inclusion criteria outlined *a priori* in Box 10 and Box 11. These studies were assessed to determine the cost-effectiveness of using VHL genetic testing in patients with a clinical diagnosis of VHL syndrome or in their family members.

Box 10 Inclusion criteria for identification of studies relevant to assessment of the cost-effectiveness of genetic testing for VHL syndrome (index patient)

Research question	
Is VHL genetic testing cost-effective when used as an addition to clinical diagnostic approaches in the diagnosis of patients presenting with symptoms suggestive of VHL syndrome?	
Selection criteria	**Inclusion criteria**
Study design	All relevant articles on economic models and trial-based economic evaluations, including the study designs listed in the 'Intervention' column of Table 13
Population	Patients presenting with one or more clinical features suggestive of VHL syndrome
Intervention	VHL genetic testing to diagnose *VHL* gene mutations, **and** clinical diagnosis from family history, clinical history, tests including CT, MRI, ultrasound, hearing test, eye exam, blood tests and other tests as appropriate, to identify any signs of disease other than presenting complaint
Comparator(s)	Clinical diagnosis from family history, clinical history, tests including CT, MRI, ultrasound, hearing test, eye exam, blood tests and other tests as appropriate to identify any signs of disease other than presenting complaint
Outcomes	Cost-effectiveness outcomes (cost, cost per relevant health outcome (eg LYG, QALY, DALY))
Search period	1993 – May 2011
Language	Non-English language articles were excluded unless they to provided a higher level of evidence than the English language articles identified

CT = computed tomography; DALY = disability-adjusted life year; LYG = life-years gained; MRI = magnetic resonance imaging; QALY = quality-adjusted life year; VHL = von Hippel-Lindau

Box 11 Inclusion criteria for identification of studies relevant to assessment of the cost-effectiveness of genetic testing for VHL mutations (family members)

Research question	
Is VHL genetic testing cost-effective when used as a triage test for lifelong screening of family members of patients who are positive for a VHL mutation?	
Selection criteria	**Inclusion criteria**
Study design	All relevant articles on economic models and trial-based economic evaluations, including the study designs listed in the 'Intervention' column of Table 13
Population	Clinically unaffected first- or second-degree family members of patients with clinically diagnosed VHL syndrome and/or a diagnosed VHL genetic abnormality
Intervention	VHL genetic testing to screen for *VHL* gene mutations **±** clinical testing (CT, MRI, ultrasound, hearing test, eye exam and blood tests) **and** routine lifelong screening for neoplasms using CT, MRI, ultrasound, hearing test, eye exam and blood tests
Comparator(s)	Clinical testing **and** routine lifelong screening for neoplasms using CT, MRI, ultrasound, hearing test, eye exam and blood tests
Outcomes	Cost-effectiveness outcomes (cost, cost per relevant health outcome (eg LYG, QALY, DALY))
Search period	1993 – May 2011
Language	Non-English language articles were excluded unless they provided a higher level of evidence than the English language articles identified

CT = computed tomography; DALY = disability-adjusted life year; LYG = life-years gained; MRI = magnetic resonance imaging; QALY = quality-adjusted life year; VHL = von Hippel-Lindau

Is VHL genetic testing cost-effective?

No relevant cost-effectiveness or cost-utility analysis assessing VHL genetic testing was identified in the literature. There was 1 cost–benefit analysis investigating the implications of multidisciplinary programs using clinical screening ± genetic testing for the management of VHL disease for the period of 1982–91 in Newfoundland, Canada (Green 1996). Another 4 cost analyses were also identified by the systematic review (Atuk et al 1998; Catapano et al 2005; Gläsker et al 1999; Hes 2000). A profile of key characteristics including study design and location, level and quality of evidence was developed for each study selected, and details are listed in Appendix G.

In the article by Green (1996), costs and benefits were compared between i) the *status quo* situation (at that time) of not using screening investigations in the management of patients and family members diagnosed with, or at risk of, VHL syndrome (Program 1); ii) the use of clinical screening only (Program 2); and iii) the use of genetic testing plus clinical screening (Program 3). As Program 1 is no longer relevant to current clinical practice, only the results of Program 2 and Program 3 are summarised and presented in Table 30.

Table 30 Costs and benefits of programs for management of VHL disease with clinical screening with or without genetic testing

	Program 2	Program 3
Program description	Education and counselling	Education and counselling Genetic testing
	Clinical screening for all affected individuals and first-degree family members	Clinical screening for gene carriers
	Treatment of early disease[a]	Treatment of early disease[a]
Costs	Geneticist's salary (education, counselling and coordination of program)	Geneticist's salary (same as Program 2)
	Secretary's salary (administration of program)	Secretary's salary (less than Program 2) Genetic testing
	Clinical screening for affected and all first-degree family members[b]	Clinical screening for gene carriers only (less than Program 2)
	Early treatment[a]	Early treatment[a] (same as Program 2)
Benefits	Information	Information
	Early diagnosis and treatment[a]	Early diagnosis and treatment[a]
	Reduced morbidity and mortality[a]	Reduced morbidity and mortality[a]
	Much reduced anxiety[a]	Even greater reduced anxiety[a]
		Reassurance for non-carriers
		Knowledge for reproductive planning

[a] Compared with Program 1 (without clinical/genetic screening); [b] Including healthcare costs (set of appointments), out-of-pocket expenses for family (travel and accommodation) and anxiety at time of screening
Source: Green (1996)

For Program 2, annual clinical screening was offered to all patients diagnosed with VHL syndrome and their first-degree family members (at 50% risk of having the VHL mutation) until at least 50 years of age. Meanwhile, the genetic testing in Program 3 was offered to all first-degree relatives and the clinical screening to those who were VHL mutation carriers. It is noted that second-degree family members were not considered in either program. This is not consistent with either the current or proposed clinical pathway as described in the 'Approach to assessment' section of this assessment report, and has, therefore, resulted in an underestimate of the cost and benefit implications of VHL genetic testing.

The cohort in the cost–benefit analysis in Green (1996) consisted of 78 patients with VHL syndrome or at-risk family members who participated in a screening program between 1982 and 1991 in Newfoundland, Canada. The analysis was performed using a societal perspective, so that both monetary and non-monetary costs and benefits to VHL patients, their family members and the healthcare system were considered.

The direct costs of the clinical screening in Program 2 consisted of the investigation costs, the administration costs, the costs of treatment and out-of-pocket expenses. The costs of Program 3 were estimated from those of Program 2, assuming that genetic analysis had been introduced in 1982 as the first-step screening test for the same VHL families as involved in Program 2. The financial implications of death and disability (eg bilateral blindness and neurological or other medical disabilities that prevented normal employment) associated with delayed diagnosis and treatment of VHL syndrome were estimated on the basis of legal awards or insurance payments for loss of future wages due to death or similar disabilities of individuals of the same age but from the general public.

There was no attempt to convert all costs and benefits (ie improved medical and/or psychosocial outcomes) into monetary units, given the acknowledged difficulties in valuing life and disability. Records of previously affected family members were retrospectively reviewed, and affected or unaffected members of VHL families participating in the screening programs were interviewed to collect data on the psychosocial costs and consequences of screening programs, for example anxiety about VHL disease and/or of screening investigations.

The quality of the cost–benefit analysis performed by Green (1996) was assessed using a 10-item checklist for a sound economic evaluation (Drummond et al 2005), as shown in Table 31.

Table 31 Assessment of the cost–benefit analysis by Green (1996)

Checklist	Appraisal
Was a well-defined question posed in an answerable form?	Yes
Was a comprehensive description of the competing alternatives given?	Yes
Was the effectiveness of the programs or services established?	No. A comparison of the clinical effects of Program 3 and Program 2 in terms of mortality and morbidity was not based on a clinical study, but was presumed by the author
Were all the important and relevant costs and consequences for each alternative identified?	Not enough information to determine
Were costs and consequences measured accurately in appropriate physical units?	Not enough information to determine
Were costs and consequences valued credibly?	Not enough information to determine
Were costs and consequences adjusted for differential timing?	No discounting method was used
Was an incremental analysis of costs and consequences of alternatives performed?	No
Was allowance made for uncertainty in the estimates of costs and consequences?	No statistical analysis and/or sensitivity analysis was performed
Did the presentation and discussion of study results include all issues of concern to users?	No

Overall, the quality of the cost–benefit analysis was low. Data regarding clinical effects were collected from an observational study for Program 2 but not for Program 3. Thus, the effectiveness of genetic testing plus clinical screening compared with clinical screening alone was not established on the basis of clinical studies, but it was assumed by the author that the additional use of genetic screening in clinical practice would not change the mortality rates and the proportions of patients disabled by VHL syndrome. In addition, there was insufficient detail on which to determine whether the relevant costs and benefits were fully identified, accurately measured or credibly valued. Apart from the above two major limitations, the cost–benefit analysis was also flawed due to not using a method of discounting to convert 'future' costs and consequences to their values at the time of analysis; a lack of incremental analysis of costs and benefits; the absence of statistical and/or sensitivity analysis taking into account the uncertainties relating to the variables used in the analysis; and lack of discussion of the consistency, generalisability and applicability of the results.

The results of the cost–benefit analysis, as reported by Green (1996), are presented in Table 32.

Table 32 Comparison (in Canadian dollars) of costs and benefits of programs for management of VHL disease

	Program 2	Program 3
Direct		
Geneticist's salary	C$90,000	C$90,000
Secretary's salary	C$53,000	C$33,000
Genetic testing[a]	C$0	C$10,850
Clinical screening for affected		
Set of appointments	C$123,500	C$123,500
Out-of-pocket expenses	C$17,000	C$17,000
Clinical screening for at risk		
Set of appointments	C$89,280	C$20,160
Out-of-pocket expenses	C$4,000	C$1,000
Treatment	C$483,000	C$483,000
Disabilities	C$0[b]	C$0[b]
Total	C$859,780	C$778,510
Indirect		
Deaths	–	–
Disabilities	–	–
Anxiety	++	+
Reduced family size	++	+

[a] The unit cost of genetic testing for VHL mutation was C$350.
[b] None of the participants were disabled by the VHL syndrome. All patients returned to work after treatment.
Source: (Green 1996)

No cost for disability was identified, as none of the participants were disabled due to complications associated with VHL disease, nor was the cost for death included in the cost–benefit analysis. Although one patient died and three patients developed unilateral blindness in the clinical screening group, these cases were caused by delayed investigation and treatment prior to 1982 (the year when Program 2 was implemented) and therefore were not included in the cost–benefit implications. The treatment costs were identical between the two groups, suggesting that the introduction of genetic testing for detection of mutations in the *VHL* gene is unlikely to change the treatment of VHL syndrome. The total direct costs for the management of VHL disease in the 78 patients or family members seen between 1982 and 1991, using genetic screening plus clinical testing (Program 3), were estimated to be C$778,510, which was about C$81,000 less than the costs of Program 2 (clinical screening only). The cost saving was attributable to the reduction in the number of at-risk family members that required clinical screening. In addition, the use of genetic screening resulted in beneficial effects on psychosocial outcomes, for example anxiety caused by VHL syndrome or the VHL screening programs and the loss of family members due to VHL-related deaths.

In the study by Hes et al (2000) the cost saving associated with the reduction of lifelong screening in the Netherlands in 2000 was estimated. Using a disease prevalence of

1:40,000, the hypothetical number of Dutch patients with VHL syndrome would be 400. The authors assumed that 100 VHL patients had been identified and that the remaining 300 patients were identified among 200 first-degree family members with a 50% chance of having VHL syndrome (100) and 800 second-degree family members at 25% risk for the disease (200). Therefore, the populations requiring clinical screening were 400 patients with genetic testing available and 1,100 (100 + 200 + 800) persons in the absence of genetic analysis. Annual VHL screening included specialist consultations, radiological examinations (CNS and abdomen), biochemical urine tests and blood tests. The total costs were estimated to be €525 per person per year (Table 33). It was indicated that genetic testing for VHL mutation resulted in a cost saving of €367,500 (525 x 1,100 – 525 x 400) in the Netherlands in 2000, which was related to the avoidance of unnecessary clinical screening of the family members of VHL patients with negative genetic testing results. The financial implications of genetic screening for VHL mutations, as estimated by Hes et al (2000), did not take into account the costs of the genetic test or its interpretation and associated genetic counselling. As DNA analysis cost €600 in the Netherlands in 2000, the introduction of genetic testing for VHL mutations incurred an extra €292,500 (600 x 1,100 – 367,500) in the first year. The additional costs would have been offset in the second year, and the testing was proposed to save €367,500 for each year thereafter.

Table 33 Clinical monitoring of persons at risk of VHL syndrome

Item	Cost per person per year
Consultations	
Ophthalmologist	€35
Neurologist	€80
Internist	€80
Radiological monitoring	
MRI of CNS and abdomen[a]	€195
Abdominal ultrasound[a]	€35
Biochemical urine tests (urea, creatinine, VMA, norepinephrine, metanephrine, adrenaline and noradrenaline)	€75
Blood tests (blood count, creatinine, urea and electrolytes)	€25
Total annual costs	€525

[a] Radiological monitoring examinations were performed every 2 years; therefore, prices were halved for these two investigations.
CNS = central nervous system; MRI = magnetic resonance imaging; VMA = vanillylmandelic acid; VHL = von Hippel-Lindau
Source: (Hes et al 2000)

Table 34 summarises the costs of genetic testing and clinical screening reported in the remaining included studies. Although the unit costs for genetic testing varied between studies, they consistently cost less than half of the annual cost for clinical screening investigations.

Table 34 Costs of genetic testing and clinical screening in various studies

	Catapano et al (2005)		Gläsker et al (1999)	Atuk et al (1998)
Country	Italy		Germany	USA
Year	2005		1999	1998
Genetic testing	DHPLC	DNA sequencing, Southern blotting and FISH	Southern blotting and SSCP	Direct DNA sequencing
Cost of genetic testing per proband	€250 €280 if sequencing required	€750	€960 €1,070 if sequencing required	US$260
Cost of genetic screening per family member	€120		€290	Unknown
Clinical screening investigations	Physical examination 24-hour urinary test (catecholamine and metanephrines) Ophthalmological examination Upper abdominal ultrasound MRI of neuraxis Audiogram MRI of inner ear		MRI of the brain MRI of the spinal canal MRI of the abdomen Ophthalmological examination Fluorescent angiography of the retina 24-hour urinary catecholamine excretion	Ophthalmological examination Urinary catecholamine measures
Cost of annual clinical screening per person	€1 400		€2 570	US$650

DHPLC = denaturing high-performance liquid chromatography; DNA = deoxyribonucleic acid; FISH = fluorescence in-situ hybridisation; MRI = magnetic resonance imaging; SSCP = single strand conformation polymorphism

Overall, none of the identified papers provided an estimate of the economic implications of genetic testing for VHL mutations in an Australian setting. The applicability of the results is further limited due to the outdated data, given that the genetic/clinical investigations and their costs have changed as technology has developed (Green 1996). Nevertheless, the available evidence does give an indication that the cost savings associated with the use of VHL genetic testing are related to the exclusion of family members who do not have a VHL mutation from unnecessary lifelong clinical monitoring.

Summary of cost-effectiveness

No relevant cost-effectiveness or cost-utility analysis was identified evaluating the cost-effectiveness of the use of genetic testing for VHL mutations in addition to usual clinical diagnosis in patients suspected of having VHL syndrome, or when used as a triage test for lifelong screening of family members.

One study compared the costs and benefits of the use of clinical screening only with genetic testing plus clinical screening, and 4 studies reported on the costs of clinical screening and VHL genetic testing, and found cost savings attributable to the reduction in the number of at-risk family members that required clinical screening.

Green (1996) also found that the use of genetic screening resulted in beneficial psychosocial outcomes, for example reduced levels of anxiety associated with the use of VHL screening programs and an associated reduction in the likelihood of early death of family members.

However, the applicability of these findings to an Australian setting is likely to be limited; therefore, their utility was primarily to inform the decision-analytic modelling that has been conducted according to the perspective of the Australian health system.

Economic evaluation

Comparative effectiveness and costs

Based on the systematic review presented in this report, no direct evidence was found to support the claim of improved effectiveness of genetic testing for a mutation in the *VHL* gene over clinical testing alone. However, VHL genetic testing, if performed with direct sequencing and a method for detecting large deletions, can be highly accurate and is likely to result in a change in management for family members who are negative for the VHL mutation. Consequently, this may result in changes to the cost of management. Potential cost and effectiveness changes are reported in Table 35.

Table 35 Main differences in clinical management if genetic testing is used in combination with clinical testing

Clinical diagnosis is incorrect; patients are diagnosed sooner than they would ordinarily be if using a clinical diagnosis	**Effectiveness** Earlier monitoring may lead to earlier treatment and improved health outcomes. **Costs** Earlier detection of some manifestations due to earlier monitoring may result in less costly procedures or interventions. Earlier detection may result in a period of monitoring that has no impact on the overall outcome of the disease (if awaiting a clinical diagnosis resulted in no adverse outcomes).
VHL genotyping may provide insight into the likely manifestation (phenotype) of VHL syndrome	**Effectiveness** Patients may be spared uncomfortable or unsafe monitoring investigations. **Costs** Resources can be used to target more likely manifestations of VHL syndrome.
Asymptomatic family members can have their VHL genetic status verified	**Effectiveness** Family members may avoid the impact upon quality of life associated with lifelong screening or the psychological impact of not knowing one's genetic status. **Costs** Avoiding the lifelong monitoring of family members who do not have the VHL mutation represents a substantial cost saving.
Early diagnosis of asymptomatic family members who would not currently be screened	**Effectiveness** Family members who would not normally be screened (because they are third-degree or more distant from the clinically diagnosed VHL patient) may be appropriately monitored and receive improved health outcomes. **Costs** The additional monitoring will represent an additional cost.

Incorrect clinical diagnosis

If VHL genetic testing is available, a small proportion of patients referred for further investigation but with insufficient symptoms to make a clinical diagnosis will be correctly identified as carrying the *VHL* gene mutation. In this case, although the clinical diagnosis was incorrect, it is likely that the patient will eventually be diagnosed with VHL syndrome in the absence of genetic verification because additional symptoms will emerge. In this population, the incremental effectiveness of genetic testing is the benefit associated with

any additional monitoring (annual screening) occurring between when VHL syndrome could have been genetically verified until it was conclusively clinically diagnosed.

There is a clear benefit associated with monitoring in patients with VHL syndrome. Early detection and treatment of retinal angiomas while they remain asymptomatic will result in improved visual outcomes compared with treatment once they become symptomatic (Kreusel et al 2006). Similarly, fewer patients with VHL syndrome died over 5 years who received screening than those who did not receive screening, although this finding was not statistically significant due to the small sample size (Rasmussen et al 2010).

However, there are several problems when interpreting this data for an economic analysis. First, while it is clear that patients who are not diagnosed with VHL syndrome but who are identified as having a VHL mutation will receive additional monitoring, it is unclear whether this monitoring will be of benefit. Eventually, almost all patients with a VHL mutation will become symptomatic, and monitoring prior to this will only carry benefit if quality or length of life could be improved by an intervention that occurs earlier than when the symptoms manifest. Some symptoms of VHL syndrome will benefit more than others from early detection, and therefore there may be either great or little value associated with early diagnosis. It is also unclear whether patients who are referred for assessment of VHL syndrome-like symptoms, but who do not meet the full criteria for a clinical diagnosis of VHL syndrome, would be followed up at all. According to MESP advice, patients with features indicative of VHL syndrome would be followed up.

In the case of misdiagnosed patients (clinically negative but with a *VHL* gene mutation), they are likely to be a very small proportion of all patients suspected of having VHL syndrome. Given the uncertainty surrounding the magnitude of the likely benefit, it is conservative to assume that the incremental effectiveness of genetic testing in this situation would be trivial, and thus to exclude it from consideration in the economic analysis.

Genotype may inform phenotype

In patients who are clinically diagnosed with VHL syndrome, genetic verification has the benefit of identifying the genotype of patients, which can be correlated with VHL phenotype. VHL syndrome type 1 tends to be more commonly associated with renal cell carcinoma and CNS haemangioblastomas but not phaeochromocytoma. VHL syndrome type 2 is more commonly associated with phaeochromocytoma as well as other manifestations of a VHL mutation. Theoretically, patients with certain phenotypes could have monitoring tailored to match the most likely manifestations of the disease. This may be particularly the case for patients who are phenotypically type 2C, in whom phaeochromocytomas are the only likely manifestation of VHL syndrome. However, no guidelines could be found suggesting that an approach to monitoring that is tailored for

VHL phenotype would occur, and clinical expert advice suggests that changes in monitoring are unlikely to occur based on phenotype. Consequently, it is unclear whether knowing the genotype would alter current clinical management, nor whether such an alteration would result in a change in effectiveness or cost. As a consequence, this issue has not been considered in the economic model.

Diagnosis of family members

Current clinical practice in Australia is to offer monitoring to first- and second-degree family members of patients who are clinically diagnosed with VHL syndrome. If the VHL mutation is inherited, approximately 50% of first-degree relatives and 25% of second-degree relatives will also carry the mutation. If the VHL genetic mutation is *de novo*, no family members will carry the mutation (or only the children of a *de novo* case).

In the absence of genetic testing, first- and second-degree relatives are offered monitoring. If all first- and second-degree relatives accept, this would be a costly undertaking. If genetic testing is available, family members who may have inherited the VHL mutation are tested. This may stop at the mother and father if a *de novo* mutation is detected, or may extend across several generations. Importantly, assuming family members accept genetic testing, the VHL mutation can be tracked so that all family members that have inherited it are diagnosed regardless of how genetically distant.

Family members who have not inherited the VHL mutation are spared the imposition of monitoring and any negative psychological impact of believing that they could be carrying a genetic disorder. Asymptomatic family members who carry the genetic mutation may be more agreeable to monitoring if they have genetic confirmation. As mentioned, appropriate long-term monitoring improves quality and length of life in patients with VHL syndrome. However, it is unclear whether the additional monitoring before onset of symptoms will improve long-term outcomes compared with monitoring that commences with the onset of symptoms.

The number of family members who will be offered screening will be far higher in the absence of genetic testing than if it is available. In an ideal scenario, in which all family members who are offered monitoring accept surveillance, genetic testing will result in a substantial cost saving. Within a few years, the cost savings from avoiding unnecessary monitoring would offset the cost of genetic testing. However, in a more grounded scenario that accounts for a proportion of family members refusing monitoring, the cost of genetic testing may take longer to recoup, in particular if there are few costs involved in waiting for the first manifestation of VHL syndrome to begin monitoring. The costs associated with genetic testing and lifelong monitoring form the basis of the presented economic evaluation.

Diagnosis of extended family members

If the VHL mutation has been inherited for several generations (yet remained clinically undetected or misdiagnosed), it is possible that relatives of a patient with VHL syndrome more distant than second-degree may have inherited the gene. In the absence of genetic testing, these relatives would not be offered monitoring. If genetic testing is available (which will allow cascade testing), these patients will be positive for the familial VHL mutation and will be offered monitoring, potentially improving disease outcomes.

Summary

There are many non-quantifiable cost savings and benefits associated with the introduction of VHL genetic testing. The magnitude of these costs and benefits are uncertain and are primarily driven by increasing monitoring among patients and family members who may require it. The largest problem in determining potential benefits is that it is unclear how effective monitoring is, or how great is the harm associated with delayed diagnoses in patients who are not monitored.

It is likely that, once a patient with VHL syndrome is clinically diagnosed, they will accept monitoring (or if inclined not to accept monitoring, would continue not to do so even with additional genetic verification), and therefore the benefit of monitoring associated with earlier diagnosis should only be measured until the first clinical symptom or manifestation. If the first manifestation is an incurable renal cell carcinoma, the benefit of monitoring may be significant. If the first manifestation is far more benign, there may be no benefit to monitoring at all, and the earlier diagnosis will have only resulted in the added burden and cost of the monitoring between diagnosis and manifestation. These cost-effectiveness differences are uncertain and will only apply to a very small proportion of patients (1.4% of patients are determined to be clinically negative but are found to have a VHL mutation), and have not been considered in this evaluation.

There are, however, quantifiable cost savings associated with the avoidance of monitoring in family members who do not carry the VHL mutation. The magnitude of the cost saving will be dependent on the proportion of family members of a patient clinically diagnosed with VHL syndrome who would accept monitoring, and for how long they would persevere with monitoring prior to becoming non-compliant.

Unfortunately, no direct evidence comparing genetic testing plus clinical diagnosis with clinical diagnosis alone was identified that reported a change in patient or family health outcomes. This is unsurprising given that the primary role of genetic testing in patients with suspected VHL syndrome is confirmatory, and genetic testing in family members is to avoid unnecessary monitoring rather than to instigate monitoring. Consistent with this, and on the advice from the PASC, the presented economic evaluation has not included a consideration of the differences in health outcomes (ie an assessment of

comparative treatment effectiveness) as a consequence of VHL genetic testing. However, it is acknowledged that in some (few) cases genetic testing will result in appropriate monitoring—and potentially improved health outcomes—when a clinical diagnosis alone would not.

Due to the multiple uncertainties in the economic evaluation, several inputs have been varied in sensitivity analyses to explore the cost comparison of VHL genetic testing. The population and circumstances for using VHL genetic testing are summarised in Table 36.

Table 36 Proposed PICO for using VHL genetic testing in the Australian population

Population	Intervention	Comparator	Type of analysis	Outcome measure
Individuals suspected of having VHL syndrome	Clinical diagnosis plus genetic testing	Clinical diagnosis alone	Cost comparison	Incremental cost of lifetime management of VHL disease per individual
Asymptomatic family members of individual suspected of having VHL syndrome	Genetic testing	No test	Cost comparison	Incremental cost of lifetime management of VHL disease per members of family of individual suspected of having VHL syndrome

Structure of economic model

This economic model is a cost comparison of the management of one patient referred with suspected VHL syndrome and their asymptomatic family members in the presence and absence of genetic testing. The model is separated into two sections—the costs of the diagnosis and monitoring of an individual who presents with symptoms suggestive of VHL syndrome (the target population for the genetic test), and the costs incurred in the subsequent screening and monitoring of first- and second-degree relatives.

Short-term model

Patients present with symptoms suggestive of VHL syndrome and are diagnosed using clinical testing or a combination of clinical and genetic testing. Once their VHL genetic status has been decided, patients are then offered monitoring. In the base case, monitoring for VHL disease is accepted by 100% of individuals (they are all presumed to be symptomatic) diagnosed with VHL syndrome. This assumption may be inaccurate; however, a refusal of monitoring in symptomatic patients may incur detriments in quality or length of life and added costs involved with later diagnoses of VHL-related manifestations. Therefore, to assume all patients will accept monitoring in the short term may be conservative.

Family members of patients who are diagnosed with VHL syndrome are offered monitoring (in the clinical testing only arm), or genetic testing and then monitoring based upon the outcome of the genetic test (in the genetic testing arm). A proportion of

family members in the genetic testing arm will refuse the genetic test, although they will still be offered monitoring according to current practice. As family members are all asymptomatic, it is assumed that a proportion of those refusing the genetic test will also refuse monitoring.

As shown in Figure 8, individuals suspected of having VHL syndrome are given clinical testing in combination with genetic testing, or clinical testing alone. Patients are then offered monitoring based upon the information gained from the testing, and enter a monitoring or no-monitoring state based upon their acceptance or refusal of monitoring. In the base case, monitoring is assumed to be accepted in 100% of cases.

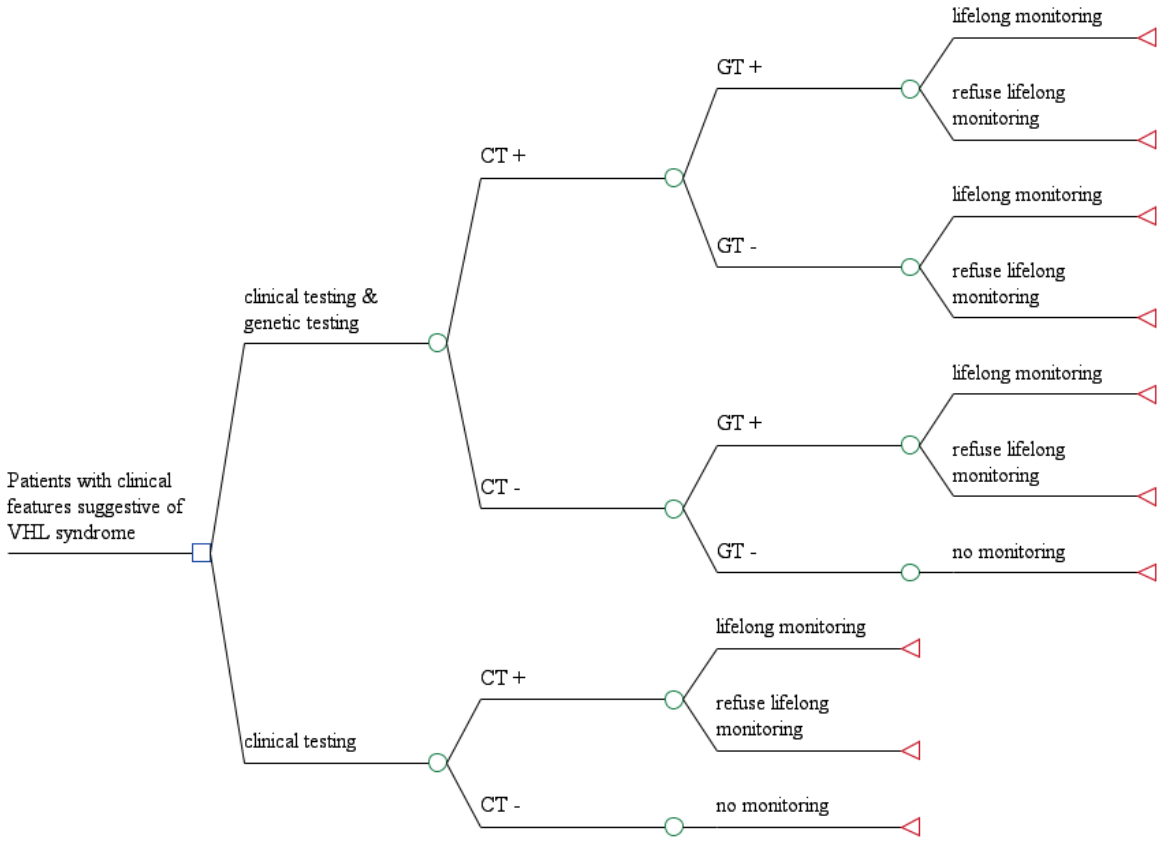


Figure 8 Determining the monitoring status of the individual patient suspected of having VHL syndrome

Developed in TreeAge Pro 2011
CT = clinical testing, GT = genetic testing

The modelling of the impact on first- and second-degree relatives is dependent upon the eventual clinical diagnosis of the individual presenting for investigation, and whether a genetic test was performed.

For individuals who are clinically diagnosed with VHL syndrome (Figure 9):

1. If the genetic test results are unknown, all first- and second-degree relatives are offered lifelong monitoring (a proportion will refuse).

2. However, if the genetic status is known, and the individual has an identifiable VHL mutation, family members are offered a genetic test. Those who accept the test will be offered lifelong monitoring if they are positive and will require no monitoring if they are negative. It is this outcome that will drive the cost savings of the genetic testing arm of the model. Those who refuse the genetic test will be offered lifelong monitoring as per current clinical practice. It is possible that the refusal of the test and subsequent refusal of monitoring may be correlated, but this has not been modelled. According to expert opinion, there will be patients who will accept monitoring but will refuse genetic testing.

3. Among patients who are clinically diagnosed with VHL syndrome for which there is no identifiable genetic mutation, genetic testing would not be offered to relatives. However, it is possible that the VHL syndrome is hereditary despite the lack of genetic diagnosis (ie mutation has yet to be identified), and relatives would still be offered lifelong monitoring. This could change in the near future as the sensitivity of genetic testing improves, such that patients who are clinically diagnosed with VHL syndrome who have no detectable genetic mutation may be considered clinical misdiagnoses, and monitoring of the individual or family may not be offered (expert opinion). This may further reduce the number of individuals and family members who are inappropriately being monitored, and benefit the genetic testing arm of the model.

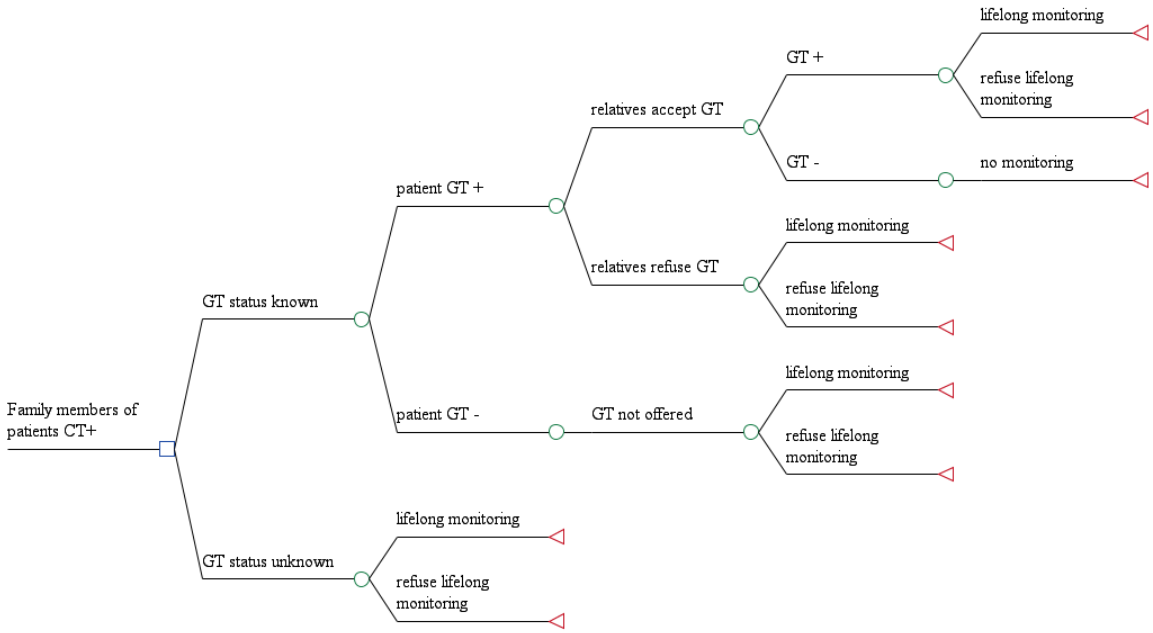


Figure 9 Determining the monitoring status of first- and second-degree relatives of an individual clinically diagnosed with VHL syndrome

Developed in TreeAge Pro 2011
CT = clinical testing, GT = genetic testing

For patients who are not clinically diagnosed with VHL syndrome (Figure 10):

1. If the genetic status of the individual is unknown, no relatives will be offered monitoring.

2. If the genetic status of the individual is negative, no relatives will be offered genetic testing or monitoring.

3. If the genetic status of the individual is positive, the relatives will be offered genetic testing, which they may accept or refuse, and monitoring (unless they are found to be genetically negative), which, again, they may accept or refuse.

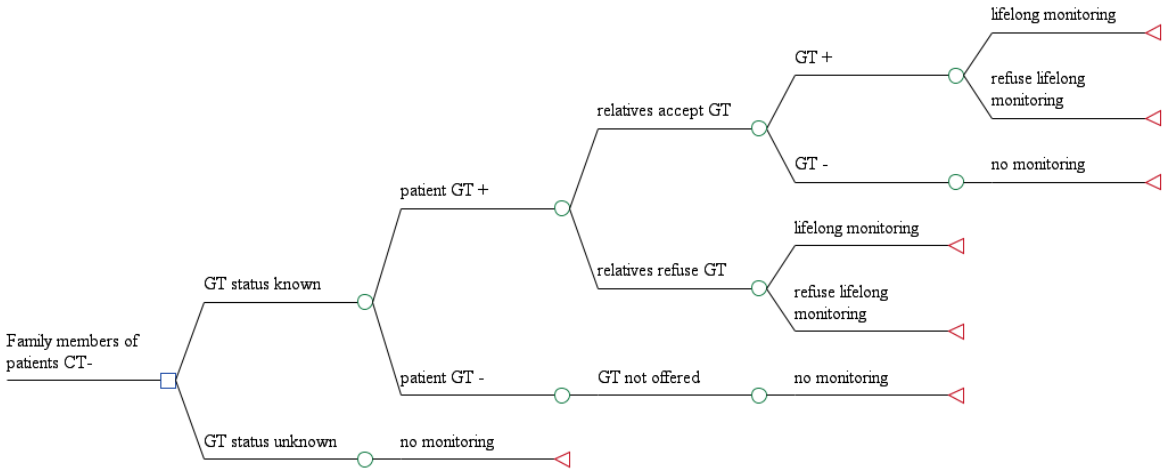


Figure 10 Determining the monitoring status of first- and second-degree relatives of an individual with suspected but not clinically diagnosed with VHL syndrome

Developed in TreeAge Pro 2011
CT = clinical testing, GT = genetic testing

Long-term model

Once monitoring status has been decided, both individuals and family members are entered into the long-term Markov model (a simplified schematic is shown in Figure 11). Patients and family members who accept monitoring and are carrying a VHL mutation will remain in a monitoring health state for life. Those who accept monitoring but who are not carrying a mutation will cease monitoring at the age of 70 years. Those who do not accept monitoring will remain in a non-monitoring state, although if they are carrying a VHL mutation, they may transit to a monitoring state if they become symptomatic. The likelihood of becoming symptomatic is based upon a transition probability estimated from a study involving VHL patients (Poulsen et al 2010).

Once in the long-term model, costs associated with monitoring are accrued while in the monitoring health state and no costs are accrued while in the no-monitoring health state. There may be additional costs associated with treatments for VHL symptoms arising in patients who are not being monitored; however, these patients will be roughly

equally prevalent in both arms of the model, and therefore costs have not been considered. The cycle length of the Markov model is 1 year. Some family members will be diagnosed or monitored from a very young age, and therefore the model time horizon is set at 100 years. The model was constructed in Microsoft Excel. A 5% discount rate was applied to costs.

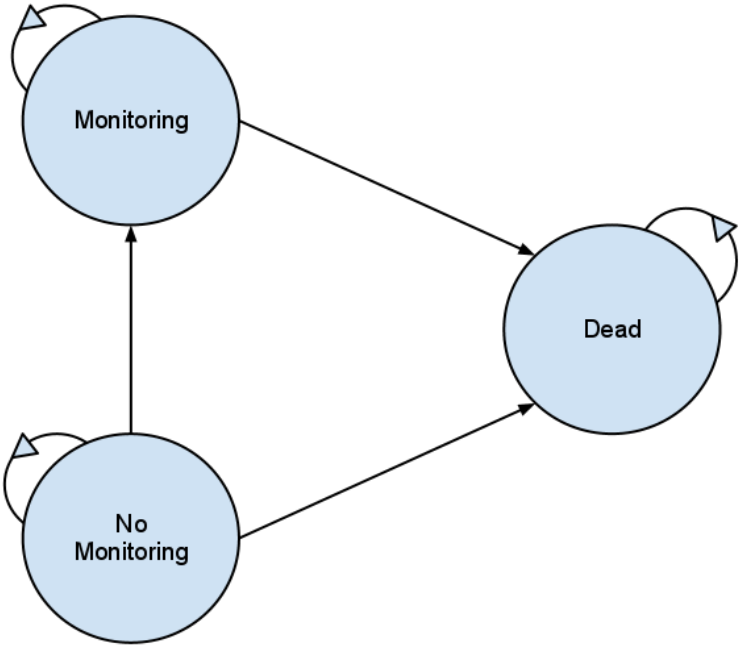


Figure 11 Long-term model capturing the monitoring costs of individuals suspected of having VHL syndrome, and their first- and second-degree relatives

Inputs to the economic evaluation

The inputs and transition probabilities for the economic evaluation were derived predominantly from a systematic review of the literature, although supplemented by expert opinion where necessary (Table 37, Table 38, and Table 39)

Table 37 Genetic test characteristics

	Estimate	Range	Source
Sensitivity (%)	89.8	70.0–100	Mean and range of data from systematic review (see Table 58)
Specificity (%)	95.8	50.0–100	Mean and range of data from systematic review (see Table 59)
Prevalence (%)	66.0	27.6–96.3	Mean and range of data from systematic review (see Table 60)

Table 38 Population characteristics

	Base case input	Alternative value	Source
Average age	26 years		Maher et al (1990)
Monitoring take-up among clinically diagnosed individuals	100%		Base: ideal
Monitoring take-up among asymptomatic family members	40%	100%	Base: Rasmussen et al 2010 Alternative: ideal
Genetic testing take-up in family members	60%	100%	Base: Rasmussen et al 2010 Alternative: ideal
Number of 1st- and 2nd-degree relatives (of patients with a known VHL mutation)	11.5	5	Pathology Services Table Committee 2010, supported with data from Garceau et al 2008 *average number of living relatives (1st- and 2nd-degree) = 12.49* Alternative: speculative
VHL mutation rate among relatives	26%		Rasmussen et al (2010), supported by data from Garceau et al 2008
Age (years) at which test negative individuals cease monitoring	70	40	Expert opinion (almost 100% penetrance by age 70 years, so compliance with monitoring may fall as patients get older). Alternative: speculative

Sources: Garceau et al (2008); Maher et al (1990); Pathology Services Table Committee (2010); Poulsen et al (2010); Rasmussen et al (2010)

Table 39 Transition probabilities used in the long term model

	From state	To state	Transition probability	Source / assumption
Test –ve	No monitoring	Monitoring	0	Patient will not become symptomatic, and therefore will never transit to a monitoring state
	No monitoring	Dead	Australian Life Tables	Same mortality risk as the general population
	Monitoring	Dead	Australian Life Tables	Same mortality risk as the general population
	Monitoring	No monitoring	<70 years = 0 70 years = 1	Expert opinion
Test +ve	No monitoring	Monitoring	0–19 years = 0.005 20–39 years = 0.05 40+ years = 0.1	Based on likelihood of becoming symptomatic (Poulsen et al 2010); see Figure 12
	No monitoring	Dead	Australian Life Tables	Patient who does not become symptomatic will have the same mortality as the general population
	Monitoring	Dead	Australian Life Tables	Patient who is monitored will have the same mortality as the general population

Source: Poulsen et al (2010)

The transition of patients or family members who do not have a genetically identifiable mutation from the no-monitoring to a monitoring health state is not possible in the model. In the case that a patient has a mutation in the *VHL* gene that cannot be detected by current genetic testing, this transition would remain possible. However, the literature states that the VHL mutation detection rate when using a combination of

genetic testing methods (Southern blot analysis, MLPA, gene sequencing) approaches 100%, and it is unclear whether patients who are clinically diagnosed with VHL syndrome yet have no detectable genetic mutation are misdiagnoses or have a mutation that has not yet been described. If the former, the assumption used in the model is that the patient and family members who decide against monitoring will not transit to a monitoring state as they have no chance of manifesting symptoms. Any patients with true VHL syndrome who cannot be genetically verified will exist in both arms of the model, and will be offered monitoring in both arms, and this simplification of the model will have no impact upon the final incremental costs reported. Genetically positive individuals or family members (with or without symptoms) who are not offered or refuse monitoring are likely to have a higher mortality rate than those who accept monitoring (Rasmussen et al 2010). This has been discussed earlier and is difficult to quantify; however, so as not to disadvantage genetic testing in the model, all patients who become symptomatic with VHL syndrome who are in a no-monitoring state are assumed to transit to a monitoring state.

The cost inputs used for the model are provided in Table 40 and Table 41.

Table 40 Costs associated with monitoring patients or family members with possible VHL syndrome

	Cost	Source	Frequency
0–4 years			
Eye/retinal exam	$82.30	MBS item 104	Annually
Annual total	*$82.30*		
5–14 years			
Eye/retinal exam	$82.30	MBS item 104	Annually
Physical/neurological assessment	$82.30	MBS item 104	Annually
Urine or blood sample test	$40.20	MBS item 66779	Annually
Annual total	*$204.80*		
15+ years			
Eye/retinal exam	$82.30	MBS item 104	Annually
Physical/neurological assessment	$82.30	MBS item 104	Annually
Urine or blood sample test	$40.20	MBS item 66779	Annually
Abdominal ultrasonography	Unit cost $111.30 cost per year $55.65	MBS item 55036	Every second year
MRI with gadolinium of brain and spine	Unit cost $492.80 cost per year $246.40	MBS item 63111	Every second year
CT scan of abdomen	Unit cost $360.00 cost per year $180.00	MBS item 56407	Every second year
Annual total	*$686.85*		

Note: Investigations required were determined on advice from the MESP.
MRI = magnetic resonance imaging; CT = clinical testing

Table 41 Costs associated with the genetic testing and genetic counselling of individuals or family members with possible VHL syndrome

	Cost	Source
Genetic counselling	$253.90	MBS item 132
Genetic testing of an individual	$600.00	From the protocol (proposed fee)
Genetic testing of family members	$340.00	From the protocol (proposed fee)

Model assumptions

Prevalence

The prevalence of VHL syndrome has been estimated from the studies in Table 17. Studies involving populations of patients suspected of having VHL syndrome or who present with CNS haemangioblastoma or retinal haemangioblastoma were analysed for the number of clinical diagnoses of VHL syndrome. Of 868 patients, 573 (66%) were clinically diagnosed with VHL syndrome. It is unclear how translatable these data are to the Australian setting; therefore, the prevalence of VHL syndrome among patients referred for further investigation has been varied in sensitivity analyses. The calculation of prevalence is presented in Table 60 (in Appendix I).

Average age

Because the model requires costing of lifelong monitoring, the average age of a patient when diagnosed is required, so as to cost the duration of monitoring. The average age at which VHL syndrome manifests is 26 years (Lonser et al 2003; Maher et al 1990). As life expectancy of patients with VHL syndrome is similar to that of the general population (Nordstrom-O'Brien et al 2010), life expectancy of both individuals and family members with a VHL mutation has been estimated using the 2007–09 Australian life tables (Australian Bureau of Statistics 2010a). There are no data on the average age of relatives and they are therefore assumed to be spread over the entire age range (this has been modelled by assuming that relatives' ages are distributed according to the Australian population pyramid (Australian Bureau of Statistics 2010b). Family members therefore have an average age of 37 years. While first- and second-degree relatives of an individual who is clinically diagnosed with VHL are likely to be of all ages, younger family members are more likely to be identified as having inherited the VHL gene, for the simple reason that older family members are likely to have already become symptomatic if they had a VHL mutation, and thus effectively be a 'patient'. Therefore, while genetic testing may be offered to all family members, it is much less likely that a VHL mutation will be found through familial cascade testing in patients older than 40 years of age (Figure 12). To represent this in the model is difficult, but it is likely that its effect would not be substantial and would simply represent a shift in the average age of those who are able to avoid monitoring.

Number of relatives

The average number of relatives of a patient who is suspected of having VHL disease is assumed to be 11.5 (Pathology Services Table Committee 2010). The majority of the costs associated with VHL disease will be monitoring in first- and second-degree relatives; therefore, this value has been varied across scenarios. This figure is supported by a US study in which the average number of living first- and second-degree relatives was 12.49 (Garceau et al 2008). This was roughly made up of 5 first-degree and 7.5 second-degree relatives.

Likelihood of an inherited VHL mutation in relatives

Rasmussen et al (2010) reported that 26% of tested family members had inherited the VHL mutation. This is consistent with the theoretical calculation of gene transmission. Based on 5 first-degree and 7.5 second-degree relatives (Garceau et al 2008), who have a 50% and 25% chance, respectively, of inheriting a germ-line VHL mutation, in 80% of cases (non *de novo* cases; Evans et al 2010) we would expect 3.5 relatives (28%) to have inherited it.

Genetic testing uptake

It has been assumed that all patients who are referred for investigation of VHL syndrome will accept genetic testing. There will obviously be a number who refuse, but this number is likely to be small. If patients do refuse genetic testing, then the costs and outcomes associated with genetic testing will be diluted.

It is likely, and has been modelled, that a proportion of family members who are offered genetic testing will refuse. In the base case, this is estimated to be 40% (Rasmussen et al 2010). Irrespective of whether they accept genetic testing or not, patients who are eligible for monitoring are offered monitoring. This means that, for patients who refuse genetic testing, lifelong monitoring will be offered as would ordinarily be done in the absence of genetic testing. It has been assumed that monitoring compliance in family members is unrelated to whether they accept or refuse genetic testing. While no comparative data of monitoring compliance was found, it is difficult to accept that family members who refuse genetic testing will agree to monitoring with the same compliance as those who are genetically confirmed as carrying a VHL mutation, although expert opinion supports that some patients will be happy to receive monitoring while refusing genetic testing. It is more likely that monitoring compliance would be greater among family members who would agree to genetic testing were it available, and are certain that they are carrying a VHL mutation.

Consequently, it is possible that, while genetic testing will help avoid lifelong monitoring among family members who are not VHL mutation carriers, it may also increase monitoring among family members who are definite carriers.

Increasing monitoring may have a positive impact on patient outcomes (Rasmussen et al 2010). The absence of evidence on whether genetic testing improves monitoring compliance, and the difficulty in extracting evidence of monitoring effectiveness for VHL patients who are yet to manifest VHL symptoms (rather than for VHL patients who have already been identified clinically), inhibits the assessment of benefits associated with improved monitoring as a consequence of genetic testing.

Lifelong monitoring compliance

Patients who are clinically diagnosed with VHL syndrome are assumed to accept and be compliant with monitoring. It is likely that some patients will be less compliant with monitoring and this is likely to vary over time; however, it is unclear how this non-compliance would affect the model. While non-compliance would result in cost savings by avoiding resource use for monitoring, it may result in greater expenditure and potential life-years lost associated with a delayed diagnosis of a VHL-related neoplasm. Furthermore, it is unclear whether genetic testing would impact upon compliance with VHL monitoring; therefore, it would be conservative to assume that compliance would be similar in both arms.

Currently, patients who are clinically diagnosed with VHL syndrome but who are not found to have the genetic mutation are offered lifelong monitoring. The families of these patients are also offered monitoring but not genetic testing.

All first- and second-degree relatives of patients who are clinically diagnosed with VHL syndrome will be offered lifelong monitoring, while those family members who are shown not to have inherited the VHL mutation are spared monitoring in the genetic testing arm. As previously mentioned, only 60% of family members will actually accept genetic testing, and all those who do not accept it will be offered monitoring. The base case of the model assumes that 40% of patients will be compliant with monitoring (Rasmussen et al 2010).

It is likely that, once an individual with a VHL mutation becomes symptomatic, compliance with monitoring will rise. For family members who initially refuse monitoring, it is possible that they will become symptomatic in time (if they are carrying the VHL mutation) and may commence monitoring. The rate at which they will return to monitoring has been estimated from Poulsen et al (2010). As in Figure 12, the likelihood of manifesting symptoms is not linear. Transition probabilities of 0.5% between 0 and 19 years of age, 5% between 20 and 39 years of age and 10% after the age of 40 years have been used to estimate the likelihood of manifesting symptoms, according to Poulsen et al (2010). These transition probabilities have been applied to family members with the VHL mutation who initially refuse monitoring but will transition to the monitoring health state.

In family members who accept monitoring but do not have a VHL mutation, it is assumed that they will eventually cease monitoring at age 70 years (expert opinion). It is unlikely that family members would continue to be compliant with monitoring if they have not manifested symptoms of VHL disease by then.

The data used for genetic testing uptake and monitoring compliance is derived from VHL families in Mexico (Rasmussen et al 2010); however, it is unclear whether these data are transferrable to the Australian setting. Improved genetic counselling and government reimbursed health care may increase these rates, so an ideal scenario of 100% testing uptake and 100% monitoring compliance has been presented.

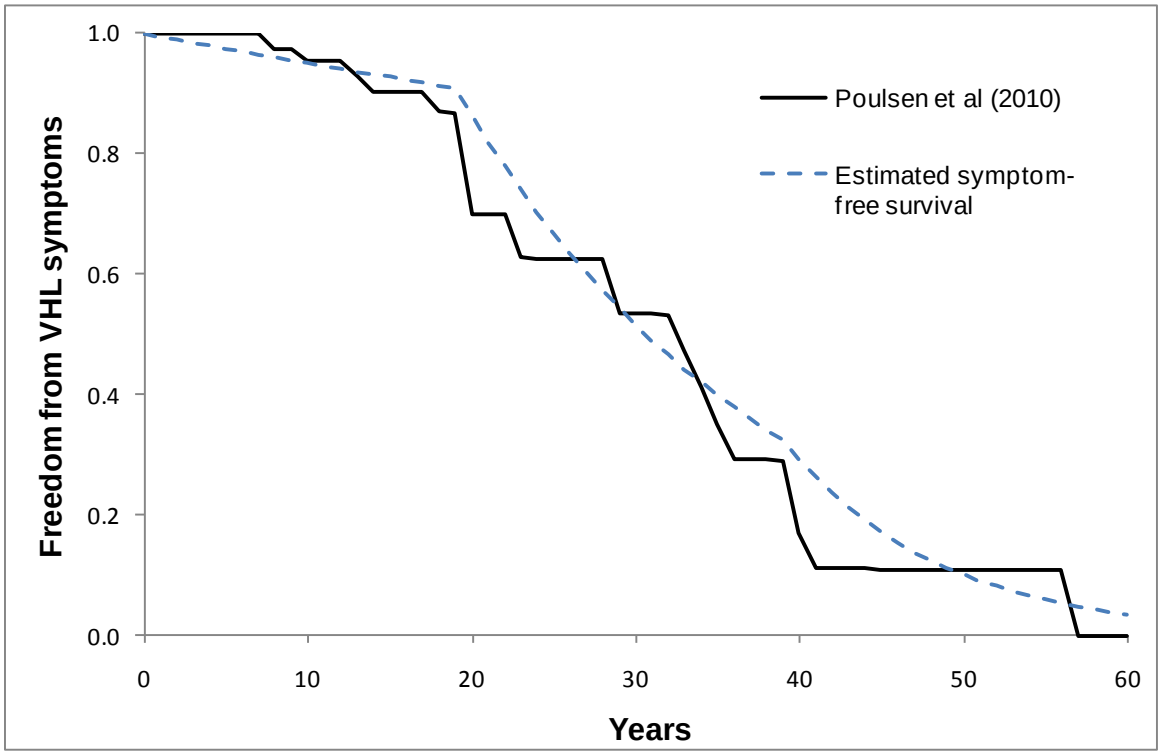


Figure 12 **Modelled transition of asymptomatic patients with a VHL mutation to symptomatic based upon the observed freedom from VHL-related symptoms reported by Poulsen et al (2010)**

Results

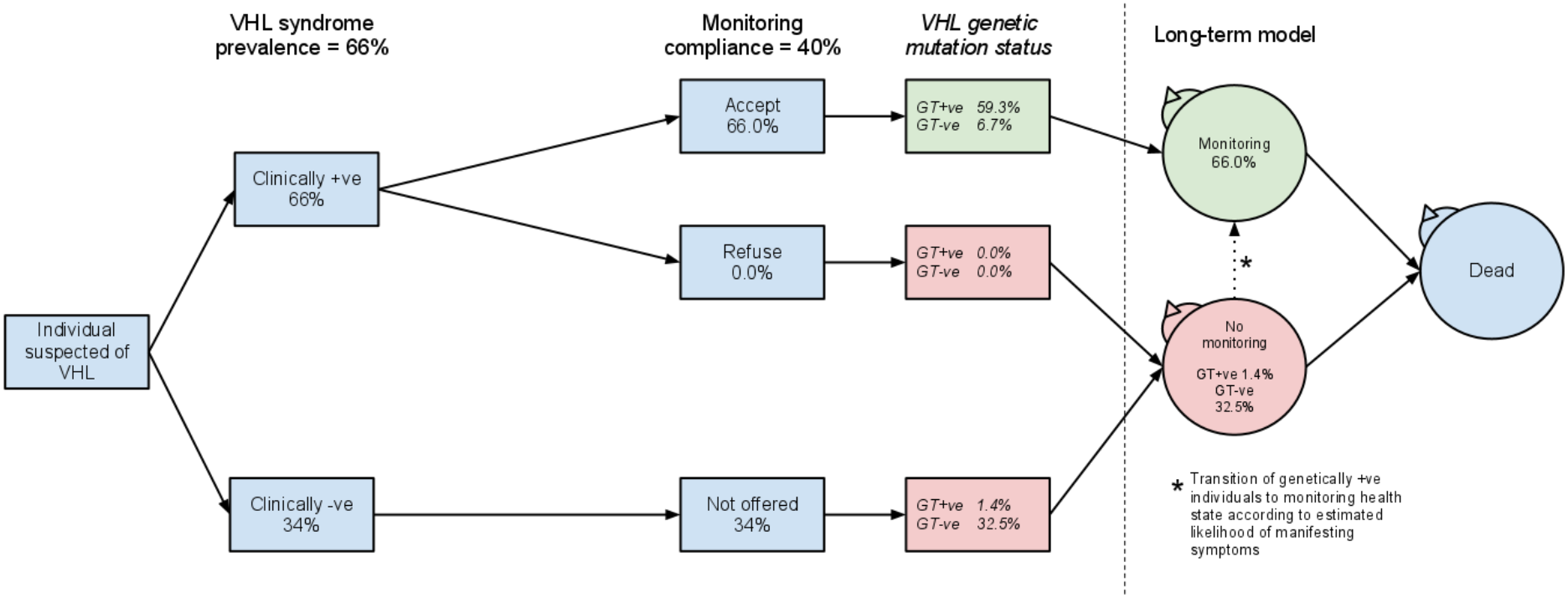
Due to the complexity of the model, schematics showing the delivery of individuals and family members to the long-term Markov model have been provided. The numbers in these schematics are derived from the base case.

Individuals suspected of having VHL syndrome

As a consequence of the high correlation between the genetic test and clinical diagnosis, there is very little difference between the proportions of individuals who receive monitoring for VHL syndrome whether a genetic test to detect VHL mutations is available or not. This is reflected in very similar costs between the two arms of the model for

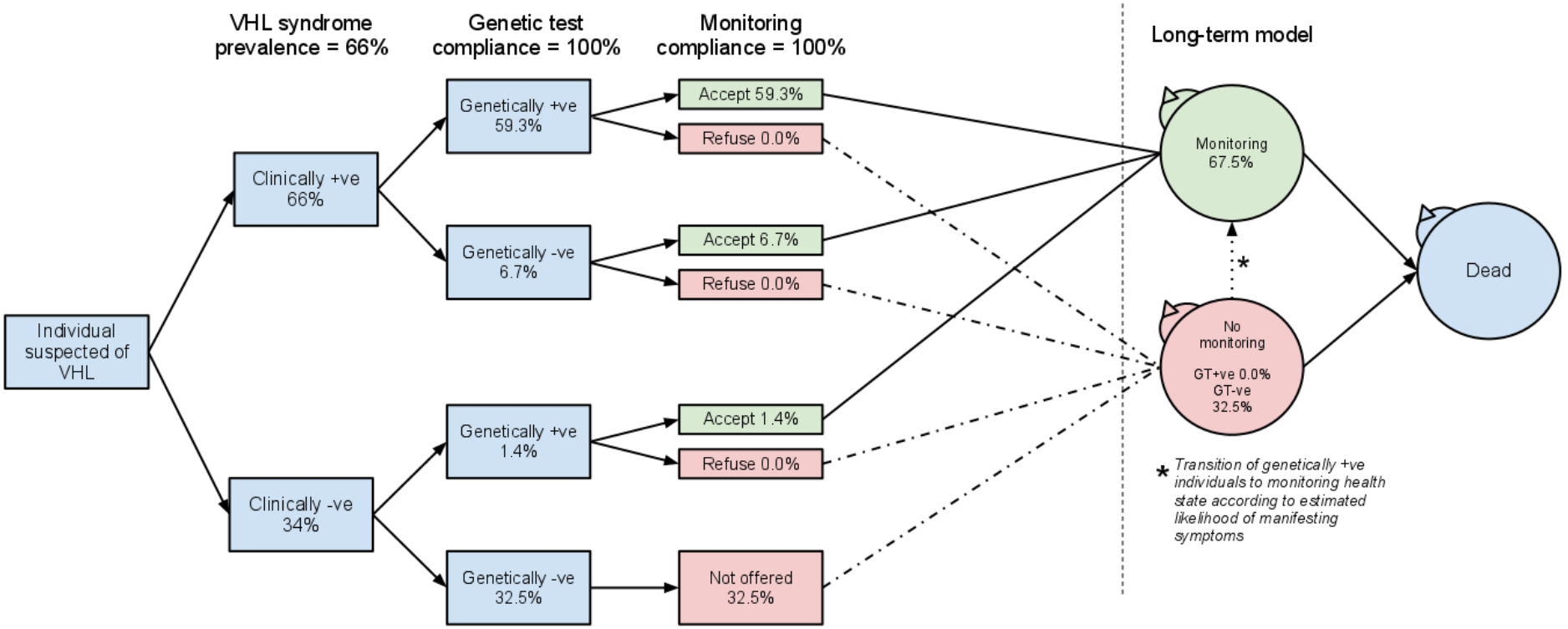
individual patients. There is a small proportion of patients (1.4%) who will be diagnosed positive with the genetic test that would be missed with clinical testing alone (Figure 13 and Figure 14); however, the model assumes that more than 98% of these will become symptomatic before dying, and so spend some time in the monitoring health state. As no consequence of delayed monitoring has been costed, the removal of these patients from monitoring results in a small incremental saving in the clinical-testing-only arm. This is less than $100 in discounted costs and is inconsequential to the model.

Figure 13 Delivery of individuals suspected of having VHL syndrome to the long-term model in the absence of genetic testing



+ve = positive, -ve = negative, GT = outcome of the genetic test for the *VHL* gene (note: in models with clinical testing only this outcome is unknown and is represented in italics, however the genetic mutation status will inform the appropriateness of monitoring in the clinical and genetic arms of the models)

Figure 14 Delivery of individuals suspected of having VHL syndrome to the long term model with genetic testing



+ve = positive, -ve = negative, GT = outcome of the genetic test for the *VHL* gene (note: in models with clinical testing only this outcome is unknown and is represented in italics, however the genetic mutation status will inform the appropriateness of monitoring in the clinical and genetic arms of the models)

Family members of patients suspected of having VHL syndrome

Among family members, the impact of introducing genetic testing is clear. Once again, a small proportion of family members will be diagnosed that would not ordinarily be diagnosed with clinical testing alone (0.4%). However, this proportion is overshadowed by the number of patients in the no-monitoring health state that arrive there because they refuse monitoring; this is approximately equal between the arms. The only clear difference between the two arms is the proportion of patients who are being monitored. In the clinical-diagnosis-only arm, 26.4% of family members are in the monitoring state (Figure 15), compared with 16.2% in the genetic testing arm (Figure 16). This reduction in monitoring rate is achieved without compromising the overall proportion of VHL mutation positive patients receiving monitoring. In both arms approximately 9.5% of patients who are VHL mutation positive begin in the no-monitoring health state.

These percentages are perhaps difficult to interpret because a proportion of families (32.5%) are related to individuals who are not clinically diagnosed with VHL syndrome or who do not have a VHL mutation in the model, and therefore would be inappropriate to monitor irrespective of the introduction of genetic testing. It may be more informative to compare the proportion of patients who are receiving monitoring that should be receiving monitoring, and the proportion who are being inappropriately monitored. This is presented in Table 42.

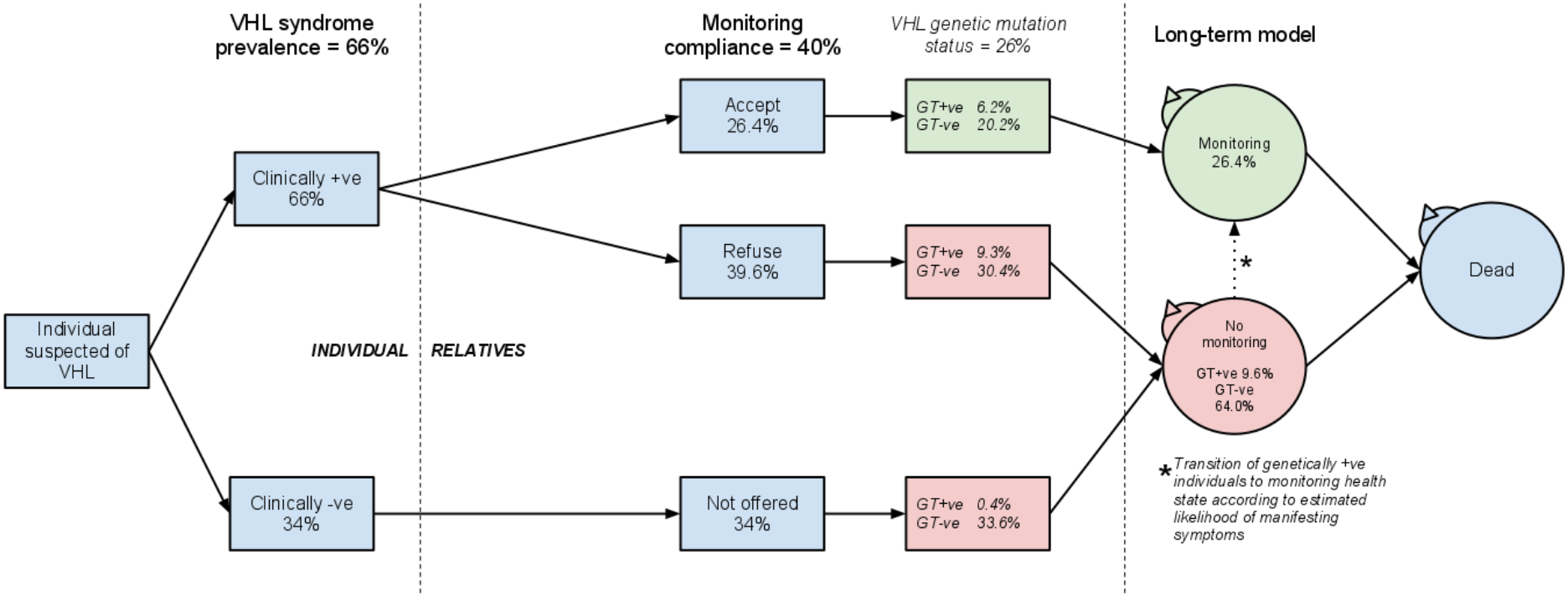
Table 42 Appropriateness of monitoring in individuals and families with and without VHL genetic testing

	Clinical only	Clinical and genetic	*Difference*
Individuals			
Proportion of those who should receive monitoring who are being monitored[a]	97.9%	100%	*2.1%*
Proportion receiving monitoring who should not be receiving monitoring[b]	0%	0%	*0%*
Families			
Proportion of those who should receive monitoring who are being monitored[a]	39.3%	40.0%	*0.7%*
Proportion receiving monitoring who should not be receiving monitoring[b]	66.5%	44.4%	*–22.1%*

[a] Individuals who are clinically or genetically positive—family members who are genetically positive or are relatives of individuals who were clinically diagnosed but without identifying a VHL mutation

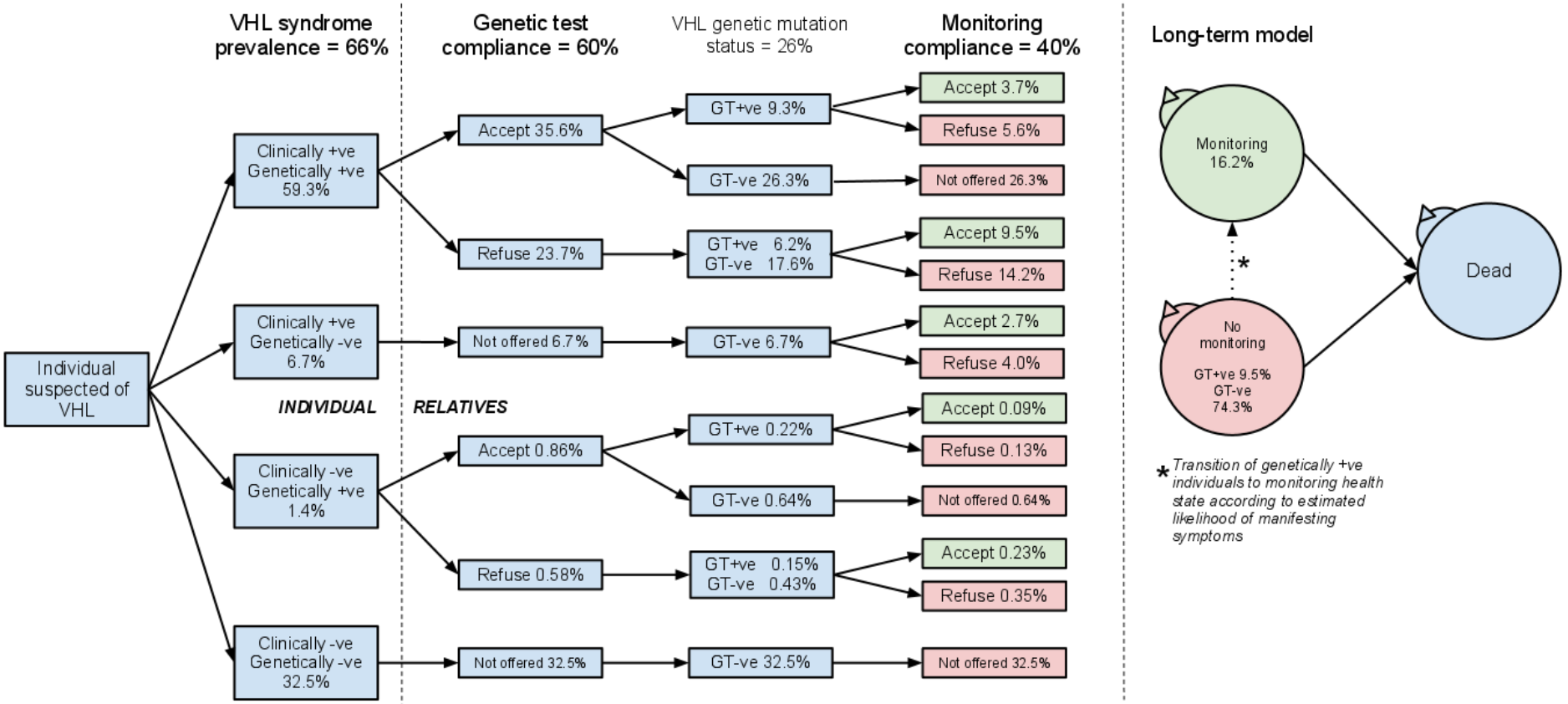
[b] Individuals who are genetically and clinically negative—family members of genetically and clinically negative individuals, and genetically negative family members of genetically positive individuals

Figure 15 Delivery of first and second degree relatives of individuals suspected of having VHL syndrome to the long term model in the absence of genetic testing



+ve = positive, -ve = negative, GT = outcome of the genetic test for the *VHL* gene (note: in models with clinical testing only this outcome is unknown and is represented in italics, however the genetic mutation status will inform the appropriateness of monitoring in the clinical and genetic arms of the models)

Figure 16 Delivery of first and second degree relatives of individuals suspected of having VHL syndrome to the long term model with genetic testing



+ve = positive, -ve = negative, GT = outcome of the genetic test for the *VHL* gene (note: in models with clinical testing only this outcome is unknown and is represented in italics, however the genetic mutation status will inform the appropriateness of monitoring in the clinical and genetic arms of the models)

Long-term model

As presented in Figure 13 and Figure 14, the introduction of genetic testing increases initial monitoring in individuals suspected of VHL syndrome by about 1.4%. This is due to the fact that clinical testing only misses a very small number of patients who have a *VHL* gene mutation. Furthermore, it has been modelled that those patients who are 'missed' by clinical diagnosis alone will eventually be monitored once they manifest symptoms. Consequently, the incremental cost of introducing genetic testing for individuals is primarily due to the cost of the genetic test and genetic counselling. As these costs occur prior to any discounting in the model, there is little difference in the overall incremental cost with ($949) or without ($1,001) discounting, as reported in Table 43.

Table 43 Undiscounted and discounted costs of the management of individuals suspected of having VHL syndrome and their families using clinical testing alone compared with genetic testing and clinical testing

Undiscounted Costs	Patients			Families		
	Clinical Only	Clinical and genetic	*Difference*	Clinical Only	Clinical and genetic	*Difference*
Monitoring	$25,532	$25,679	*$147*	$95,760	$70,777	*–$24,983*
Genetic testing	$0	$600	*$600*	$0	$1,425	*$1,425*
Genetic counselling	$0	$254	*$254*	$0	$1,774	*$1,774*
Total	$25,532	$26,533	*$1,001*	$95,760	$73,975	*–$21,784*

Discounted Costs	Patients			Families		
	Clinical Only	Clinical and genetic	*Difference*	Clinical Only	Clinical and genetic	*Difference*
Monitoring	$8,805	$8,900	*$95*	$38,836	$26,940	*–$11,896*
Genetic testing	$0	$600	*$600*	$0	$1,425	*$1,425*
Genetic counselling	$0	$254	*$254*	$0	$1,774	*$1,774*
Total	$8,805	$9,754	*$949*	$38,836	$30,138	*–$8,697*

	Combined undiscounted			Combined discounted		
	Clinical Only	Clinical and genetic	*Difference*	Clinical Only	Clinical and genetic	*Difference*
Total	$121,292	$100,509	*–$20,783*	$47,641	$39,892	*–$7,749*

The incremental cost of managing family members of patients suspected of having VHL syndrome is substantial. As reported in Table 42, there is very little difference between the two arms in the proportion of family members who should be monitored and who are actually monitored. However, 66.5% of family members who are receiving monitoring have a negligible risk (equal to whole-population risk) of manifesting with VHL-related neoplasms when only clinical testing is available, compared with 44.4% when genetic and clinical testing is available. Genetic testing and genetic counselling

will, on average, add an additional cost of $1,425 and $1,774 per family of an individual suspected of having VHL syndrome. However, the discounted savings associated with avoiding the monitoring in family members who are not at risk of developing VHL syndrome is approximately $11,896.

The overall incremental discounted cost of clinical testing with genetic testing compared with clinical testing alone is $7,749, favouring the genetic testing arm. Importantly, this is based upon a modest uptake of genetic testing (60%) and monitoring (40%) among family members. As increases in testing or monitoring will increase the incremental savings of genetic testing, this may represent a conservative estimate.

The estimation of incremental cost of genetic testing with clinical testing compared with clinical testing alone is sensitive to the following variables:

- sensitivity and specificity of the genetic test
- prevalence of the VHL mutation among patients suspected of having VHL syndrome
- uptake of genetic testing among family members
- uptake of monitoring among family members
- cost of monitoring
- number of relatives (at risk of developing VHL syndrome).

The incremental cost of genetic testing with clinical testing compared with clinical testing alone has been recalculated in sensitivity analyses (Table 44).

Table 44 Combined costs of the management of individuals suspected of having VHL syndrome and their families using clinical testing alone, compared with genetic testing with clinical testing using alternative inputs

	Combined undiscounted			Combined discounted		
	Clinical Only	Clinical and genetic	*Difference*	Clinical Only	Clinical and genetic	*Difference*
Base case	$121,292	$100,509	*–$20,783*	$47,641	$39,892	*–$7,749*
Lowest estimate for sensitivity (70%) and specificity of the test (50%)	$128,148	$120,718	*–$7,430*	$49,843	$49,008	*–$835*
Lowest estimate of prevalence (27.6%)	$53,056	$46,201	*–$6,855*	$20,554	$18,593	–$1,962
All genetically negative family members who agree to monitoring cease monitoring at age 40 years	$80,434	$79,899	*–$536*	$28,694	$30,461	$1,768
Lower number of at-risk relatives to be tested (5)	$67,167	$58,697	*–$8,470*	$25,691	$22,858	–$2,833
Genetic testing is accepted by 100%	$121,292	$84,020	*–$37,272*	$47,641	$32,525	–$15,116
Monitoring among those offered increases to 100%	$203,637	$145,379	*–$58,258*	$88,137	$62,544	–$25,593
Cost of monitoring doubles	$242,584	$196,965	*–$45,619*	$95,282	$75,732	–$19,550

The results of the sensitivity analyses reveal that, in most situations, the added costs associated with genetic testing are offset by the costs of inappropriate monitoring of family members that are avoided. Therefore, genetic testing for a mutation in the *VHL* gene will most likely result in a cost saving.

Financial analysis

Likely number of genetic tests per year

As the result is definitive, VHL genetic testing would only need to be performed once for each patient, using duplicate sampling as recommended by the RCPA in their 2007 position statement titled *Sample requirements for medical genetic testing: do genetic tests demand a different standard?* (Royal College of Pathologists of Australasia 2007).

The lack of an Australian registry for patients with VHL syndrome and their family members means that the number of patients with VHL syndrome and the number of VHL mutation carriers in Australia are unknown. A recent study estimated the prevalence, birth incidence and *de novo* mutation rate in the UK from a UK family genetic register service (Evans et al 2010). If we assume the same rates occur in Australia, approximately 1/91,000 people have a germ-line VHL mutation, with a birth incidence of 1/42,000 births and a *de novo* mutation rate of 21%.

Based on Australia's current (2011) population of 22,683,000 and an estimated prevalence of 1/91,000, there are approximately 249 people with VHL syndrome in Australia. If we assume that there are currently 300,000 live births per year in Australia and a birth incidence of 1/42,000, there are 7 babies born with a VHL mutation per year. If we assume there are 200,000–300,000 immigrants per year, with a prevalence of 1/91,000, there are 2–3 immigrants with a VHL mutation entering the country per year.

It is not possible to work out if there is a large backlog of patients requiring VHL genetic testing should the test be listed on the MBS. Assuming constant usage of the test since 2007, about 480 diagnostic VHL tests have been conducted to date. How many of these tests were conducted on patients with a clinical diagnosis of VHL syndrome and how many tested positive is unknown. Based on unpublished South Australian data, it is estimated that approximately 25% of the tests may have been positive, thus indicating that only half of the patients with VHL syndrome have been tested.

However, from the birth and immigration data above, we expect at least 10 new cases per year, with 8 being familial and 2 *de novo* cases (21%). If we assume that 3 of the familial cases are immigrants, we have 5 'new' cases per year that will present with symptoms and require diagnostic VHL genetic testing. We also have 5 familial cases that would require predictive testing to determine if the VHL mutation has been inherited.

If we take into account the prevalence data presented in Table 17 in the 'Results of assessment' section, we expect 2 of the 5 'new' VHL cases to present with a CNS haemangioblastoma, 2 with a retinal haemangioblastoma, and 1 with another neoplasm such as phaeochromocytoma or renal cell carcinoma. It is thought that 20–30% of CNS haemangioblastomas are due to VHL syndrome, and therefore 10 CNS haemangioblastoma cases must be tested to get 2 that have a VHL mutation. Sporadic retinal haemangioblastomas occur less frequently in younger patients; therefore, in the best case scenario we may expect only the 2 VHL mutation carriers to be referred for genetic testing. A patient referred with phaeochromocytoma has a 10% probability of having a VHL mutation (Erlic et al 2009), so 10 patients would need to be tested to identify the VHL mutation carrier; and, currently, few patients presenting with only renal cell carcinoma have a germ-line VHL mutation. Thus, we would expect to test at least 22 patients to identify the 5 new cases, a yield of 23% positive test results.

If the apparent availability of the VHL genetic test improves awareness among specialists, referrals for genetic testing may increase. It is also possible that all or most patients presenting with a single VHL-related neoplasm may be tested in the future. One study suggests that only 4% of patients presenting with a single CNS haemangioblastoma and no other symptoms have a germ-line VHL mutation (Woodward et al 2007). This could substantially increase the number of CNS haemangioblastoma

patients that are tested for a VHL mutation per year. According to Neumann et al (1998), only 2% of patients with a renal cell carcinoma had a germ-line VHL mutation, also potentially increasing the number of tests that could be undertaken each year.

Only 49 relatives were tested for a VHL mutation in 2006 and 2007, and it is impossible to predict how many relatives in Australia have been tested to date. It is likely that many at-risk relatives have not yet been tested, potentially creating a backlog for predictive VHL genetic testing. However, in the long term, there will be 5 familial first-degree relatives born with a VHL mutation, which suggests that at least 10 first-degree relatives will require testing every year to obtain 5 positive results. In addition, the 2 *de novo* cases and the 3 new cases occurring in immigrants would have first-degree relatives requiring testing. Immigrants are unlikely to have a large number of family members in Australia, and only immediate family members of *de novo* cases require testing. Thus, we can assume 3 relatives per index case, suggesting that, in total, there would be a maximum of 25 relatives requiring predictive VHL genetic testing each year. However, as only 66% are expected to agree to testing, according to a study by Rasmussen (2010), approximately 17 of these relatives will actually receive VHL genetic testing.

It is unlikely that the increase in genetic testing will result in a substantial increase in the number of patients diagnosed with a VHL mutation. It is more likely that the increased use of the test will merely result in a reduced yield. Due to the lack of data on the likely future use of the genetic test, it has been assumed that there will be a doubling of usage over the next 5 years (Table 45) following a listing on the MBS. It is important to recognise that this level of usage would result in a far lower yield (< 10%) than was reported in the literature, and therefore may reflect a very conservative approach. The use of predictive genetic testing, however, will remain unchanged as this is related to the number of new diagnoses of VHL syndrome. In 2006 and 2007, 20 and 29 predictive tests, respectively, were performed. It has been assumed that 30 tests per year are performed for the financial impact analysis.

Table 45 Number of genetic tests, VHL mutation positive diagnoses and patients avoiding monitoring over the next 5 years

Estimated number of events per year	2012	2013	2014	2015	2016
Diagnostic tests	80	100	120	140	160
Predictive tests	30	30	30	30	30
New VHL mutation positive diagnoses	10	10	10	10	10
Cumulative monitoring avoided:[a]					
50% reduction in monitoring	10	20	30	40	50
75% reduction in monitoring	30	60	90	120	150

[a] It is assumed that all patients who are diagnosed with a positive VHL mutation will receive monitoring, and that monitoring will be reduced in suspicious cases that have been ruled out for a VHL mutation.

Unit costs

The costs considered in this financial impact analysis are those of genetic testing (diagnostic and predictive), genetic counselling and monitoring. The cost of monitoring will be different depending upon the age of the patient who is being monitored and, for simplicity, all patients are assumed to be adults. It is important to note that the cost of monitoring would be incurred in the absence of genetic testing and is not a consequence of the introduction of genetic testing. Furthermore, due to the ability to confidently rule out patients suspected of having VHL syndrome and family members of patients with a VHL mutation, a proportion of patients will be able to avoid monitoring that would not be able to do so were the test unavailable.

These costs have previously been outlined in Table 40 and Table 41. They are summarised in Table 46.

Table 46 Unit costs associated with the introduction of genetic testing for VHL syndrome

	Cost	Source
Genetic testing of an individual	$600.00	From the protocol (proposed fee)
Genetic testing of family members	$340.00	From the protocol (proposed fee)
Genetic counselling	$253.90	MBS item 132
Annual monitoring costs of adults with suspected VHL syndrome	$686.85	MBS items listed in Appendix C

The costs associated with the introduction of genetic testing are difficult to represent. The financial impact has been estimated on new VHL cases only; therefore, the number of identified VHL patients will accumulate over the time period considered (Table 45). Additionally, the number of patients able to avoid monitoring (due to the absence of a VHL mutation) will also be cumulative, and savings from avoided monitoring will therefore be modest at first and increase over time. Both a 50% and a 75% reduction in monitoring have been considered (Table 49). Little or no reduction in monitoring among individuals suspected of having VHL syndrome was considered in the cost comparison because there was little difference between the likelihood of clinical or genetic diagnoses found in the literature. However, it is likely that the numbers suspected to have VHL syndrome are greater in Australian clinical practice, and therefore the number who may receive some form of monitoring may be increased. This remains an area of uncertainty.

Unit costs separated by payer

The setting in which the genetic testing and monitoring is undertaken will determine who is responsible for the cost (Table 47). If the genetic test is listed on the MBS, there may be an increase in referrals from the private health system. It has been assumed that 25% of services will be performed in the private healthcare system, and in these cases the patient, or private insurance, will reimburse the proportion of the costs not

covered by the MBS. In the public health system it is assumed that the state/territory governments, rather than patients, will cover the costs not borne by the MBS. For genetic counselling, 75% of the fee is assumed to be reimbursed by the MBS in both the private and public sectors, with the patient and the state/territory government covering the 25% gap. However, while it is likely that the specialist (clinical geneticist) will provide genetic counselling in private, it is unclear how prevalent this may be in the public sector, where genetic counsellors (who are not medical specialists) are employed. It is likely that a proportion of patients will be provided genetic counselling in the public sector and the state/territory government will absorb the costs, at a saving to the MBS. Due to the inability of genetic counsellors to receive payment from the MBS (and are therefore funded by the state/territory governments), there may be an incentive to use specialists for genetic counselling over genetic counsellors, despite the higher cost associated with the service.

Table 47 Unit costs for genetic tests, counselling and annual monitoring separated by MBS, other government or patient

Based on 75% of services delivered in the public sector	Cost	MBS	Other government	Patient/ insurer
Genetic testing of an individual	$600.00	$450.00	$112.50	$37.50
Genetic testing of family members	$340.00	$255.00	$63.75	$21.25
Genetic counselling	$253.90	$190.43	$47.61	$15.87
Annual monitoring costs of adults with suspected VHL syndrome	$686.85	$515.14	$128.78	$42.93

Total cost to the Australian healthcare system overall

Total healthcare costs incorporate all direct costs associated with the introduction of genetic testing for a VHL mutation. These costs are assumed to be for testing, counselling and monitoring of patients (Table 48). Patients will be monitored whether VHL genetic testing is listed on the MBS or not, and a proportion of patients will not be offered monitoring following genetic testing who may have been monitored without genetic testing. The costs associated with monitoring are therefore costs avoided or savings.

Table 48 Expected number of diagnostic and predictive tests, and annual cost of testing and counselling for patients suspected of having VHL syndrome and their family members

Costs associated with the introduction of genetic testing for suspected VHL syndrome	2012	2013	2014	2015	2016
Diagnostic testing (n)	80	100	120	140	160
Predictive testing (n)	30	30	30	30	30
Diagnostic genetic testing	$48,000	$60,000	$72,000	$84,000	$96,000
Predictive genetic testing	$10,200	$10,200	$10,200	$10,200	$10,200
Genetic counselling	$27,929	$33,007	$38,085	$43,163	$48,241
Total	$86,129	$103,207	$120,285	$137,363	$154,441

Based upon 80 diagnostic tests, increasing linearly to 160 in 2016, and 30 predictive tests and genetic counselling for all persons requiring tests, the overall cost of genetic testing to the Australian healthcare system ranges from $86,129 in 2012 to $154,441 in 2016. The increase in cost is largely driven by the increase in diagnostic testing that may occur if case finding among patients with symptoms suspicious for VHL syndrome becomes more frequent. If the test becomes available on the MBS, the threshold to send someone for a test may fall, and the number of tests may therefore increase. There is, however, no evidence that the incidence of VHL syndrome should increase, hence the steady number of predictive tests of family members.

It is unclear what proportion of patients would avoid monitoring if the genetic test becomes available (Table 49). The economic evaluation base case assumed a 40% reduction in monitoring among family members on the basis of a negative mutation test, but did not assume any reduction in monitoring of individuals who were suspected of having VHL syndrome.

Table 49 Costs avoided due to reductions in monitoring following the introduction of VHL genetic testing

Costs associated with monitoring following the introduction of genetic testing for suspected VHL syndrome	2012	2012	2012	2012	2012
Number avoiding monitoring:					
50%	10	20	30	40	50
75%	30	60	90	120	150
Costs of monitoring averted:					
50%	$6,869	$13,737	$20,606	$27,474	$34,343
75%	$20,606	$41,211	$61,817	$82,422	$103,028

Costs to the MBS

The MBS is responsible for 75% of the cost of procedures undertaken on patients in a hospital setting who are not admitted. Currently, genetic services and counselling are

likely to be located in large teaching hospitals, and it has therefore been assumed that patients will continue to receive genetic testing and counselling through these services (Table 50). Monitoring for VHL syndrome may occur outside of the hospital setting but, for ease of calculation, it has been assumed that these services are provided within a hospital setting. This assumption will have only a minor impact upon the cost to the MBS.

Table 50 Annual cost of VHL genetic testing and counselling, and expected cost savings associated with reductions in monitoring

Testing and counselling	2012	2013	2014	2015	2016
MBS	$64,597	$77,405	$90,214	$103,022	$115,831
Other government	$16,149	$19,351	$22,553	$25,756	$28,958
Patient/insurer	$5,383	$6,450	$7,518	$8,585	$9,653
Offset monitoring 50%					
MBS	–$5,151	–$10,303	–$15,454	–$20,606	–$25,757
Other government	–$1,288	–$2,576	–$3,864	–$5,151	–$6,439
Patient/insurer	–$429	–$859	–$1,288	–$1,717	–$2,146
Offset monitoring 75%					
MBS	–$15,454	–$30,908	–$46,362	–$61,817	–$77,271
Other government	–$3,864	–$7,727	–$11,591	–$15,454	–$19,318
Patient/insurer	–$1,288	–$2,576	–$3,864	–$5,151	–$6,439
Total costs (50% reduction in monitoring)					
MBS	$59,445	$67,103	$74,760	$82,417	$90,074
Other government	$14,861	$16,776	$18,690	$20,604	$22,518
Patient/insurer	$4,954	$5,592	$6,230	$6,868	$7,506
Total	**$79,261**	**$89,470**	**$99,680**	**$109,889**	**$120,099**
Total costs (75% reduction in monitoring)					
MBS	$49,143	$46,497	$43,851	$41,206	$38,560
Other government	$12,286	$11,624	$10,963	$10,301	$9,640
Patient/insurer	$4,095	$3,875	$3,654	$3,434	$3,213
Total	**$65,524**	**$61,996**	**$58,469**	**$54,941**	**$51,414**

If considering the introduction of genetic testing separately from the cost incurred (and saved) by monitoring of patients with VHL syndrome, it is expected that the MBS will absorb costs between $65,000 (based on current test usage) and $116,000 (based on a doubling of current usage for diagnostic tests). With the introduction of genetic testing, 10 patients with a VHL mutation will require monitoring each year; however, some patients will avoid monitoring who are found to not be carrying a VHL mutation. If genetic testing results in a reduction of 50% in monitoring, 10 patients would have avoided monitoring, resulting in a saving to the MBS of about $5,000 in the first year of genetic testing and over $25,000 by 2016 (at which time 50 patients would be avoiding monitoring each year). Clearly, within a short period of time, the cost savings of

monitoring would far exceed the costs associated with VHL genetic testing, even if the use of the diagnostic test were to double (Table 51).

Table 51 Expected cost of VHL genetic testing over 5 years if listed on the MBS

Total expenditure over the next 5 years (2012–16)	50% reduction in monitoring	75% reduction in monitoring
MBS	$373,798	$219,257
Other government	$93,450	$54,814
Patient/insurer	$31,150	$18,271
TOTAL	$498,398	$292,343

If genetic testing for a mutation in the *VHL* gene is listed on the MBS, it is expected that it will cost the Australian healthcare system up to $500,000 over the next 5 years, with the MBS responsible for about $380,000. It is important to consider that much of this expenditure will also occur in the absence of MBS listing of the VHL genetic test, with state/territory governments or individual patients paying for the test. Listing on the MBS may increase the use of the VHL genetic test; however, this increase in usage may be appropriate and offset by the clinical investigations that may have taken place in its stead.

Discussion

Safety

Although the likelihood of adverse events as a consequence of VHL genetic testing is low, it is recognised that there are some risks associated with taking a peripheral blood sample. Venepuncture can lead to bruising, pain, nerve damage and arterial puncture (Lavery & Ingram 2005).

Even though there were no reports in the literature of adverse events arising from the genetic testing procedure, it can potentially cause psychological harms such as anxiety while awaiting results. While a negative result may have a positive impact and offer peace of mind to the individual, a positive test result may cause psychological harms that require treatment, such as increased anxiety and depression (Trepanier et al 2004; Levy & Richard 2000). A positive test result may even lead to life modifications, including reproductive intentions (Levy & Richard 2000).

False negative or false positive test results may also cause psychological harms, and possibly physical harms, due to delayed or inappropriate treatment. However, in the case of VHL genetic testing, false positives are unlikely to be an issue as these patients are probably true carriers of a VHL mutation, but a positive clinical diagnosis (an imperfect reference standard) could not be given. The small number of patients with false negative test results (when dual testing methodologies to detect VHL mutations are used) will still receive annual screening when they transition to symptomatic status, which will minimise any potential harms.

For these reasons, it is recommended that testing should only be performed after appropriate genetic counselling has been provided to the patient by a genetic counselling service or by a clinical geneticist on referral, with further counselling as necessary upon receipt of the test results.

Effectiveness

No direct evidence was identified that compared health outcomes following a clinical diagnosis and VHL genetic testing in patients suspected of having VHL syndrome with health outcomes following a clinical diagnosis of VHL syndrome alone. Direct evidence was also not identified to assess the effectiveness of VHL genetic testing when used as a triage test for lifelong screening of family members.

Given that the annual screening protocol is identical for all patients diagnosed with VHL syndrome, irrespective of their VHL mutation status, the lack of comparative data was predictable. The non-comparative data obtained highlighted the health benefits of

annual screening in a population that had been genetically tested, but any incremental benefit from the test could not be determined.

As clinical diagnosis is still the gold standard for identifying patients with VHL syndrome, the genetic test does not provide any additional benefit for these patients. As reflected in the management algorithm for use of VHL genetic testing (Figure 4), patients that are clinically diagnosed with VHL syndrome but have no detectable mutation in the *VHL* gene will continue to be screened annually. The value of VHL genetic testing of the index case is really only to identify at-risk family members, and perhaps to better tailor screening methods according to phenotypic expression.

Diagnostic accuracy data for the index case

In the absence of informative direct evidence, a linked evidence approach was undertaken to provide an assessment of the effectiveness of using genetic testing in either the diagnosis of VHL syndrome in symptomatic patients or the identification of family members carrying the VHL mutation. The first part of the linkage sought to determine the diagnostic accuracy of the different VHL mutation testing methodologies.

The sensitivity of genetic testing was highly variable between studies (3.9–100%; Table 19), largely due to the different methodologies used. Small changes to the *VHL* gene were detected by two different DNA sequencing methodologies. After PCR amplification of all three VHL exons, the PCR products were either used directly for DNA sequencing, or were pre-screened using various methods to compare their physical properties with PCR products obtained from normal control VHL DNA. In the latter case, only the PCR products that had different properties to the normal PCR products were then sequenced. Large deletions or rearrangements, involving all or part of the *VHL* gene, were detected using methods such as Southern blotting and MLPA. Many studies used both DNA sequencing and deletion detection methodologies.

These studies were also divided into three study population groups (the third having three subgroups): i) patients presenting with one or more VHL-associated neoplasms who could potentially have VHL syndrome—representative of the full spectrum of patients expected to undergo genetic testing to diagnose VHL syndrome; ii) patients who had already been clinically diagnosed with VHL syndrome—the absence of patients with a negative clinical diagnosis results in a lack of data for determining test specificity for this group; and iii) patients who were diagnosed with a specific VHL-associated neoplasm [a) CNS haemangioblastoma, b) retinal haemangioblastoma or c) phaeochromocytoma] with or without a clinical diagnosis of VHL syndrome—also representative of the type of patients expected to undergo VHL genetic testing, albeit different subgroups.

Interestingly, studies conducted with phaeochromocytoma patients with or without a clinical diagnosis of VHL syndrome showed different sensitivity results to most other studies. Seven out of 8 studies had a sensitivity of 100% for detecting VHL mutations in all except one patient with a clinical diagnosis of VHL syndrome, using DNA sequencing methodologies, compared with a sensitivity of 44.4–91.4% in studies involving other patient groups. As phaeochromocytoma usually results from an altered pVHL (due to an amino acid substitution) caused by a missense VHL mutation that is detected by DNA sequencing, and as large deletions of the *VHL* gene (not detectable by DNA sequencing) are not expected in this patient group, a higher sensitivity is consistent with the expectations for this patient group.

On the whole, the results suggested that direct DNA sequencing of the PCR products from all three exons of the *VHL* gene is more successful at identifying small errors than sequencing of only those PCR products that have altered physical properties, compared with a control PCR product from a normal *VHL* gene (median 76.9%, range 44.4–91.4, compared with 66.9%, range 51.8–87.5), with false negative rates of 24.9% and 40.5%, respectively. In fact, the false negative rate of 24.9% for direct DNA sequencing studies correlates with the 20–30% of VHL families that have large germ-line deletions that are only detectable using deletion detection methodologies (Ciotti et al 2009). This also explains the low median sensitivity (median 17.4%, range 3.9–36.6) for studies that used deletion detection methodologies.

The sensitivity improved when a DNA sequencing methodology was combined with a deletion detection methodology. The greatest improvement, to 100% (range 70–100), occurred when direct DNA sequencing and a deletion detection methodology were combined. Currently, all laboratories that test the *VHL* gene in Australia offer direct DNA sequencing (3/3 laboratories), and this is usually combined with a deletion detection methodology such as MLPA (2/3 laboratories), suggesting that most diagnostic laboratories should be able to correctly identify nearly all patients that carry a germ-line VHL mutation. In fact, GeneReviews on VHL syndrome states that the detection rate for VHL mutations is nearly 100% (Gene Tests 1993; Schimke et al 2000). Nevertheless, according to this review, these methods were still associated with a false negative rate of 10.2% in the included studies, suggesting that either some patients are clinically misdiagnosed with VHL syndrome when they do not have the condition or, more likely, detection of a germ-line mutation is not yet possible for some patients with VHL syndrome.

There are several possible reasons for not detecting a VHL mutation. Some patients will have somatic genetic mosaicism, where a VHL mutation will be present in particular embryonic cell lineages, such as the kidneys and adrenal glands. These patients may develop VHL-associated neoplasms in these regions but, if the mutation is not present in

peripheral blood cells, the mutation will not be detected using standard genetic testing protocols. As genetic testing of somatic neoplastic and surrounding normal tissue becomes more widespread, there is accumulating evidence to suggest that somatic mosaicism is more prevalent than previously believed. Alternatively, the mutation may not be detected because it occurs outside the region being tested; for example, it could be a splicing mutant deep within an intron, or in the promoter region of the *VHL* gene. It could also be missed because it is in the primer region and is not amplified for sequencing, or it does not involve an MLPA probe. On the other hand, there may be epigenetic modifications of the *VHL* gene via processes such as DNA methylation, resulting in gene inactivation. Another possibility is that the mutation may lie in another gene that either affects the function of the VHL protein or has similar downstream effects to the loss of functional VHL protein (expert advice of MESP clinical expert).

The median specificity in all studies that involved patients with either a positive or negative clinical diagnosis of VHL syndrome varied little, and was high for all genetic testing methodology groups (94.9–100%). The false positive rate also varied little (between 0% and 5.2%), indicating that few patients who did *not* meet the criteria for clinical diagnosis of VHL syndrome were found to have an underlying VHL mutation. However, it is highly likely that these patients with 'false positives' actually did have the first manifestations of VHL syndrome and that the disease had not yet progressed sufficiently to obtain a positive clinical diagnosis. The high positive predictive values (97.1–100%) indicate that a patient with a positive test result has a very high probability of having a true germ-line VHL mutation. The negative predictive value is low for deletion detection methodologies (17.1%), as expected due to the small proportion of patients that have large germ-line deletions. However, the negative predictive value for the methodology corresponding to current laboratory standards (the dual test methodology) had a median negative predictive value of 100%, indicating that patients with a negative test result are unlikely to have an undetected germ-line VHL mutation.

The median 100% sensitivity and specificity values for studies that used direct DNA sequencing plus a deletion detection method (corresponding to current laboratory standards) do not correlate with the observed average 10.2% false negative and 4.2% false positive rates. To provide an explanation for this observation, individual studies comprising these groups were evaluated separately. Indeed, the majority of studies had 100% values with no false positives or false negatives (10/17 and 6/8 studies, respectively).

Surprisingly, the 2 largest studies included for determining the sensitivity of the VHL genetic test had very different results. Stolle et al (1998) reported a sensitivity of 100% (0% false negatives), compared with 79.0% (21% false negatives) reported by Maher et al (1996). Stolle et al (1998) claimed that the improved sensitivity was attributable to

the use of quantitative Southern blotting (which takes into account the intensity of the signal), which detected 36.6% of the VHL mutations in this study. In fact, this was the largest proportion of large deletions detected in any study that provided data for the use of a deletion detection method alone, and was almost double the 18.8% detected by Southern blotting in the study by Maher et al (1996). Stolle et al (1998) claimed that 8.6% (8/93) of VHL patients in their study had a deletion of the entire *VHL* gene that was detected using quantitative Southern blotting but not using normal Southern blotting. It is uncertain how quantitative Southern blotting compares with MLPA, the current standard laboratory method for detecting large deletions in the *VHL* gene.

The largest study included for determining the specificity of the VHL genetic test had a specificity of 96.7%, which correlates with the observed false negative rate of 4.2% (Hes et al 2007). A specificity of less than 100% was expected due to the ability of the genetic test to detect a VHL mutation in patients not yet clinically diagnosed.

The case series that reported on the diagnostic yield of genetic testing for VHL mutations were separated into eight distinct study population groups, and involved either an unknown number of patients with a clinical diagnosis of VHL syndrome or patients without a positive clinical diagnosis (patients with a clinical diagnosis of VHL were excluded). The genetic testing methods used varied between studies, but all included DNA sequencing with or without pre-screening of the PCR products. Southern blotting or MLPA were used to detect large deletions in some of these studies. However, the impact of any differences between genetic testing methods on the diagnostic yield cannot be determined in most cases due to the small number of studies for each methodology.

The studies that provided diagnostic yield data for VHL genetic testing of patients diagnosed with phaeochromocytomas were divided into three groups. Patients with familial phaeochromocytomas but no other symptoms for syndromic diseases (eg as VHL or MEN 2) had a 45.8% probability of having a germ-line VHL mutation. This was much higher than in phaeochromocytoma patients with or without syndromic diseases (eg VHL or MEN 2) and in patients with apparently sporadic phaeochromocytomas, who had a 10.2% and 6.5% probability of having a germ-line VHL mutation, respectively. Thus, approximately half of all patients who present with a family history of phaeochromocytomas alone carry a VHL mutation corresponding to type 2C VHL syndrome, and approximately 1 in 10 patients with either syndromic or sporadic phaeochromocytomas have a germ-line VHL mutation.

The overall diagnostic yields for genetic testing of patients with sporadic CNS and retinal haemangioblastomas, pancreatic neuro-endocrine tumours and renal cell carcinomas were 5.1% (5/98), 0% (0/27), 1.0% (1/101) and 1.6% (3/187), respectively. As retinal

haemangioblastomas are a common first manifestation of VHL disease (according to Poulsen et al (2010) in 27% of patients), the lack of VHL mutations identified in patients with sporadic retinal haemangioblastomas is probably due to the small size of the 2 studies.

The body of evidence included in this assessment report was appraised according to NHMRC methodological guidelines (NHMRC 2008). This appraisal considered the evidence-base, in particular the number of studies and their methodological quality, the homogeneity of the studies' results, the clinical relevance of the effectiveness data, the generalisability of the evidence to the MBS target population, and the applicability of the evidence to the Australian healthcare system. Table 52 presents the results of appraisal of the evidence for the diagnostic accuracy of testing for *VHL* gene mutations in patients presenting with VHL-related neoplasms, who may or may not have VHL syndrome.

Table 52 Body of evidence assessment matrix for diagnostic accuracy of VHL genetic testing in the diagnosis of VHL syndrome

Component	A Excellent	B Good	C Satisfactory	D Poor
Evidence-base[a]			Level III studies with low risk of bias, or level I or II studies with moderate risk of bias	
Consistency[b]		Most studies consistent and inconsistency may be explained		
Clinical impact	N/A			
Generalisability		Population(s) studied in the body of evidence are similar to the target population		
Applicability		Applicable to Australian healthcare context with few caveats		

Note: For an explanation of this table see 'Assessment of the body of evidence' on page 62
N/A = not applicable

Diagnostic accuracy data for first- or second-degree family members

As the germ-line VHL mutation has already been identified in the index case, pre-symptomatic genetic testing of family members should be highly accurate and should not be affected by the methodologies used. Thus, all relatives tested should be correctly identified as VHL mutation carriers or not, unless there is human error such as contamination of samples. In comparison, annual clinical screening protocols can only identify relatives presenting with early signs of disease; thus, clinical screening provides an imperfect reference standard for pre-symptomatic genetic testing.

Not surprisingly, all studies involving relatives of patients with a known germ-line VHL mutation had a sensitivity of 100%, with no false negatives, as all relatives who show symptoms of VHL syndrome would have inherited the familial germ-line VHL mutation. Conversely, a high false positive rate would be anticipated, as VHL genetic testing can identify relatives who have inherited the familial germ-line VHL mutation before the manifestation of clinical signs of disease. This was reflected in the median specificity of studies involving either first-degree relatives or both first- and second-degree relatives, which had a median of 78% (range 50.0–100) and 85.0% (range 42.9–100) and a false positive rate of 23.5% and 16.9%, respectively.

The specificity and false positive rates are also very dependent on the age of the relatives being tested. The older the relatives, the more likely it is that some clinical signs of disease would have been detected by clinical screening. This would result in increased specificity and a lower false positive rate. In fact, Bender et al (2001) found that the penetrance of clinical signs of VHL syndrome in patients carrying a germ-line VHL mutation was 48.0% at 35 years of age and 88.0% at 70 years. The difference in the timeframe required for a clinical versus a genetic diagnosis also affects the median positive predictive values for first-degree relatives (69.4% [range 33.3–100]) and for first- and second-degree relatives (47.8% [range 20.0–100]). The positive predictive value is higher for first-degree relatives compared with first- and second-degree relatives, as more first-degree relatives would be expected to inherit a VHL mutation (50% probability of inheriting the germ-line VHL mutation compared with 25% for second-degree relatives). Predictably, the negative predictive value for VHL genetic testing was 100% for all studies, as a relative with a negative VHL genetic test result should not develop VHL syndrome.

The likelihood of either first-degree or both first- and second-degree relatives inheriting a germ-line VHL mutation was 36.0% and 38.1%, respectively. This is lower than the 50% of first-degree relatives predicted to inherit the VHL mutation, probably due to other symptomatic members of the family having been tested previously or older family members dying from VHL-related causes without a diagnosis of VHL syndrome. The similar results for studies involving either first-degree or both first- and second-degree relatives is likely due to the larger representation of siblings, parents and children (first-degree relatives) compared with grandparents, aunts, uncles, nieces, nephews and cousins (second-degree relatives). These results suggest that approximately 4 out of 10 relatives that undergo VHL genetic testing will be identified as carriers of the familial VHL mutation. However, if only asymptomatic relatives are considered, 26.8% of first-degree relatives and 22.4% of first- and second-degree relatives inherited the familial VHL mutation. Thus, only 2 first-degree relatives and 3 first- or second-degree relatives out of 10 without any symptoms indicative of VHL syndrome will be identified as VHL mutation carriers.

Table 53 provides an overall assessment of the body of evidence relating to the diagnostic accuracy of genetic testing for *VHL* gene mutations in relatives of patients with a known mutation.

Table 53 Body of evidence assessment matrix for diagnostic accuracy of genetic testing for *VHL* gene mutations in relatives of patients with a known mutation

Component	A Excellent	B Good	C Satisfactory	D Poor
Evidence-base[a]			Level III studies with low risk of bias, or level I or II studies with moderate risk of bias	
Consistency[b]		Most studies consistent and inconsistency may be explained		
Clinical impact	N/A			
Generalisability		Population(s) studied in the body of evidence are similar to the target population		
Applicability		Applicable to Australian healthcare context with few caveats		

Note: For an explanation of this table see 'Assessment of the body of evidence' on page 62
N/A = not applicable

Patient management

Some evidence was identified regarding patient management following a diagnosis of VHL syndrome using genetic testing in combination with clinical diagnosis, but none provided a direct comparison between patients with a known VHL mutation and those that had not been tested. Therefore, due to the lack of an appropriate comparator group in these studies, no conclusions can be made about the *incremental* change in patient management (ie the clinical impact) from genetic testing.

Knowledge of a specific germ-line VHL mutation in a patient with a clinical diagnosis of VHL syndrome is not expected to alter patient management significantly. However, it may provide some information about the types of neoplasms that are likely to develop in a particular patient. Patients with VHL type 1 syndrome are more likely to develop renal cell carcinoma and CNS haemangioblastomas without phaeochromocytoma, and are more likely to have germ-line VHL mutations predicted to inactivate the VHL protein. This includes large deletions and nonsense mutations predicted to result in a truncated protein. Patients with VHL type 2 syndrome are more likely to develop phaeochromocytoma with or without other VHL-associated neoplasms, and are more likely to have germ-line missense mutations predicted to produce altered full-length

pVHL. In particular, a missense mutation at codon 167 is associated with a high risk of phaeochromocytoma (53% and 82% at 30 and 50 years, respectively) (Ho et al 2003). Thus, management of patients with type 1 or type 2 VHL syndrome could be tailored to ensure early detection of the neoplasms most likely to occur.

Although no difference in patient management is expected for those presenting with the same VHL-associated neoplasms based on VHL mutation status, early detection of neoplasms via routine screening is expected to affect long-term patient outcomes. Thus, a patient presenting with early stages of *de novo* VHL syndrome, who may not be offered screening due to insufficient evidence for a positive clinical diagnosis, would commence screening earlier if a VHL mutation was identified. This may reduce any morbidity that may have been associated with an undetected neoplasm becoming symptomatic.

On the other hand, the VHL genetic test is expected to change patient management for asymptomatic relatives when used as a triage test for lifelong screening. Relatives with a negative genetic test result would not require lifelong screening, saving potential anguish and unnecessary use of healthcare resources. Economic modelling suggested that the proportion of relatives that receive unnecessary screening would fall from 67% to 44% after predictive VHL genetic testing (Table 42). Routine screening programs can then be targeted towards relatives who have inherited the VHL mutation, so that any new neoplasms are detected and treated early to prevent serious morbidity and/or mortality outcomes.

Several studies investigated the likelihood of patients agreeing to have the VHL genetic test and continue screening. The proportion of patients with (88.0%) and without (97.0%) retinal manifestations that agreed to genetic testing was quite high (Dollfus et al 2002), especially when compared with the number of at-risk relatives of patients with VHL syndrome and a known VHL mutation who agreed to genetic testing (58.5–65.8%; (Evans et al 1997; Rasmussen et al 2010). Interestingly, relatives aged over 20 years (94.9%) were more likely to undergo genetic testing than children aged less than 5 years (0%), indicating that parents do not wish to test very young children. Their reluctance is probably due to the burden this knowledge may place on the child, and tended to diminish with increasing age of the child (33.3% of children aged 5–9 years and 50.0% of children aged over 10 years were tested).

Only 38.9% (14/36) of patients with a VHL mutation continued screening after 5 years (Rasmussen et al 2010). Symptomatic patients were significantly more likely to continue screening after 5 years than asymptomatic patients (57.9% compared with 17.6%; OR = 5 [95% CI 1.2, 20.3]; p = 0.02). Patients who have had a neoplasm detected are more aware of the personal risks involved in discontinuation of screening, and are less

complacent than asymptomatic family members who have inherited a VHL mutation (who may not adhere to routine annual screening programs until they actually have their first neoplasm).

Table 54 provides an overall assessment of the body of evidence relating to the change in management associated with genetic testing for *VHL* gene mutations in patients and their asymptomatic relatives.

Table 54 Body of evidence assessment matrix for effectiveness of genetic testing at influencing management of patients with VHL syndrome and asymptomatic relatives with a *VHL* gene mutation

Component	A Excellent	B Good	C Satisfactory	D Poor
Evidence-base[a]				Level IV studies, or level I to III studies with high risk of bias
Consistency[b]		Most studies consistent and inconsistency may be explained		
Clinical impact			Moderate—family members	Slight or restricted—patients
Generalisability		Population(s) studied in the body of evidence are similar to the target population		
Applicability		Applicable to Australian healthcare context with few caveats		

Note: For an explanation of this table see 'Assessment of the body of evidence' on page 62

Economic considerations

The use of VHL genetic testing in combination with clinical testing for the identification of VHL syndrome in the Australian population would likely result in a cost saving to the Australian healthcare system compared with clinical testing alone. Using modest estimations of genetic testing uptake and disease monitoring compliance, genetic testing reduces the proportion of family members who are receiving monitoring from 26% to 16% without impacting upon the number of family members receiving monitoring who should be monitored. After the cost of testing and counselling to the index case and family is incorporated into the costs, the use of genetic testing results in a $7,749 cost saving (discounted) over the lifetime of each patient and their first- and second-degree relatives.

A cost comparison is only meaningful if there is no difference in effectiveness between the two management strategies. While no data were reported for comparative safety or

effectiveness of genetic testing with clinical testing compared with clinical testing alone, a conclusion of similar effectiveness is likely to be conservative in this instance. The knowledge of genetic status may improve monitoring compliance among those who require it, may improve the quality of life and reduce testing-related adverse events in those who have not inherited the gene by allowing them to avoid monitoring, and will result in a small increase in monitoring among those who require it (in clinically negative yet genetically positive patients), and thus improve their health outcomes. Therefore, assumptions of equal effectiveness and the application of a cost comparison are likely to be conservative.

The model used to compare costs of the genetic and clinical testing with clinical testing alone is markedly different to the likely use of genetic testing in Australian clinical practice. The economic analysis compares the costs of testing and lifetime monitoring of one individual (index case) and their first- and second-degree relatives. However, as VHL syndrome is a rare disease, it is likely that most VHL families will be known and only *de novo* cases will be discovered when a person becomes symptomatic. In this situation only a few family members will be genetically tested, and subsequent family members will be tested in childhood. Given the small number of estimated cases per year, and the likelihood that these cases are children of families already known to have a VHL mutation, the number of genetic tests each year is likely to be small.

Current usage of VHL genetic testing is unknown, although Australian data from 2006–07 suggests that about 80 diagnostic tests are done annually. This is higher than expected given that the anticipated number of new VHL cases (*de novo* cases or inherited of previously unknown VHL families) in Australia is likely to be less than 10. Therefore, 80 diagnostic tests either represents testing of a backlog, in which case the number of tests would have fallen since 2007, or a far higher case finding rate than was reported in the literature. In the absence of more accurate data, 80 tests a year is assumed to be current usage; with expected usage estimated to double, this may represent an overestimation and therefore a conservative approach in calculating costs.

It is anticipated that the listing of the VHL genetic test on the MBS will result in an annual cost to the MBS for diagnostic and predictive tests and genetic counselling of $65,000, increasing to $116,000 (undiscounted) if diagnostic testing doubles over the next 5 years. The cost to the Australian healthcare system (including state/territory governments and patient contributions) is about $154,000 per year if the rate of diagnostic testing doubles.

Compared with a situation in which genetic testing is not being done, fewer VHL patients and family members will require monitoring. The precise number of patients suspected of having VHL syndrome, or their family members who can avoid monitoring through

genetic confirmation, is unknown. In an ideal situation, 50% of all first-degree and 25% of all second-degree relatives could avoid monitoring; however, a proportion will not accept monitoring regardless of the genetic test results. Two costings have been performed in which genetic testing results in a 50% and 75% reduction in required monitoring, respectively. Consequently, there is a cost saving to the MBS and the Australian healthcare system that increases each year (as more individuals are spared monitoring). By year 5, based on only 10 patients per year avoiding monitoring, the Australian healthcare system will be saving $34,000 annually. The cost savings associated with averted monitoring will eventually exceed the costs associated with genetic testing and counselling.

Importantly, as is obvious from 2006–07 genetic testing data, testing for VHL already occurs despite it not being listed on the MBS. Therefore, the listing of this test on the MBS may not increase costs to the Australian healthcare system, but rather shift costs borne currently by the state/territory governments or individuals to the MBS. The availability of the test on the MBS may therefore improve access and address important equity issues.

Conclusions

Conclusions with respect to comparative safety

Even with a lack of evidence, the likelihood of adverse events as a consequence of VHL genetic testing are low, but it is recognised that there are some risks associated with genetic testing. Taking a peripheral blood sample can lead to bruising, pain, nerve damage and arterial puncture (Lavery & Ingram 2005).

An adverse positive genetic test could potentially cause psychological harms such as anxiety and depression that require treatment, or lead to life modifications including reproductive intentions (Trepanier et al 2004; Levy & Richard 2000).

False negative or false positive test results may also cause psychological harms, and possibly physical harms, due to delayed or inappropriate treatment. However, in the case of VHL genetic testing, patients with a false positive test result are probably true carriers of a VHL mutation, in whom a positive clinical diagnosis could not be given at the time. Few patients should receive a false negative test result when tested using dual test methods, and they will still receive annual monitoring, minimising any potential harms.

Conclusions with respect to effectiveness of VHL genetic testing

There were no data available to determine the direct health impact of including genetic testing as part of the current diagnostic strategy for patients suspected of VHL syndrome and their relatives. However, by linking evidence on the accuracy of VHL testing in individuals with change in management data, it is clear that most of the benefits from testing will accrue from reducing the need to monitor for VHL-associated neoplasms in asymptomatic family members who test negative for the mutation.

Testing in patients with symptoms of VHL syndrome

The current worldwide standard VHL genetic testing methods of direct DNA sequencing of PCR products from all three exons of the *VHL* gene, plus a method to detect large deletions of the *VHL* gene such as MLPA, appear to be the most accurate of the modalities available.

Despite being highly accurate, with median sensitivity, specificity, positive and negative predictive values of 100%, there is a false negative rate of 10.2%. This suggests that detection of a germ-line mutation is not yet possible for some patients with VHL

syndrome. Thus, VHL genetic testing should not be used as a standalone test for the diagnosis of VHL syndrome in symptomatic patients, but as a confirmatory test.

The false positive rate of 4.2% was expected, as there will always be a few patients with an underlying VHL mutation who do *not* meet the clinical criteria for VHL syndrome because their disease has not yet progressed sufficiently to obtain a positive clinical diagnosis.

Genetic diagnosis of a VHL mutation was more accurate in patients with phaeochromocytoma than in any other patient group. This was most likely due to the high degree of correlation between the risk of developing phaeochromocytoma and the presence of a missense VHL mutation (a single nucleotide change detected by DNA sequencing). Thus, a negative VHL genetic test would effectively rule out a diagnosis of VHL syndrome in these patients.

No difference in patient management is expected for patients presenting with the same VHL-associated neoplasms, irrespective of the method of diagnosis. Nevertheless, knowledge of a specific germ-line VHL mutation that indicates a VHL syndrome type in a patient with a clinical diagnosis of VHL syndrome may provide some information about the types of neoplasms that are likely to develop. Thus, management of patients could be tailored to ensure early detection of the neoplasms most likely to occur.

Predictive VHL genetic testing in relatives

The diagnostic accuracy of genetic testing within family members was uniformly high and did not vary with the genetic testing methodology. This was expected—once a VHL mutation has been identified in an index case, their close relatives need only be tested for that specific mutation, using a testing methodology able to detect that type of mutation.

Overall, we reported that approximately 4 out of 10 of all first- and second-degree relatives, and 2–3 out of 10 asymptomatic first- and second-degree relatives who undergo VHL genetic testing were identified as carriers of the familial VHL mutation. Younger relatives are more likely to receive a positive genetic test before any clinical signs of disease can be detected by clinical screening.

The VHL genetic test is expected to change patient management for asymptomatic relatives when used as a triage test for lifelong screening. Relatives with a negative genetic test result would not require lifelong screening, saving potential anguish and unnecessary use of healthcare resources. Lifelong screening programs can then be targeted towards relatives who have inherited the VHL mutation and are likely to develop VHL-associated neoplasms.

Compliance with VHL genetic testing

A greater proportion of symptomatic patients (88.0–97.0%) agreed to genetic testing compared with at-risk relatives (58.5–65.8%). Relatives aged over 20 years were more likely to undergo genetic testing than children aged less than 5 years, as parents are reluctant to have very young children genetically tested.

Only 38.9% of patients with a VHL mutation continued screening after 5 years, with symptomatic patients more likely to continue than asymptomatic patients.

Health benefits

Health benefits are derived from reduced morbidity and mortality due to annual screening for early detection of newly developed neoplasms. However, the annual screening protocol is identical for all patients clinically diagnosed with VHL syndrome, irrespective of their VHL mutation status.

It is therefore unclear if the addition of VHL genetic testing to clinical diagnosis has any impact on the health outcomes of patients with VHL syndrome or relatives at risk of the disease other than—hypothetically—through increased compliance with monitoring.

There is the possibility that people with the mutation could eventually receive monitoring that is tailored to their genetic phenotype (reducing the need for some tests). However, the main benefit appears to be that people without the mutation, who are asymptomatic, can be effectively ruled out from lifelong monitoring for VHL-associated neoplasms.

Conclusions with respect to the economic considerations

The degree of savings involved with a reduction in lifelong monitoring will be largely contingent upon the uptake of genetic testing among family members and the compliance with monitoring when required. In the absence of direct evidence, a cost comparison was performed showing a difference in health outcomes from the comparison of diagnostic strategies. If only 60% of family members accept genetic testing and 40% accept monitoring, the introduction of genetic testing will result in a discounted net saving of $7,749 over the lifetime of one individual and their first- and second-degree relatives. Substantial uncertainties regarding the use of genetic testing in the Australian setting will limit the applicability of these conclusions.

Genetic testing for VHL is already available and is funded by state/territory governments or by individuals. Therefore, there is unlikely to be a substantial pool of patients who are clinically diagnosed with VHL who have not also received genetic testing. Therefore, the listing of the VHL genetic test (both diagnostic and predictive) on the MBS will not

necessarily result in an increase in costs to the Australian healthcare system, but rather a shift in who is responsible for the cost. Currently, it is estimated that about 80 VHL diagnostic tests are occurring per year in Australia. This is far greater than the number of estimated VHL diagnoses and likely represents case finding in patients with neoplasms that are suspicious for VHL. Currently, diagnostic and predictive VHL testing, combined with genetic counselling, is estimated to cost $86,129 per year. However, if specialists become more familiar with VHL, or the listing of the VHL genetic test on the MBS increases awareness, the threshold to send someone for a test may fall and the number of diagnostic VHL genetic tests may increase. If the demand for the VHL diagnostic test doubles, the annual cost of all VHL genetic testing and counselling will increase to $154,441 per year.

Importantly, genetic testing may allow family members (and perhaps individuals suspected of having VHL syndrome who may be confirmed as not carrying a VHL genetic mutation) to avoid lifelong monitoring. Theoretically, more than 50% and 75% of all first- and second-degree family members, respectively, of patients with an inherited VHL genetic mutation may avoid lifelong monitoring, which is estimated to cost $687 annually (or double the cost of the predictive genetic test for family members). The cost offset due to avoided monitoring has been calculated assuming that no one has avoided monitoring up to this point; therefore, savings would be slowly accrued at first but would overtake the cost of genetic testing in the future. Based upon a 50% reduction in monitoring (estimated at 10 persons per year), by year 5, 50 persons would be avoiding monitoring each year who would have been receiving monitoring had they not had access to the genetic test. This is an estimated cost saving of $34,343 per year. It is important to realise that hundreds of individuals initially suspected of having VHL and family members of patients with VHL are currently not receiving monitoring due to negative genetic testing, and these savings are not represented in the financial analysis.

When cost and cost savings are considered together, based on a doubling of case finding (160 diagnostic tests per year) and a 50% reduction in monitoring, the cost to the Australian healthcare system would be $120,099, with the MBS responsible for $90,074.

Appendix A MSAC terms of reference and membership

The Medical Services Advisory Committee (MSAC) is an independent scientific committee comprising individuals with expertise in clinical medicine, health economics and consumer matters. It advises the Minister for Health and Ageing on whether a new medical service should be publicly funded based on an assessment of its comparative safety, effectiveness, cost-effectiveness and total cost, using the best available evidence. In providing this advice, the MSAC may also take other relevant factors into account. This process ensures that Australians have access to medical services that have been shown to be safe and clinically effective, as well as representing value for money for the Australian healthcare system.

The MSAC is to:

- advise the Minister for Health and Ageing on medical services including those that involve new or emerging technologies and procedures, in relation to:
    - the strength of evidence in relation to the comparative safety, effectiveness, cost-effectiveness and total cost of the medical service;
    - whether public funding should be supported for the medical service and, if so, the circumstances under which public funding should be supported;
    - the proposed Medicare Benefits Schedule (MBS) item descriptor and fee for the service where funding through the MBS is supported;
    - the circumstances, where there is uncertainty in relation to the clinical or cost-effectiveness of a service, under which interim public funding of a service should be supported for a specified period, during which defined data collections under agreed clinical protocols would be collected to inform a re-assessment of the service by the MSAC at the conclusion of that period;
    - other matters related to the public funding of health services referred by the Minister
- advise the Australian Health Ministers' Advisory Council (AHMAC) on health technology assessments referred under AHMAC arrangements.

The MSAC may also establish subcommittees to assist it to effectively undertake its role. The MSAC may delegate some of its functions to its Executive subcommittee.

The membership of the MSAC at the September 2011 meeting comprised a mix of clinical expertise covering pathology, nuclear medicine, surgery, specialist medicine and general practice, plus clinical epidemiology and clinical trials, health economics, consumers, and health administration and planning:

Member *(Executive listed first followed by members in alphabetical order)*	**Expertise or affiliation**
Professor Robyn Ward (Chair)	Medical Oncology
Associate Professor Frederick Khafagi (Deputy Chair)	Nuclear Medicine
Professor Jim Butler (Chair, Evaluation subcommittee)	Health Economics
Associate Professor John Atherton	Cardiology
Associate Professor Michael Bilous	Anatomical Pathology
Professor Jim Bishop AO	Commonwealth Chief Medical Officer (*ex-officio member*)
Associate Professor Kirsty Douglas	General Practice/Research
Professor Kwun Fong	Thoracic Medicine
Professor Paul Glasziou	Evidence-based health care
Mr Scott Jansson	Pathology
Professor David Little	Orthopaedics
Mr Russell McGowan	Consumer Health Representative
Professor David Roder	Health medicine/epidemiology
Associate Professor Bev Rowbotham	Haematology
Dr Graeme Suthers	Genetics/Pathology
Mr David Swan	AHMAC Representative (*ex-officio member*)
Professor Ken Thomson	Radiology
Dr Christine Tippett	Obstetrics/Gynaecology
Associate Professor David Winlaw	Paediatric Cardiothoracic Surgery
Dr Caroline Wright	Colorectal Cancer/Surgery

Appendix B MESP members and evaluators

Members of the Medical Expert Standing Panel for application 1153: VHL genetic testing

Member	Expertise
Assoc Prof Bruce Bennetts	Genetic Medicine
Dr Marion Harris	Oncology
Ms Kerryn Weekes	Senior Scientist (Clinical Genetics Laboratory)
Winthrop Prof Jon Emery	Genetic Medicine and Cancer Diagnosis

Evaluators

Name	Organisation
Dr Judy Morona	Research Officer, Adelaide Health Technology Assessment
Mr David Tamblyn	Research Officer, Adelaide Health Technology Assessment
Ms Vivian Liufu	Research Officer, Adelaide Health Technology Assessment
Mr Ben Ellery	Research Officer, Adelaide Health Technology Assessment
Ms Skye Newton	Team Leader, Adelaide Health Technology Assessment
Dr Shuhong Wang	Research Fellow, Adelaide Health Technology Assessment
Ms Tracy Merlin	Manager Director, Adelaide Health Technology Assessment

Appendix C MBS items required to monitor patients for signs of VHL disease

Commonly occurring types of healthcare resources that are required to diagnose and monitor patients presenting with a neoplasm associated with VHL syndrome or with a family history of VHL syndrome are listed below.

Identifier	Description	Quantity provided
MBS item number 23	**LEVEL B CONSULTATION AT CONSULTING ROOMS** Professional attendance at consulting rooms by a general practitioner (not being a service to which any other item in this table applies) lasting less than 20 minutes, including any of the following that are clinically relevant: a) taking a patient history; b) performing a clinical examination; c) arranging any necessary investigation; d) implementing a management plan; e) providing appropriate preventive health care; in relation to 1 or more health-related issues, with appropriate documentation.	**Fee:** $34.90 **Benefit:** 100% = $34.90
MBS item number 104	**SPECIALIST, REFERRED CONSULTATION—SURGERY OR HOSPITAL** (Professional attendance at consulting rooms or hospital by a specialist in the practice of his or her specialty where the patient is referred to him or her) **INITIAL** attendance in a single course of treatment, not being a service to which ophthalmology items 106, 109 or obstetric item 16401 apply.	**Fee:** $82.30 **Benefit:** 75% = $61.75 85% = $70.00
MBS item number 105	Each attendance **SUBSEQUENT** to the first in a single course of treatment	**Fee:** $41.35 **Benefit:** 75% = $31.05 85% = $35.15
MBS item number 110	**CONSULTANT PHYSICIAN (OTHER THAN IN PSYCHIATRY), REFERRED CONSULTATION—SURGERY OR HOSPITAL** (Professional attendance at consulting rooms or hospital by a consultant physician in the practice of his or her specialty (other than in psychiatry) where the patient is referred to him or her by a medical practitioner) - **INITIAL** attendance in a single course of treatment	**Fee:** $145.20 **Benefit:** 75% = $108.90 85% = $123.45
MBS item number 116	- Each attendance (other than a service to which item 119 applies) **SUBSEQUENT** to the first in a single course of treatment	**Fee:** $72.65 **Benefit:** 75% = $54.50 85% = $61.80
MBS item number 132	**CONSULTANT PHYSICIAN (OTHER THAN IN PSYCHIATRY) REFERRED PATIENT TREATMENT AND MANAGEMENT PLAN - SURGERY OR HOSPITAL** Professional attendance of at least 45 minutes duration for an initial assessment of a patient with at least two morbidities (this can include complex congenital, developmental and behavioural disorders), where the patient is referred by a medical practitioner, and where a) assessment is undertaken that covers:	**Fee:** $253.90 **Benefit:** 75% = $190.45 85% = $215.85

Identifier	Description	Quantity provided
	- a comprehensive history, including psychosocial history and medication review; - comprehensive multi or detailed single organ system assessment; - the formulation of differential diagnoses; and b) a consultant physician treatment and management plan of significant complexity is developed and provided to the referring practitioner that involves: - an opinion on diagnosis and risk assessment - treatment options and decisions - medication recommendations Not being an attendance on a patient in respect of whom, an attendance under items 110, 116 and 119 has been received on the same day by the same consultant physician. Not being an attendance on the patient in respect of whom, in the preceding 12 months, payment has been made under this item for attendance by the same consultant physician.	
MBS item number 133	**CONSULTANT PHYSICIAN (OTHER THAN IN PSYCHIATRY) REVIEW OF REFERRED PATIENT TREATMENT AND MANAGEMENT PLAN - SURGERY OR HOSPITAL** Professional attendance of at least 20 minutes duration subsequent to the first attendance in a single course of treatment for a review of a patient with at least two morbidities (this can include complex congenital, developmental and behavioural disorders), where a) a review is undertaken that covers: - review of initial presenting problem/s and results of diagnostic investigations - review of responses to treatment and medication plans initiated at time of initial consultation comprehensive multi or detailed single organ system assessment, - review of original and differential diagnoses; and b) a modified consultant physician treatment and management plan is provided to the referring practitioner that involves, where appropriate: - a revised opinion on the diagnosis and risk assessment - treatment options and decisions - revised medication recommendations Not being an attendance on a patient in respect of whom, an attendance under item 110, 116 and 119 has been received on the same day by the same consultant physician. Being an attendance on a patient in respect of whom, in the preceding 12 months, payment has been made under item 132 by the same consultant physician, payable no more than twice in any 12 month period.	**Fee:** $127.10 **Benefit:** 75% = $95.35 85% = $108.05
MBS item number 66779	**PATHOLOGY** Adrenaline, noradrenaline, dopamine, histamine, hydroxyindoleacetic acid (5HIAA), hydroxymethoxymandelic acid (HMMA), homovanillic acid (HVA), metanephrines, methoxyhydroxyphenylethylene glycol (MHPG), phenylacetic acid (PAA) or serotonin quantitation - 1 or more tests	**Fee:** $40.20 **Benefit:** 75% = $30.15 85% = $34.20
MBS item number 55036	**ULTRASOUND SCAN OF ABDOMEN**, including scan of urinary tract when undertaken but not being a service associated with the service described in item 55600 or item 55603, where: a) the patient is referred by a medical practitioner for ultrasonic examination not being a service associated with a service to which an item in Subgroups 2 or 3 of this Group applies; b) the referring medical practitioner is not a member of a group of practitioners of which the providing practitioner is a member; and c) the service is not performed with item 55038, 55044 or 55731 on the same patient within 24 hours (R)	**Fee:** $111.30 **Benefit:** 75% = $83.50 85% = $94.65

Identifier	Description	Quantity provided
MBS item number 56407	**COMPUTED TOMOGRAPHY** - scan of upper abdomen only (diaphragm to iliac crest) with intravenous contrast medium and with any scans of upper abdomen (diaphragm to iliac crest) prior to intravenous contrast injection, when undertaken, not being a service to which item 56307, 56507, 56807 or 57007 applies (R) (K) (Anaes.)	**Fee:** $360.00 **Benefit:** 75% = $270.00 85% = $306.00
MBS item number 63111 *(if abnormality detected on ultrasound)*	**MAGNETIC RESONANCE IMAGING** (including Magnetic Resonance Angiography if performed), performed under the professional supervision of an eligible provider at an eligible location where the patient is referred by a specialist or by a consultant physician - **scan of head and cervical spine** for: - tumour of the central nervous system or meninges (R) (Contrast) (Anaes.)	**Fee:** $492.80 **Benefit:** 75% = $369.60 85% = $421.60

Source: Department of Health and Ageing (2011)
MBS = Medicare Benefits Schedule

Appendix D Search terms used for literature searches

Table 55 Search terms for VHL genetic testing (direct evidence)

Element of clinical question	Suggested search terms
Population	**Embase.com** 'von hippel 196lindau disease'/exp OR 'von hippel lindau' OR 'vhl' OR 'vhl gene' OR 'vhl mutation' **PubMed** von Hippel Lindau disease[MeSH] OR von Hippel Lindau[Text Word] OR (VHL[Text Word] AND (gene*[Text Word] OR mutat*[Text Word]))
Intervention/test	AND **Embase.com** 'diagnosis'/exp OR 'diagnosis' OR 'genetic screening'/exp OR 'genetic screening' OR 'genetic test' OR 'genetic testing' OR 'molecular test' OR 'molecular testing' OR 'DNA screening'/exp OR 'DNA screening' OR 'DNA test' OR 'DNA testing' OR 'sequence analysis'/exp OR 'sequence analysis' OR 'genetic procedures'/exp OR 'genetic procedure' **PubMed** Diagnosis[MeSH] OR diagnos*[Text Word] OR genetic testing[MeSH] OR genetic test[Text Word] OR genetic test*[Text Word] OR molecular diagnostic techniques[MeSH] OR molecular test[Text Word] OR molecular test*[Text Word] OR genetic screening[Text Word] OR gene[Text Word]
Comparator	N/A
Outcomes	N/A
Limits	Humans, 1993 – May 2011

Table 56 Search terms for VHL genetic testing (linked evidence)

Element of clinical question	Suggested search terms
Test accuracy	**Embase.com** 'von hippel lindau disease'/exp OR 'von hippel lindau' OR 'vhl gene' OR 'vhl mutation' OR 'vhl' AND 'diagnosis, measurement and analysis'/exp OR 'sensitivity and specificity'/exp OR 'sensitivity' OR 'specificity' OR 'accuracy' OR 'diagnostic error'/exp OR 'false negative' OR 'false positive' OR 'predictive value' OR 'likelihood ratio' **PubMed** von Hippel Lindau disease[MeSH] OR von Hippel Lindau[Text Word] OR (VHL[Text Word] AND (gene*[Text Word] OR mutat*[Text Word])) AND Diagnostic Techniques[MeSH] OR Sensitivity and Specificity[MeSH] OR sensitive*[Text Word] OR specific*[Text Word] OR Diagnostic Errors[MeSH] OR accuracy[Text Word] OR false negative[Text Word] OR false positive[Text Word] OR predictive value*[Text Word] OR likelihood ratio*[Text Word]
Change in management of patients identified with VHL mutation	**Embase.com** 'von hippel lindau disease'/exp OR 'von hippel lindau' OR 'vhl gene' OR 'vhl mutation' OR 'vhl' AND 'therapy'/exp OR 'therapy' OR 'disease management'/exp OR 'management' OR 'patient care' OR 'treatment' OR 'therapy' OR 'surveillance' OR 'monitoring' OR 'screening' **PubMed** von Hippel Lindau disease[MeSH] OR von Hippel Lindau[Text Word] OR (VHL[Text Word]

Element of clinical question	Suggested search terms
	AND (gene*[Text Word] OR mutat*[Text Word])) AND Patient Care Management[MeSH] OR manage*[Text Word] OR therap*[Text Word] OR treat*[Text Word] OR surveillance[Text Word] OR monitor*[Text Word]
Ethical issues	**Embase.com** Search 1 'genetic screening'/mj OR 'genetic test' OR 'genetic testing'/mj OR 'molecular test' OR 'molecular testing' OR 'dna screening'/mj OR 'dna test' OR 'dna testing' OR 'sequence analysis'/mj OR 'genetic procedure' AND 'patient right'/mj OR 'patient autonomy'/mj OR 'personal autonomy'/mj OR 'autonomy' OR 'social justice'/mj OR 'access to information'/mj OR 'bioethics'/mj OR 'informed consent'/mj OR 'privacy'/mj OR 'confidentiality'/mj Search 2 'genetic screening':ti OR 'genetic test':ti OR 'genetic testing':ti OR 'molecular test':ti OR 'molecular testing':ti OR 'dna screening':ti OR 'dna test':ti OR 'dna testing':ti OR 'sequence analysis':ti OR 'genetic procedure':ti OR 'genetic screening':ab OR 'genetic test':ab OR 'genetic testing':ab OR 'molecular test':ab OR 'molecular testing':ab OR 'dna screening':ab OR 'dna test':ab OR 'dna testing':ab OR 'sequence analysis':ab OR 'genetic procedure':ab AND 'patient right':ti OR 'patient autonomy':ti OR 'personal autonomy':ti OR 'autonomy':ti OR 'social justice':ti OR 'access to information':ti OR 'bioethics':ti OR 'informed consent':ti OR 'privacy':ti OR 'confidentiality':ti OR 'patient right':ab OR 'patient autonomy':ab OR 'personal autonomy':ab OR 'autonomy':ab OR 'social justice':ab OR 'access to information':ab OR 'bioethics':ab OR 'informed consent':ab OR 'privacy':ab OR 'confidentiality':ab **PubMed** von Hippel Lindau disease[MeSH] OR von Hippel Lindau[Text Word] OR (VHL[Text Word] AND (gene*[Text Word] OR mutat*[Text Word])) OR genetic testing[MeSH] OR gene* test*[Text Word] OR molecular diagnostic techniques[MeSH] OR molecular test*[Text Word] OR DNA test*[Text Word] OR gene* screen*[Text Word] AND Personal Autonomy[MeSH] OR Social Justice[MeSH] OR Bioethical Issues[MeSH] OR Bioethics[MeSH] OR Informed consent[MeSH] OR Third-Party Consent[MeSH] OR genetic counselling[Text Word] OR genetic education[Text Word] OR autonomy[Text Word] OR privacy[Text Word] OR consent[Text Word] OR confidentiality[Text Word]
Limits	Humans, 1993 – May 2011

Table 57 Search terms for additional databases for economic evaluation of VHL genetic testing

Element of clinical question	Suggested search terms
Cost-effectiveness	**Embase.com** 'von hippel lindau disease'/exp OR 'von hippel lindau' OR 'vhl gene' OR 'vhl mutation' OR 'vhl' **PubMed** von Hippel Lindau disease[MeSH] OR von Hippel Lindau[Text Word] OR (VHL[Text Word] AND (gene*[Text Word] OR mutat*[Text Word]))
Limits	Humans, 1993 – May 2011

Appendix E Health Technology Assessment Agency websites

AUSTRALIA	
Australian Safety and Efficacy Register of New Interventional Procedures – Surgical (ASERNIP-S)	http://www.surgeons.org/Content/NavigationMenu/Research/ASERNIPS/default.htm
Centre for Clinical Effectiveness	http://www.southernhealth.org.au/cce
Centre for Health Economics, Monash University	http://www.buseco.monash.edu.au/centres/che/
AUSTRIA	
Institute of Technology Assessment / HTA unit	http://www.oeaw.ac.at/ita
CANADA	
Agence d'Evaluation des Technologies et des Modes d'Intervention en Santé (AETMIS)	http://www.aetmis.gouv.qc.ca/site/home.phtml
Alberta Heritage Foundation for Medical Research (AHFMR)	http://www.ahfmr.ab.ca/publications.html
Alberta Institute of Health Economics	http://www.ihe.ca/
The Canadian Agency for Drugs And Technologies in Health (CADTH)	http://www.cadth.ca/index.php/en/
Canadian Health Economics Research Association (CHERA/ACRES) – Cabot database	http://www.mycabot.ca
Centre for Health Economics and Policy Analysis (CHEPA), McMaster University	http://www.chepa.org
Centre for Health Services and Policy Research (CHSPR), University of British Columbia	http://www.chspr.ubc.ca
Health Utilities Index (HUI)	http://www.fhs.mcmaster.ca/hug/index.htm
Institute for Clinical and Evaluative Studies (ICES)	http://www.ices.on.ca
Saskatchewan Health Quality Council (Canada)	http://www.hqc.sk.ca
DENMARK	
Danish Centre for Evaluation and Health Technology Assessment (DACEHTA)	http://www.sst.dk/english/dacehta.aspx?sc_lang=en
Danish Institute for Health Services Research (DSI)	http://dsi.dk/english/
FINLAND	
Finnish Office for Health Technology Assessment (FINOHTA)	http://finohta.stakes.fi/EN/index.htm
FRANCE	
The Haute Autorité de santé (HAS) - or French National Authority for Health	http://www.has-sante.fr/portail/jcms/c_5443/english?cid=c_5443
GERMANY	
German Institute for Medical Documentation and Information (DIMDI) / HTA	http://www.dimdi.de/static/en/index.html
Institute for Quality and Efficiency in Health Care (IQWiG)	http://www.iqwig.de
THE NETHERLANDS	
Health Council of the Netherlands Gezondheidsraad	http://www.gezondheidsraad.nl/en/
Institute for Medical Technology Assessment (Netherlands)	http://www.imta.nl/

NEW ZEALAND	
New Zealand Health Technology Assessment (NZHTA)	http://nzhta.chmeds.ac.nz/
NORWAY	
Norwegian Knowledge Centre for the Health Services	http://www.kunnskapssenteret.no
SPAIN	
Agencia de Evaluación de Tecnologias Sanitarias, Instituto de Salud "Carlos III"l/Health Technology Assessment Agency (AETS)	http://www.isciii.es/
Andalusian Agency for Health Technology Assessment (Spain)	http://www.juntadeandalucia.es/
Catalan Agency for Health Technology Assessment (CAHTA)	http://www.gencat.cat
SWEDEN	
Center for Medical Health Technology Assessment	http://www.cmt.liu.se/?l=en&sc=true
Swedish Council on Technology Assessment in Health Care (SBU)	http://www.sbu.se/en/
SWITZERLAND	
Swiss Network on Health Technology Assessment (SNHTA)	http://www.snhta.ch/
UNITED KINGDOM	
National Health Service Health Technology Assessment (UK) / National Coordinating Centre for Health Technology Assessment (NCCHTA)	http://www.hta.ac.uk/
NHS Quality Improvement Scotland	http://www.nhshealthquality.org/
National Institute for Clinical Excellence (NICE)	http://www.nice.org.uk/
The European Information Network on New and Changing Health Technologies	http://www.euroscan.bham.ac.uk/
University of York NHS Centre for Reviews and Dissemination (NHS CRD)	http://www.york.ac.uk/inst/crd/
UNITED STATES	
Agency for Healthcare Research and Quality (AHRQ)	http://www.ahrq.gov/clinic/techix.htm
Harvard School of Public Health	http://www.hsph.harvard.edu/
Institute for Clinical and Economic Review (ICER)	http://www.icer-review.org/
Institute for Clinical Systems Improvement (ICSI)	http://www.icsi.org
Minnesota Department of Health (US)	http://www.health.state.mn.us/htac/index.htm
National Information Centre of Health Services Research and Health Care Technology (US)	http://www.nlm.nih.gov/hsrph.html
Oregon Health Resources Commission (US)	http://egov.oregon.gov/DAS/OHPPR/HRC/about_us.shtml
Office of Health Technology Assessment Archive (US)	http://fas.org/ota
U.S. Blue Cross/ Blue Shield Association Technology Evaluation Center (Tec)	http://www.bcbs.com/blueresources/tec/
Veteran's Affairs Research and Development Technology Assessment Program (US)	http://www.research.va.gov/default.cfm

Appendix F Literature sources

The *VHL* gene has only been described in the literature after 1993; therefore, the search period was restricted from 1993 (or if inception of the database was later, from that date) until May 2011.

Bibliographic databases

Electronic database	Time period
Cochrane Library – including, Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effects, the Cochrane Central Register of Controlled Trials (CENTRAL), the Health Technology Assessment Database, the NHS Economic Evaluation Database	1993 – May 2011
Web of Science – Science Citation Index Expanded	1993 – May 2011
Current Contents	1998 – May 2011
Embase.com (including Embase and Medline)	1993 – May 2011
PubMed	1993 – May 2011
CINAHL	1993 – May 2011
EconLit	1993 – May 2011
PsycINFO (for ethical issues only)	1993 – May 2011

Additional sources of literature

Source	Location
Internet	
NHMRC - National Health and Medical Research Council (Australia)	http://www.health.gov.au/nhmrc/
US Department of Health and Human Services (reports and publications)	http://www.os.dhhs.gov/
New York Academy of Medicine Grey Literature Report	http://www.nyam.org/library/greylit/index.shtml
Trip database	http://www.tripdatabase.com
Current Controlled Trials metaRegister	http://controlled-trials.com/
National Library of Medicine Health Services/Technology Assessment Text	http://text.nlm.nih.gov/
U.K. National Research Register	http://www.update-software.com/National/
Google Scholar	http://scholar.google.com/
Hand searching (journals from 2010–11)	
Studies other than those found in regular searches	Library or electronic access
Expert clinicians	MSAC Medical Expert Standing Panel (MESP)
Pearling	
All included articles had their reference lists searched for additional relevant source material	

Additional databases searched for economic evaluations

Electronic database	Time period
Cost-effectiveness Analysis (CEA) Registry	1993 – June 2011
Database of Abstracts of Reviews of Effects or Reviews of Effects (DARE)	1993 – June 2011
Health Technology Assessment database	1993 – June 2011
NHS Economic Evaluation Database (NHS EED)	1993 – June 2011
European Network of Health Economics Evaluation Databases (EURONHEED)	1993 – June 2011
Paediatric Economic Database Evaluation (PEDE)	1993 – December 2009[a]
Search terms used: von hippel lindau disease OR von hippel lindau OR vhl gene OR vhl mutation OR vhl	

[a] The Paediatric Economic Database Evaluation contains citations from January 1980 to December 2009. Articles published after 2009 cannot be retrieved from this database.

Specialty websites

VHL Family Alliance	http://www.vhl.org/
The VHL mutations database	http://www.umd.be/VHL/
GeneTests	http://www.ncbi.nlm.nih.gov/sites/GeneTests/?db=GeneTests
Laboratories offering clinical testing for VHL syndrome	http://www.ncbi.nlm.nih.gov/sites/GeneTests/lab/clinical_disease_id/2171?db=genetests
The Royal College of Pathologists of Australasia Catalogue of Genetic Tests and Laboratories	http://genetictesting.rcpa.edu.au/
Genetics Home Reference Von Hippel-Lindau syndrome	http://ghr.nlm.nih.gov/condition/von-hippel-lindau-syndrome
Cancer.Net Von Hippel-Lindau Syndrome	http://www.cancer.net/patient/Cancer+Types/Von+Hippel-Lindau+Syndrome
eMedicine - von Hippel-Lindau Disease	http://emedicine.medscape.com/article/950063-overview
Cancer Council Australia Types of family cancer	http://www.cancer.org.au//aboutcancer/familycancers/typesfamilycancer.htm

Appendix G Studies included in this review

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
(Akcaglar et al 2008) Uludag University, Gorukle, Turkey	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 31 kindred of 4 VHL patients with synchronous VHL disease and RCC, and a confirmed deletion of the *VHL* gene, located on the short arm of chromosome 3	*Inclusion:* Close relatives of 1 of 4 patients with synchronous VHL disease and RCC *Exclusion:* Not stated	*Intervention*: Moorehead karyotyping method, showing deletions on the short arm of chromosome 3 *Comparator (for deletion positive kindred):* Detailed clinical screening (details not provided)	Diagnostic accuracy	N/A
	Case series Level IV diagnostic evidence CX P1 Q3	N = 31 kindred of 4 VHL patients with synchronous VHL disease and RCC, and a confirmed deletion of the *VHL* gene, located on the short arm of chromosome 3	*Inclusion:* Close relatives of 1 of 4 patients with synchronous VHL disease and RCC *Exclusion:* Not stated	*Intervention*: Moorehead karyotyping method	Diagnostic yield	N/A
(AlFadhli et al 2004) Kuwait University, Kuwait	Case series Level IV diagnostic evidence CX P1 Q3	N = 9 close relatives of proband with clinical diagnosis of VHL	*Inclusion:* Close relatives of proband that are at risk of VHL syndrome *Exclusion:* Not stated	*Intervention*: SSCP and DNA sequencing	Diagnostic yield	N/A
(AlFadhli et al 2008) Kuwait University, Kuwait	Case series Level IV diagnostic evidence CX P1	N = 33 family members n = 13 with clinical diagnosis of VHL n = 20 asymptomatic family members	*Inclusion:* Member of an extended VHL family with Arabian and Persian genetic admixture *Exclusion:*	*Intervention*: SSCP and DNA sequencing	Diagnostic yield	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
	Q3		Not stated			
(Amar et al 2005) Assistance Publique-Hôpitaux de Paris; France	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 314 patients with a PH or a functional PGL n = 9 patients with VHL n = 47 patients with other familial syndromes	*Inclusion:* Patients with PH or a functional PGL recruited from several clinical centres *Exclusion:* Not stated	*Intervention:* DNA sequencing *Comparator:* Hereditary PH syndromes were diagnosed as described by Gimenez-Roqueplo et al (2003)	Diagnostic accuracy	N/A
(Atuk et al 1998) University of Virginia Health Sciences Centre, Charlottesville, Virginia, USA	Comparative study Level III-2 diagnostic evidence CX P1 Q1	N = 13 members of a VHL family followed since 1964 n = 6 affected members n = 7 unaffected members	*Inclusion:* Members of a large kindred (descendants of 3 siblings) that had been followed since 1964 *Exclusion:* Not stated	*Intervention:* DNA sequencing *Comparator:* Screening by measuring blood pressure, urinary norepinephrine, epinephrine, metanephrines and vanillylmandelic acid concentrations, and by ophthalmoscopy	Diagnostic accuracy Costs	N/A
(Bar et al 1997) Hadassah University Hospital and Medical School, Jerusalem, Israel	Case series Level IV diagnostic evidence CX P1 Q3	N = 27 patients with sporadic PHs (no personal or familial history of syndromic disease)	*Inclusion:* Not stated *Exclusion:* Not stated	*Intervention*: SSCP and DNA sequencing	Diagnostic yield	N/A
(Bender et al 1997) Albert-Ludwigs-University, Freiburg, Germany	Case series Level IV diagnostic evidence CX P2, P1 Q3	N = 4 patients with PHs and no other signs of VHL disease N = 5 first-degree relatives of 2 index cases with known VHL mutations	*Inclusion:* All cases with thoracic PH treated at our institutions over the past 2 decades *Exclusion:* Not stated	*Intervention*: DNA sequencing	Diagnostic yield	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
(Bender et al 2000) Albert-Ludwigs-University, Freiburg, Germany	Comparative study Level III-2 diagnostic evidence CX P1 Q1	N = 38 patients with PH n = 21 diagnosed with VHL n = 17 sporadic cases	*Inclusion:* All cases with thoracic PH treated at the institutions over the past 2 decades *Exclusion:* Not stated	*Intervention*: Southern blotting, SSCP and DNA sequencing *Comparator:* Thorough history and physical examination for clinical diagnosis of VHL, including direct ophthalmoscopy and MRI of the brain, spinal cord and abdomen	Diagnostic accuracy	N/A
(Bender et al 2001) Albert-Ludwigs-University, Freiburg, Germany	Comparative study Level III-2 diagnostic evidence CX P1 Q1	N = 125 patients n = 64 patients belonging to 15 families with a known VHL c.505 T/C mutation n = 10 subjects with apparently sporadic VHL-associated tumours, with the c.505 T/C mutation n = 51 first-degree relatives that were diagnosed as VHL c.505 T/C mutation carriers	*Inclusion:* Patients or a first-degree asymptomatic relative with the VHL c.505 T/C mutation *Exclusion:* Not stated	*Intervention*: PCR with modified primers to create restriction-site polymorphisms *Comparator:* Clinical surveillance that included direct ophthalmoscopy and gadolinium-enhanced MRI of the brain, spinal cord and abdomen, plus 24-hour urine for catecholamines	Diagnostic accuracy Penetrance	N/A
(Brauch et al 1997) Womens Hospital Eppendorf, University of Hamburg, Hamburg, Germany	Case series Level IV diagnostic evidence CX P2 Q3	N = 62 patients with sporadic PH N = 7 first-degree relatives of 2 index cases	*Inclusion:* Patients who underwent surgery, 1995–96, for sporadic PH at the Ludwig Maximilian University, the Hospital Martha-Maria in Munich, or the Benjamin Franklin University in Berlin *Exclusion:* Not stated	*Intervention*: SSCP and DNA sequencing	Diagnostic yield	N/A
(Cascon et al 2009) Hereditary	Case series Level IV diagnostic	N = 237 consecutively registered patients diagnosed with PHs or PGLs	*Inclusion:* Consecutively registered patients clinically diagnosed with functioning or	*Intervention:* Complete genetic characterisation	Diagnostic yield	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
Endocrine Cancer Group, Madrid, Spain	evidence CX P2 Q3	n = 45 patients with hereditary syndromes n = 192 patients with sporadic disease	non-functioning PHs in public Spanish hospitals, 1995–2008, for genetic testing *Exclusion:* Not stated			
(Castellano et al 2006) University of Brescia and University of Turin, Italy	Case series Level IV diagnostic evidence CX P2 Q3	N = 45 patients with PHs or PGLs n = 35 with PHs n = 7 with PGLs n = 3 with HNPs	*Inclusion:* Patients with PHs or PGLs referred to the hypertension centres of the University of Brescia and the University of Turin in the past 20 years *Exclusion:* Syndromic patients with MEN 2, VHL or neurofibromatosis features	*Intervention:* DNA sequencing	Diagnostic yield	N/A
(Catapano et al 2005) Istituto di Ricovero e Cura a Carattere Scientifico, San Giovanni Rotondo, Italy	Case series Level IV diagnostic evidence CX P2 Q3	N = 14 patients with CNS HBs	*Inclusion:* Southern Italian patients with CNS HBs that were operated on, 1993–2002, and gave informed consent *Exclusion:* Family history of VHL, other clinical manifestations of VHL disease	*Intervention:* DHPLC, DNA sequencing	Diagnostic yield Costs	N/A
(Chen et al 1995) Frederick Cancer Research and Development Center, Frederick, Maryland, USA	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 114 unrelated patients diagnosed with VHL syndrome	*Inclusion:* Affected members of apparently unrelated VHL families from the USA, Canada, Puerto Rico and Hawaii *Exclusion:* Not stated	*Intervention*: SSCP and DNA sequencing, Southern blotting *Comparator:* Diagnostic criteria for VHL as described by Hosoe et al (1990)	Diagnostic accuracy	N/A
(Chen et al 1996) Frederick Cancer Research and	Case series Level IV diagnostic evidence	N = 12 members of a large Pennsylvanian VHL type 2A PH family	*Inclusion:* Members of a large Pennsylvanian VHL type 2A PH family (#1190) of German	*Intervention*: SSCP and DNA sequencing *Comparator:*	Diagnostic yield	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
Development Center, Frederick, Maryland, USA	CX P1 Q3	n = 5 diagnosed with VHL syndrome	origin containing 19 affected subjects *Exclusion:* Not stated	Clinical diagnosis according to Chen et al (1995)		
(Cho et al 2009) Samsung Medical Center, Seoul, Korea	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 26 patients suspected of having VHL syndrome n = 15 patients with VHL diagnosis n = 11 patients with VHL-associated symptom	*Inclusion:* Unrelated patients referred to Medical Centre, October 2001 – September 2006, with suspicion of VHL disease *Exclusion:* Not stated	*Intervention*: DNA sequencing, MLPA *Comparator:* Diagnosis of VHL syndrome requires at least two HBs or a single HB in association with a visceral manifestation (RCC, PH or multiple pancreatic cysts), or a single HB, PH, multiple pancreatic cysts or RCC in a patient with family history	Diagnostic accuracy	N/A
(Choo et al 2004) National Institutes of Health, Bethesda, Maryland, USA	Case series Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 175 patients or individuals from families with confirmed VHL disease or who were at risk n = 129 patients with confirmed VHL (clinical and GT positive) n = 46 relatives (clinical and GT negative)	*Inclusion:* Participants were prospectively enrolled if they were patients or individuals from families with confirmed VHL disease or who were at risk for VHL disease based on their family history of clinical manifestations *Exclusion:* Abnormal middle ear function, history of significant noise exposure, whole-brain radiation therapy, chemotherapy, sudden hearing loss for any reason, closed head trauma with loss of consciousness	*Intervention*: The screening protocol included evaluations by an audiologist, medical geneticist, urological oncologist, ophthalmologist, neurosurgeon and neuro-otologist. Laboratory studies included standard serum chemistry, complete blood counts, thyroid panels and urinalysis, as well as 24-hour urine screening for catecholamines	Prevalence	N/A
(Choyke et al 1997) National Cancer	Case series Level IV interventional	N = 56 consecutive affected men from the VHL Clinic n = 34 VHL mutation status	*Inclusion:* Male patient with confirmed VHL; with follow-up of at least 2 years	*Intervention*: Details of genetic testing not reported	Prevalence	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
Institutes of Health, Frederick, Maryland, USA	evidence Medium quality (NHS CRD = 4/6)	known	*Exclusion:* Not stated	*Comparator:* Clinical screening including CT scans and ultrasound of the abdomen, MRI of the brain and spine, audiology and ophthalmoscopy		
(Ciotti et al 2009) University of Genova and the Azienda Ospedaliera Universitaria San Martino of Genova, Genova, Italy	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 43 patients suspected to have VHL syndrome n = 27 classic VHL cases n = 3 patients with non-classic VHL meeting diagnostic criteria n = 13 patients not meeting diagnostic criteria for VHL syndrome	*Inclusion:* 43 index cases referred to the Service of Medical Genetics, Genova, for germ-line mutation analysis in the *VHL* gene, 1995–2008 *Exclusion:* Not stated	*Intervention*: Real-time Q-PCR *Comparator:* Diagnostic criteria for classic and non-classic VHL disease, and VHL-associated disease not meeting criteria as described by Hes et al (2007)	Diagnostic accuracy	N/A
(Corcos et al 2008) Hopital Beaujon, Clichy, France	Comparative study Level III-2 diagnostic evidence CX P2 Q2	N = 35 VHL patients with pancreatic endocrine tumours from 29 families	*Inclusion:* Consecutive VHL patients with well-documented pancreatic endocrine tumours *Exclusion:* Not stated	*Intervention*: DNA sequencing, Southern blotting *Comparator:* Criteria not reported	Diagnostic accuracy	N/A
(Cotesta et al 2009) University Sapienza, Rome, Italy	Comparative study Level III-2 diagnostic evidence CX P1 Q1	N = 30 PH patients n = 4 patients with VHL n = 17 patients with other syndromes n = 9 sporadic cases	*Inclusion:* Patients with PH who were referred to the Day Hospital of Secondary Hypertension, 1992–2008 *Exclusion:* Not stated	*Intervention*: DNA sequencing *Comparator:* Personal and family history, clinical examination including blood pressure, biochemical analysis, abdominal CT or MRI scan	Diagnostic accuracy	N/A
(Crossey et al	Case series	N = 3 families with PH in	*Inclusion:*	*Intervention*:	Diagnostic	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
1995) University of Cambridge, Cambridge, UK	Level IV diagnostic evidence CX P2 Q3	more than 1 relative and with no other signs of VHL disease	Not stated *Exclusion:* Not stated	SSCP and DNA sequencing, Southern blotting	yield	
(Cruz et al 2007) da Universidade Estadual Paulista — UNESP, Botucatu, São Paulo, Brazil	Case series Level IV diagnostic evidence CX P1 Q3	N = 7 family members of 2 siblings diagnosed with PHs	*Inclusion:* Family members of 2 siblings diagnosed with PHs *Exclusion:* Not stated	*Intervention*: DNA sequencing	Diagnostic yield	N/A
(Cybulski et al 1999) Pomeranian Medical Academy, Szczecin, Poland	Comparative study Level III-2 diagnostic evidence CX P2 Q2	N = 16 patients with VHL syndrome n = 5 cases where large deletions of the *VHL* gene were identified previously by Southern blotting n = 11 cases were selected from a series of 23 unrelated VHL patients, after sequencing of the gene failed to identify a germ-line VHL mutation	*Inclusion:* Patients diagnosed with VHL syndrome and have, or are likely to have, a large deletion of the *VHL* gene *Exclusion:* Not stated	*Intervention*: Long PCR *Comparator:* Clinical diagnosis based on pedigree and clinical criteria	Diagnostic accuracy	N/A
	Case series Level IV diagnostic evidence CX P1 Q3	N = 24 relatives of 9 VHL patients with deletions identified by means of long PCR	Not reported	*Intervention*: Long PCR	Diagnostic yield	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
(Cybulski et al 2002) Pomeranian Medical Academy, Szczecin, Poland	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 34 patients diagnosed with VHL syndrome belonging to one of 34 VHL families	*Inclusion:* 1 member from 32 families that presented without PH(VHL type 1) and 2 families with PH (VHL type 2) *Exclusion:* Not stated	*Intervention*: DNA sequencing, long PCR, MLPA *Comparator:* Diagnosed on the basis of clinical criteria	Diagnostic accuracy	N/A
(De Krijger et al 2006) Josephine Nefkens Institute, Rotterdam, Netherlands	Comparative study Level III-2 diagnostic evidence CX P1 Q1	N = 10 paediatric PH patients n = 2 diagnosed with VHL	*Inclusion:* Paediatric patients with PHs selected for mutation analysis for *RET*, *VHL*, *SDHB* and *SDHD* without knowing the patient or family history *Exclusion:* Not stated	*Intervention*: DGGE, SSCP and DNA sequencing *Comparator:* Criteria not reported	Diagnostic accuracy	N/A
(Dollfus et al 2002) Hopitaux Universitaires de Strasbourg, Strasbourg, France	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 196 VHL patients n = 91 patients with ocular manifestations n = 105 patients without ocular manifestations	*Inclusion:* Patients registered in the French VHL database, 1996–99, that met the diagnostic criteria for VHL and agreed to genetic testing *Exclusion:* Patients with solitary retinal HB and no mutation detected in the *VHL* gene	*Intervention*: SSCP and DNA sequencing, Southern blotting *Comparator:* Criteria for VHL defined by the presence of two major manifestations, including at least one CNS or retinal HB, one major manifestation and a positive family history, or an isolated clinical feature with mutation in the *VHL* gene	Diagnostic accuracy	N/A
	Case series Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 211 patients registered in the French VHL database that met the diagnostic criteria for VHL n = 196 patients that agreed	*Inclusion:* Patients registered in the French VHL database, 1996–99, that met the diagnostic criteria for VHL *Exclusion:*	*Intervention*: A questionnaire inquiring about the ocular and general status of the patients was sent to ophthalmologists treating patients	Prevalence Change in manage-ment	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
		to genetic testing n = 149 patients that had a VHL mutation	Patients with solitary retinal HB and no mutation detected in the *VHL* gene	with VHL		
(Erlic et al 2009) Albert-Ludwigs-University, Freiburg, Germany	Comparative study Level III-2 diagnostic evidence CX P1 Q1	N = 1,149 index cases presenting with symptomatic PH n = 65 diagnosed with VHL	*Inclusion:* Index cases from the European-American Phaeochromocytoma Registry who presented with clinical PH at the time of registration *Exclusion:* Other members of the same family; patients who developed PH after molecular genetic testing was done; the many families in the Black Forest region in Germany, who carry an identical VHL mutation, due to a founder effect	*Intervention* MLPA, PCR-based mutation scanning *Comparator:* Based on clinical retrospective data and family history, we defined as syndromic cases all patients fulfilling the clinical criteria for the diagnosis of NF 1, VHL, and MEN 2 syndromes	Diagnostic accuracy	N/A
(Erlic et al 2010) Albert-Ludwigs-University, Freiburg, Germany	Case series Level IV diagnostic evidence CX P1 Q3	N = 101 unrelated registrants in the German NET-Registry that had ICTs of the pancreas	*Inclusion:* All NET registrants, 1 November 2005 – 31 October 2008, that had ICTs of the pancreas *Exclusion:* Not stated	*Intervention*: HPLC and DNA sequencing, MLPA	Diagnostic yield	N/A
	Case series Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 485 registrants from the VHL-Registry that underwent pancreatic imaging 52/485 had ICTs 13/485 had malignant ICTs 171/485 had pancreatic cysts	*Inclusion:* VHL registrants with a proven germ-line mutation of the *VHL* gene and ICTs of the pancreas *Exclusion:* Not stated	*Intervention* Clinical diagnosis of solid pancreatic tumours by histological confirmation and/or imaging	Prevalence	N/A
(Evans et al 1997) Paterson Institute	Case series Level IV interventional	N = 73 at-risk members of VHL families	*Inclusion:* All at-risk subjects from VHL families in	*Intervention* Method not reported	Change in manage-	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
for Cancer Research, Christie Hospital, Manchester, UK	evidence High quality (NHS CRD = 5/6)		which genetic testing has been possible *Exclusion:* Not stated		ment	
(Fisher et al 2002) Stanford University School of Medicine, Palo Alto, CA, USA	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 6 children with cerebellar HB n = 2 diagnosed with VHL n = 4 sporadic cases	*Inclusion:* Tumour registries from four paediatric referral centres (Lucile Salter Packard Children's Hospital, Children's Hospital of Los Angeles, the Johns Hopkins Hospital and Children's Hospital of Buffalo) were screened, 1990–99, for all patients aged ≤ 21 years at diagnosis of a cerebellar HB *Exclusion:* Not stated	*Intervention* DNA sequencing, Southern blotting *Comparator:* Medical records were reviewed. Patients and families were questioned about and examined for stigmata of VHL disease: retinal angioma, visceral cysts, PH, pancreatic ICT, endolymphatic sac tumour or clear-cell RCC	Diagnostic accuracy	N/A
(Franke et al 2009) University Medical Center Freiburg, Freiburg, Germany	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 308 index cases with VHL syndrome	*Inclusion:* Patients on the Freiburg VHL registry of 308 unrelated familial or sporadic VHL index cases, fulfilling either distinct clinical criteria of VHL or having a clear history of VHL in their family *Exclusion:* Not stated	*Intervention*: DNA sequencing, MLPA *Comparator:* All patients are registered with demographic data and detailed clinical data.	Diagnostic accuracy	N/A
(Garcia et al 1997) Hospital de la Santa Creu i Sant Pau, Barcelona, Spain	Case series Level IV diagnostic evidence CX P1 Q3	N = 15 family members n = 5 members with suspected VHL syndrome n = 10 asymptomatic family members	*Inclusion:* Members of a family with suspected VHL disease *Exclusion:* Not stated	*Intervention*: Restriction-site polymorphism	Diagnostic yield	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
(Gergics et al 2009) Semmelweis University, Budapest, Hungary	Comparative study Level III-2 diagnostic evidence CX P1 Q1	N = 11 patients with VHL symptoms N = 37 patients with phaeochromocytoma	*Inclusion:* A patient from 1 of 7 unrelated VHL families Unrelated patient with confirmed, sporadic unilateral PHs evaluated, 1998–2008 *Exclusion:* Not stated	*Intervention*: DNA sequencing, real-time PCR, MLPA *Comparator:* Clinical diagnosis based on medical history, physical examination, abdominal ultrasonography, CT or MRI, brain and spinal cord MRI, ophthalmologic examination and laboratory tests	Diagnostic accuracy	N/A
	Case series Level IV diagnostic evidence CX P1 Q3	N = 32 family members n = 24 family members of VHL patients n = 8 relatives of 3 VHL mutation +ve PH patients	*Inclusion:* Not reported *Exclusion:* Not stated	*Intervention*: DNA sequencing, real-time PCR, MLPA	Diagnostic yield	N/A
(Gimenez-Roqueplo et al 2003) Hopital Europeen Georges Pompidou, College de France, Paris, France	Case series Level IV diagnostic evidence CX P2 Q3	N = 84 patients with apparently sporadic PH (no family history or clinical signs of familial or syndromic disease)	*Inclusion:* Patients with apparently sporadic PH recruited as part of a cohort of patients with PHs in the COMETE network *Exclusion:* Patients with a personal or family history, or any clinical signs indicative of HNP, MEN 2A and 2B, VHL disease or NF1	*Intervention*: DNA sequencing	Diagnostic yield	N/A
(Glasker et al 1999) Albert-Ludwigs-University,	Comparative study Level III-2 diagnostic evidence CX	N = 141 patients with symptomatic HBs of the CNS n = 94 diagnosed with VHL n = 81 with VHL germ-line	*Inclusion:* All patients with HBs admitted to the hospital, 1983–98, and patients referred for genetic testing of the *VHL* gene	*Intervention*: Southern blotting, SSCP and DNA sequencing *Comparator:*	Diagnostic accuracy Costs	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
Freiburg, Germany	P1 Q1	mutations	*Exclusion:* Not stated	Clinical diagnosis: clinical information of intracranial as well as spinal findings, detailed data from ophthalmological and visceral findings, and an extensive pedigree analysis		
(Glasker et al 2001) Albert-Ludwigs-University, Freiburg, Germany	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 31 patients with CNS HB n = 18 patients with VHL disease n = 13 patients with sporadic tumours	*Inclusion:* Patients with CNS HB, who were consecutively treated 1993–97 at the Freiburg University Medical Centre *Exclusion:* Not stated	*Intervention*: SSCP and DNA sequencing, Southern blotting *Comparator:* Criteria not reported	Diagnostic accuracy	N/A
(Gläsker et al 2005) Albert-Ludwigs-University, Freiburg, Germany	Comparative study Level III-2 diagnostic evidence CX P1 Q3	N = 6 patients with CNS HBs requiring surgery n = 4 patients with VHL disease n = 2 patients with sporadic tumours	*Inclusion:* Patients with CNS HB treated, 1983–2003, at the Freiburg University Medical Centre, who exhibited an entirely extradural location of the tumour during surgery *Exclusion:* Asymptomatic spinal verve tumours not requiring surgery	*Intervention*: Method not reported *Comparator:* Criteria not reported	Diagnostic accuracy	N/A
(Glavac et al 1996) Laboratory of Molecular Pathology, Medical Faculty, Ljubljana, Slovenia	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 65 patients with VHL syndrome N = 15 asymptomatic family members with a VHL mutation	*Inclusion:* Affected members of 65 families diagnosed with VHL syndrome Asymptomatic family members from 8 large VHL families *Exclusion:* Not stated	*Intervention*: DNA sequencing, SSCP analysis, Southern blotting *Comparator:* Clinical diagnosis by standard criteria Clinical screening of VHL mutation +ve relatives	Diagnostic accuracy	N/A
	Case series	N = 50 asymptomatic family	*Inclusion:*	*Intervention*:	Diagnostic	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
	Level IV diagnostic evidence CX P1 Q3	members	Asymptomatic family members from 8 large VHL families *Exclusion:* Not stated	DNA sequencing, SSCP analysis, Southern blotting	yield	
(Gomy et al 2010) University of Sao Paulo, Ribeirao Preto, Brazil	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 10 VHL families n = 9 VHL families n = 1 sporadic VHL-associated lesion	*Inclusion:* Families that were referred to the Cancer Genetic Counselling Service and fulfilled the diagnostic criteria for VHL disease, and 1 patient with a sporadic cerebellar HB *Exclusion:* Not stated	*Intervention*: DNA sequencing, MLPA *Comparator:* Clinical diagnosis using conventional diagnostic criteria	Diagnostic accuracy	N/A
(Green 1996) Memorial University of Newfoundland, Canada	Case series Level IV diagnostic evidence CX P1 Q3	N = 28 family members with no clinical signs of VHL disease	*Inclusion:* Affected members of 65 families diagnosed with VHL syndrome Asymptomatic family members from 8 large VHL families *Exclusion:* Not stated	*Intervention*: Restriction-site polymorphism	Diagnostic yield Cost–benefit analysis	N/A
(Gross et al 1996) Hadassah University Hospital, Jerusalem, Israel	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 5 first-degree family members with VHL mutations n = 1 with signs of clinical disease n = 4 with no signs of disease	*Inclusion:* Kindred of a Jewish VHL family of Kurdish origin with 3 generations *Exclusion:* Not stated	*Intervention*: SSCP and DNA sequencing *Comparator:* Criteria not reported	Diagnostic accuracy	N/A
	Case series Level IV diagnostic evidence	N = 19 kindred from 1 family n = 4 diagnosed with VHL n = 15 asymptomatic first-	*Inclusion:* Kindred of a Jewish VHL family of Kurdish origin with 3 generations	*Intervention*: SSCP and DNA sequencing	Diagnostic yield	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
	CX P1 Q3	degree relatives	*Exclusion:* Not stated			
(Hattori et al 2006) Yokohama City University Graduate School of Medicine, Yokohama, Japan	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 31 patients n = 27 patients from 19 families (16 VHL type 1, 2 VHL type 2A and 1 VHL type 2B) n = 4 unrelated, solitary patients with single VHL manifestations	*Inclusion:* Children with PH that had attended various German hospitals and had been registered in the GPOH-MET 97 trial *Exclusion:* Not stated	*Intervention*: DNA sequencing, real-time Q-PCR *Comparator:* All individuals were clinically diagnosed with the classical VHL criteria	Diagnostic accuracy	N/A
(Hering et al 2006) Institute for Human Genetics and Anthropology, UKJ, Jena, Germany	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 14 children with PH n = 9 with VHL syndrome	*Inclusion:* Patients diagnosed with VHL syndrome or with VHL-associated manifestations *Exclusion:* Not stated	*Intervention*: DNA sequencing, MLPA *Comparator:* Criteria not reported	Diagnostic accuracy	N/A
(Hes et al 2000a) University Medical Centre, Utrecht, The Netherlands	Case series Level IV diagnostic evidence CX P2 Q3	N = 84 HB patients with a single HB N = 4 patients with multiple HBs but no other evidence of VHL disease	*Inclusion:* UK and Dutch patients with single or multiple HBs referred for DNA diagnosis, 1996–99. All patients underwent clinical screening for VHL-associated tumours with negative findings *Exclusion:* Not stated	*Intervention*: DNA sequencing, Southern blotting	Diagnostic yield	N/A
(Hes et al 2000b) University Medical Centre, Utrecht,	Comparative study Level III-2 diagnostic evidence	N = 11 patients from 1 VHL family n = 3 diagnosed with VHL	*Inclusion:* Patients from 1 family referred to the Department of Medical Genetics for	*Intervention*: Southern blotting *Comparator:*	Diagnostic accuracy	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
The Netherlands	CX P1 Q1	syndrome n = 8 asymptomatic family members	germ-line mutation analysis in the *VHL* gene *Exclusion:* Not stated	Clinical screening including ophthalmoscopy, yearly alternate MRI and ultrasonography of the abdomen, and (at various frequencies) MRI of the CNS (Hes & Feldberg 1999)		
(Hes 2000) University Medical Centre, Utrecht, The Netherlands	Case series Level IV diagnostic evidence CX P1 Q3	N = 1,100 VHL patients and family members n = 100 VHL patients n = 200 first-degree relatives n = 800 second-degree relatives	*Inclusion:* Patients diagnosed with VHL syndrome and their relatives *Exclusion:* Not stated	*Intervention*: Genetic testing plus annual screening	Cost-effective-ness	N/A
(Hes et al 2007) University Medical Center Utrecht, Utrecht, The Netherlands.	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 146 n = 30 index patients from different families with classic VHL disease n = 8 isolated patients with classic VHL disease n = 17 index patients from different families with non-classic VHL disease: 14 had multiple or familial HB in one organ system, and 3 had HB with a close relative with RCC n = 91 probands with single-organ involvement: 83 sporadic patients, 5 familial cases with PH only and 3 with hereditary RCC	*Inclusion:* 146 probands were ascertained according to the DNA eligibility criteria, 1994–2001 *Exclusion:* Not stated	*Intervention*: DNA sequencing, Southern blotting, MLPA *Comparator:* Clinical diagnosis of at least one VHL manifestation in a patient with familial VHL disease, or at least two or more HBs or a single HB in combination with a typical visceral lesion in a sporadic patient Classic VHL disease: at least three typical VHL tumours with involvement of at least two distinct organ systems Non-classic VHL disease	Diagnostic accuracy	N/A
(Ho et al 2003)	Systematic review	Narrative review: patients	*Inclusion:*	*Intervention*:	Change in	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
Canadian Coordinating Office for Health Technology Assessment, Ottawa, Canada	Level IV interventional evidence High quality (SIGN 2008)	with VHL syndrome and a diagnosed VHL mutation, and their families	457 relevant articles on inherited cancer predisposing syndromes *Exclusion:* Articles that did not meet inclusion criteria	Genetic testing using any method	manage-ment	
(Hoebeeck et al 2005) Ghent University Hospital, Ghent, Belgium	Comparative study Level III-2 diagnostic evidence CX P1 Q3	N = 17 individuals from 15 unrelated families	*Inclusion:* Mainly selected for having a (partial or entire) *VHL* gene deletion (14/17), as determined previously by Southern blotting *Exclusion:* Not stated	*Intervention*: DNA sequencing, real-time Q-PCR, Southern blotting *Comparator:* Clinical diagnosis based on a single retinal or cerebellar HB, RCC or PH, and a positive familial history, or two or more HBs, or HB combined with a further typical VHL tumour	Diagnostic accuracy	N/A
(Huang et al 2004) Renji Hospital, Shanghai Second Medical University, Shanghai, China	Comparative study Level III-2 diagnostic evidence CX P1 Q1	N = 6 VHL mutation positive asymptomatic kindred	*Inclusion:* Members of a large kindred with VHL disease *Exclusion:* Not stated	*Intervention*: DNA sequencing, Southern blotting *Comparator:* Clinical screening including ophthalmologic evaluation, MRI of the CNS, abdominal ultrasound and CT	Diagnostic accuracy	N/A
	Case series Level IV diagnostic evidence CX P1 Q3	N = 27 kindred n = 9 kindred with VHL syndrome n = 18 asymptomatic relatives	*Inclusion:* Members of a large kindred with VHL disease *Exclusion:* Not stated	*Intervention*: DNA sequencing, Southern blotting	Diagnostic yield	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
(Huang et al 2007) Cathay General Hospital, Taipei, Sijhih City, Taiwan	Case series Level IV diagnostic evidence CX P2 Q3	N = 38 subjects from three unrelated families	*Inclusion:* Subjects from three unrelated families (F01, F02 and F03) attending the Department of Surgery *Exclusion:* Not stated	*Intervention*: DNA sequencing, Southern blotting	Diagnostic yield	N/A
(Joly et al 2011) Université Paris Descartes, Paris, France	Case series Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 176 patients who had VHL disease with renal involvement and were VHL mutation positive from a total of 112 families n = 113 who had treatment for RCC	*Inclusion:* All consecutive patients with VHL referred to the hospital, January 1988 – 31 January 3 2009, who tested positive for VHL germ-line mutations *Exclusion:* Not stated	*Intervention*: Not reported *Comparator:* Patient charts were reviewed retrospectively. Data were collected on age, gender, organs affected by VHL and VHL mutation type	Effective-ness	6.3 ± 5.4 years
(Kang et al 2005) Cancer research Institute, Seoul National University, Seoul, Korea	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 15 patients n = 11 diagnosed with VHL syndrome from 7 families n = 2 cases from 1 family with PH n = 2 sporadic PH patients	*Inclusion:* Korean VHL and PH patients *Exclusion:* Not stated	*Intervention*: DNA sequencing, long-range PCR *Comparator:* Clinical diagnosis (criteria not stated)	Diagnostic accuracy	N/A
	Case series Level IV diagnostic evidence CX P1 Q3	N = 3 first-degree relatives of 1 VHL patient	Not reported	*Intervention*: DNA sequencing, long-range PCR	Diagnostic yield	N/A
(Kanno et al 1996) Yokohama City	Comparative study Level III-2 diagnostic	N = 8 individuals with VHL syndrome belonging to 1 of 5	*Inclusion:* Individuals belonging to 1 of 5 families	*Intervention*: SSCP and DNA sequencing	Diagnostic accuracy	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
University School Medicine, Yokohama, Japan	evidence CX P1 Q2	VHL families	with VHL syndrome *Exclusion:* Not stated	*Comparator:* Clinical diagnosis (criteria not stated)		
	Case series Level IV diagnostic evidence CX P1 Q3	N = 17 asymptomatic relatives	Not reported	*Intervention*: SSCP and DNA sequencing	Diagnostic yield	N/A
(Kim et al 2009b) Yonsei University College of Medicine, Seoul, Korea	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 12 patients with VHL	*Inclusion:* Patients that were diagnosed with VHL in the institute, January 1996 – July 2008 *Exclusion:* Not stated	*Intervention*: Method not reported *Comparator:* Clinical diagnosis included ophthalmoscopy, CT or ultrasonography of the abdomen, CT and MRI of the head and spine	Diagnostic accuracy	N/A
(Klein et al 2001) Dr. Margarete Fischer-Bosch-Institut für Klinische Pharmakologie, Stuttgart, Germany	Comparative study Level III-2 diagnostic evidence CX P2 Q1	N = 79 patients n = 43 unrelated VHL patients with known VHL mutations n = 36 patients with unknown VHL mutation status 20 with suspected VHL 5 diagnosed with VHL 11 with VHL-associated manifestation	*Inclusion:* Unrelated VHL patients with different previously established VHL germ-line mutations Patients with unknown VHL germ-line status referred from various physicians and human genetics departments in Europe for VHL mutation analysis *Exclusion:* Not stated	*Intervention*: DHPLC and DNA sequencing *Comparator:* Clinical diagnosis (criteria not stated)	Diagnostic accuracy	N/A
(Krawczyk et al	Case series	N = 53 patients with PH	*Inclusion:*	*Intervention*:	Diagnostic	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
2010) Skłodowska-Curie Memorial Cancer Centre, Poland	Level IV diagnostic evidence CX P1 Q3		Patients with diagnosis of PH referred for genetic evaluation *Exclusion:* Not stated	SSCP and DNA sequencing	yield	
(Kreusel et al 2000) Klinikum Benjamin Franklin, Berlin, Germany	Comparative study Level III-2 diagnostic evidence CX P1 Q1	N = 37 non-related patients presenting with capillary retinal angioma n = 29 diagnosed with VHL n = 8 sporadic retinal angioma cases	*Inclusion:* Non-related patients with retinal angiomatosis presenting in the Benjamin Franklin University Eye Clinic, Berlin, 1988–99 *Exclusion:* Not stated	*Intervention*: SSCP and DNA sequencing, Southern blotting *Comparator:* Clinical diagnosis using standard clinical criteria as described by Melmon & Rosen (1964)	Diagnostic accuracy	N/A
	Case series Level IV diagnostic evidence CX P1 Q3	N = 22 first-degree relatives (20 parents and 2 siblings) of VHL patients with VHL mutation	*Inclusion:* Not reported *Exclusion:* Not stated	*Intervention*: SSCP and DNA sequencing, Southern blotting	Diagnostic yield	N/A
(Kreusel et al 2006) Augen-Zentrum, DRK-Kliniken Westend, Berlin, Germany	Case series Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 57 consecutive patients presenting with capillary retinal angiomatosis resulting from VHL disease n = 43 patients with clinical and genetic diagnosis n = 12 with clinical diagnosis only n = 2 with genetic diagnosis only	*Inclusion:* Non-related patients with retinal angiomatosis presenting in the Benjamin Franklin University Eye Clinic, Berlin, 1988–2002, and in the Augenklinik Berlin-Marzahn, 2002–04 *Exclusion:* Not stated	*Intervention*: Ocular disease was analysed by a review of the patient's medical history, review of the medical charts, best-corrected visual acuity, slit-lamp examination, funduscopy, fundus photography and fluorescein angiography	Prevalence Effective-ness	7.3 ± 4.9 years
(Kreusel et al	Comparative study	N = 11 patients with a solitary juxtapapillary	*Inclusion:* Non-related patients who presented	*Intervention*:	Diagnostic	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
2007) Augen-Zentrum, DRK-Kliniken Westend, Berlin, Germany	Level III-2 diagnostic evidence CX P1 Q2	capillary retinal angioma n = 6 clinically diagnosed with VHL	with a solitary juxtapapillary capillary retinal angioma at 2 eye clinics in Germany, 1974–98 *Exclusion:* Not stated	Not reported *Comparator:* Clinical diagnosis using personal and family history, and medical screening including MRI of the brain and spinal cord, urine catecholamines, and abdominal sonography or CT	accuracy	
(Li et al 1998) Kurolinsku Hospital, Stockholm Sweden	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 10 unrelated patients with VHL syndrome	*Inclusion:* Patients diagnosed with VHL syndrome from unrelated families *Exclusion:* Not stated	*Intervention*: DNA sequencing, Southern blotting *Comparator:* Clinical diagnosis using standard clinical criteria as described by Melmon & Rosen (1964)	Diagnostic accuracy	N/A
(Libutti et al 2000) National Cancer Institutes of Health, Bethesda, MD, USA	Comparative study Level III-2 diagnostic evidence CX P1 Q1	N = 44 VHL patients from 36 families diagnosed with PNETs	*Inclusion:* Patients with VHL disease and a PNET who were evaluated, December 1988 – December 1999, at the Clinical Centre *Exclusion:* Not stated	*Intervention*: DNA sequencing, Southern blotting *Comparator:* Clinical diagnosis of PNET was made either by pathologic analysis of tissue specimens or by characteristic radiographic appearance on CT and MRI	Diagnostic accuracy	N/A
	Case series Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 188 VHL mutation positive families	*Inclusion:* Patients with VHL disease who were evaluated, December 1988 – December 1999, at the Clinical Centre *Exclusion:* Not stated	*Intervention*: DNA sequencing, Southern blotting *Comparator:* Clinical diagnosis of PNET was made either by pathologic analysis of tissue specimens or	Prevalence	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
				by characteristic radiographic appearance on CT and MRI		
(Magnani et al 2001) San Raffaele Clinica Molecular Biology Laboratory, Policlinico Hospital, University of Milan, Italy	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 18 patients from the San Raffaele Clinica and Policlinico Hospital n = 4 patients with family history of VHL n = 14 VHL patients with no family history of disease n = 21 first-degree relatives of 5 index cases n = 9 symptomatic or with clinical signs of disease n = 2 with no clinical signs of disease	*Inclusion:* Taken from a sample of 39 patients and their relatives from the San Raffaele Clinica and Policlinico Hospital, Milan *Exclusion:* Not stated	*Intervention*: DG-DGGE analysis and DNA sequencing, Southern blotting *Comparator:* Criteria as for Seizinger et al (1991)	Diagnostic accuracy	N/A
(Maher et al 1996) Cambridge University Department of Pathology, Cambridge, UK	Comparative study Level III-2 diagnostic evidence CX P1 Q1	N = 138 unrelated patients clinically diagnosed with VHL disease	*Inclusion:* Unrelated patients clinically diagnosed with VHL disease and that had proven retinal angioma, CNS HB, RCC, or PH *Exclusion:* Not stated	*Intervention*: Southern blotting, SSCP and heteroduplex analysis, DNA sequencing *Comparator:* Clinical diagnosis according to standard criteria as described by Maher et al (1990)	Diagnostic accuracy	N/A
(Mannelli et al 2009) University of Florence, Florence, Italy	Case series Level IV diagnostic evidence CX P2 Q3	N = 501 consecutive patients (adults and children) with PHs and/or PGLs	*Inclusion:* Consecutive patients (adults and children) with PHs and/or PGLs that visited one of the 17 endocrinology or hypertension centres of the Italian Phaeochromocytoma/Paraganglioma Network, 2003–07 inclusive *Exclusion:*	*Intervention*: SSCP and DNA sequencing, Southern blotting	Diagnostic yield	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
			Not stated			
(Manski et al 1997) National Institutes of Health, Bethesda, Maryland, USA	Comparative study Level III-2 diagnostic evidence CX P1 Q1	N = 10 patients diagnosed with VHL and endolymphatic sac tumours	*Inclusion:* Eligible participants whose brain MRIs were available for review and were diagnosed with VHL and endolymphatic sac tumour *Exclusion:* Not stated	*Intervention*: SSCP and DNA sequencing, Southern blotting *Comparator:* VHL screening included family and personal medical history, physical examination, indirect ophthalmoscopy, MRI of brain and spine, abdominal CT scan in adults, abdominal ultrasonography in children < 18 years of age and 24-hour urinary catecholamine levels	Diagnostic accuracy	N/A
	Case series Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 374 patients whose brain MRIs were available for review n = 121 patients fulfilled the strict criteria VHL n = 66 consecutive patients from the VHL clinic, without additional screening criteria were studied n = 49 patients with proven VHL	*Inclusion:* Eligible participants whose brain MRIs were available for review *Exclusion:* Not stated	*Intervention*: VHL screening included family and personal medical history, physical examination, hearing tests, MRI of brain	Prevalence	N/A
(Marcos et al 2002) National Institutes of Health, Bethesda, MD, USA	Comparative study Level III-2 diagnostic evidence CX P1	N = 25 VHL patients with histologically confirmed PNETs	*Inclusion:* Patients were identified from an electronic database containing clinical and imaging data from 450 patients with VHL disease, August 1990 – January 2001	*Intervention*: Details of genetic testing not reported *Comparator:* Clinical diagnosis included comprehensive evaluation,	Diagnostic accuracy	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
	Q1		*Exclusion:* Not stated	including physical and clinical examination, and laboratory testing		
(Martin et al 1998a) Princess Margaret hospital for Children, Perth, WA, Australia.	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 9 relatives (5 first- and 4 second-degree relatives) from 1 family n = with VHL disease n = 7 with no signs of disease	*Inclusion:* Members of a family with familial PH who carry a mutation in the *VHL* gene *Exclusion:* Not stated	*Intervention*: As described by Martin et al (1996) *Comparator:* Clinical monitoring including ophthalmological examinations	Diagnostic accuracy	N/A
(Martin et al 1998b) Princess Margaret hospital for Children, Perth, WA, Australia	Comparative study Level III-2 diagnostic evidence CX P1 Q1	N = 16 patients from Australia and New Zealand n = 14 probable VHL n = 2 isolated PHs	*Inclusion:* Patients with any clinical manifestations suggestive of VHL disease, referred to GSWA, September 1994 – December 1997 *Exclusion:* Not stated	*Intervention*: SSCP, DNA sequencing, Southern blotting *Comparator:* Clinical diagnosis (criteria not reported)	Diagnostic accuracy	N/A
(Meyer-Rochow et al 2009) Royal North Shore Hospital and University of Sydney, Sydney, Australia	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 74 patients with PH n = 6 VHL patients n = 18 patients with other familial syndromic disease n = 50 with no family history of disease	*Inclusion:* Patients with PH that were identified from the Cancer Genetics Kolling Institute of Medical Research Tumour Bank Database, 1993–2007 *Exclusion:* Not stated	*Intervention*: Genetic testing (method not reported) *Comparator:* Clinical stigmata of VHL disease determined according to the criteria of Melmon & Rosen (1964)	Diagnostic accuracy	N/A
(Mukhopadhyay et al 2002) St Bartholomew's Hospital, West Smithfield,	Comparative study Level III-2 diagnostic evidence CX P1	N = 17 patients belonging to 14 families with clinical stigmata VHL disease n = 9 patients with pancreatic cysts n = 1 patient with an islet cell	*Inclusion:* Consecutive patients with clinical stigmata VHL disease followed since 1988 *Exclusion:* Patients with a clinical diagnosis of NF1	*Intervention*: DHPLC analysis, DNA sequencing *Comparator:* Clinical information including family history, biochemistry,	Diagnostic accuracy	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
London, UK	Q2	tumour	and known carriers of *RET* germ-line mutations	radiology and histopathology		
	Case series Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 17 patients belonging to 14 families with clinical stigmata n = 9 patients with VHL disease pancreatic cysts	*Inclusion:* Consecutive patients with clinical stigmata VHL disease followed since 1988 *Exclusion:* Not stated	*Intervention*: Genetic testing (method not reported) *Comparator:* Clinical stigmata of VHL disease determined according to the criteria of Melmon & Rosen (1964)	Prevalence	N/A
(Neumann et al 1998) Albert Ludwigs University, Freiburg, Germany	Case series Level IV diagnostic evidence CX P1 Q3	N = 189 unselected sporadic RCC patients from a register of all patients surgically treated for RCC	*Inclusion:* Sporadic RCC patients: from a register of all patients surgically treated for RCC at the University of Freiburg between November 1, 1983 and October 31, 1994. *Exclusion:* Not stated	*Intervention*: SSCP and DNA sequencing, Southern Blotting	Diagnostic yield	N/A
	Case series Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 438 patients with RCC n = 63 subjects from 30 families with RCC (21/30 families had an identified VHL mutation) n = 375 patients with sporadic RCC	*Inclusion:* VHL patients: from VHL registers from institutions in Freiburg, Utrecht, Ilava and Hawaii that included families of German, Italian, Croatian, Slovakian, Dutch, Iranian and American ancestry that had been diagnosed with VHL and RCC Sporadic RCC patients: from a register of all patients surgically treated for RCC, 1 November 1983 – 31 October 1994 *Exclusion:*	*Intervention*: SSCP and DNA sequencing, Southern blotting *Comparator:* Abdominal ultrasonography, CT and MRI imaging of abdomen, brain and spine, ophthalmoscopy, 24-hour urine catecholamine assay, ultrasonography of the testes	Effective-ness	VHL patients: mean = 87.5 ± 9.2 months Sporadic RCC patients: mean = 60.3 ± 2.2 months

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
			Not stated			
(Neumann et al 1999) Albert Ludwigs University, Freiburg, Germany	Case series Level IV diagnostic evidence CX P1 Q3	N = 39 patients with PHs that underwent adrenal-sparing surgery	*Inclusion:* Patients with adrenal PH that have undergone adrenal-sparing surgery since 1985 *Exclusion:* Not stated	*Intervention*: Using current standards (Glavac et al 1996)	Diagnostic yield	N/A
	Case series Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 39 patients with PHs that underwent adrenal-sparing surgery n = 21 patients with VHL mutations n = 13 sporadic cases	*Inclusion:* Patients with adrenal PH that have undergone adrenal-sparing surgery since 1985 *Exclusion:* Not stated	*Intervention*: Using current standards (Glavac et al 1996) *Comparator:* Clinical diagnosis based on the presence of retinal angiomas or haemangiomas in the patient or a first-degree relative	Effective-ness Change in manage-ment	Mean = 73 months (range 16–179)
(Neumann et al 2002) Albert Ludwigs University, Freiburg, Germany	Case series Level IV diagnostic evidence CX P2 Q3	N = 271 patients with non-syndromic PH	*Inclusion:* Patients with PHs that were consecutively registered in the population registries of Freiburg, Germany, and Warsaw, Poland, and provided a pheripheral blood sample *Exclusion:* PHs discovered by clinical or genetic screening in asymptomatic patients, patients with NF1, patients with a family history (eg VHL, MEN 2)	*Intervention*: SSCP and DNA sequencing	Diagnostic yield	N/A
(Niemela et al 2000) Helsinki University Hospital, and the	Comparative study Level III-2 diagnostic evidence CX	N = 29 patients with retinal HB who agreed to a genetic test n = 8 with clinically definite	*Inclusion:* Consecutive patients with retinal HB treated, 1 January 1974 – 30 June 1998	*Intervention*: DNA sequencing *Comparator:* Clinical diagnosis included	Diagnostic accuracy	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
Finnish Cancer Registry, Helsinki, Finland	P1 Q2	VHL n = 21 patients with HBs but not VHL disease	*Exclusion:* Not stated	hospital records, family history, neurologic examination, enhanced MRI scan of the CNS, enhanced CT scan of the upper abdomen and ophthalmologic examination		
	Case series Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 36 patients with retinal HB n = 11 with clinically definite VHL n = 10 patients with clinically suspected VHL n = 15 patients with a single retinal HB	*Inclusion:* Consecutive patients with retinal HB treated, 1 January 1974 – 30 June 1998 *Exclusion:* Not stated	*Intervention:* Ophthalmologic examination included indirect ophthalmoscopy and Goldmann 3-mirror contact lens fundus examination	Effective-ness	Median = 10 years
(Olschwang et al 1998) Fondation Jean Dausset-CEPH, Paris, France	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 110 patients n = 92 unrelated VHL patients n = 18 patients with sporadic HB	*Inclusion:* A series of patients with sporadic HB or unrelated VHL patients referred from different regions of France *Exclusion:* Not stated	*Intervention*: DGGE and DNA sequencing, Southern blotting *Comparator:* Clinical data were collected through patient interviews and hospital notes	Diagnostic accuracy	N/A
(Pack et al 1999) National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland, USA	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 31 VHL patients n = 30 patients with a known *VHL* gene deletions n = 1 patient with a point mutation in the *VHL* gene	*Inclusion:* Patients with a clinical diagnosis for VHL disease from 17 unrelated families with known *VHL* gene deletions 1 patient with a point mutation in the *VHL* gene *Exclusion:* Not stated	*Intervention*: FISH *Comparator:* Clinical diagnosis (criteria not described)	Diagnostic accuracy	N/A
	Case series	N = 6 asymptomatic relatives	*Inclusion:* Asymptomatic relatives from 4 of the	*Intervention*:	Diagnostic	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
	Level IV diagnostic evidence CX P1 Q3		VHL patients *Exclusion:* Not stated	FISH	yield	
(Patocs et al 2004) Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary	Comparative study Level III-2 diagnostic evidence CX P2 Q3	N = 41 patients with PHs	*Inclusion:* Patients in a database including all patients with PHs evaluated at the 2nd Department of Medicine, January 1995 – July 2003 *Exclusion:* Not stated	*Intervention*: DNA sequencing *Comparator:* Clinical diagnosis based on family history and clinical manifestations of associated hereditary disorders	Diagnostic accuracy	N/A
(Patocs et al 2008) Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary	Comparative study Level III-2 diagnostic evidence CX P1 Q1	N = 22 kindred n = 6 patient with clinical signs of VHL disease n = 16 patients with no clinical signs of disease	*Inclusion:* Members of a large Hungarian VHL type 2 family spanning 5 generations *Exclusion:* Not stated	*Intervention*: DNA sequencing *Comparator:* Clinical screening included medical history, physical examination, abdominal ultrasonography, abdominal and brain CT or MRI, ophthalmologic examination, routine biochemical testing and 24-hour urinary catecholamine metabolite determinations	Diagnostic accuracy	N/A
(Pigny et al 2009) Centre de Biologie et Pathologie, CHRU de Lille,	Level IV diagnostic evidence CX P1	N = 100 patients with apparently sporadic PH N = 8 patients with a PH and a positive familial history of	*Inclusion:* All patients with an apparently sporadic PH (surgically removed and pathologically confirmed), 2002–07	*Intervention*: DNA sequencing, MLPA *Comparator:* Clinical diagnosis based on	Diagnostic yield	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
France	Q3	adrenal tumour	Patients with a PH and a positive familial history of adrenal tumour *Exclusion:* Not stated	review of medical records and family history by inquiry		
(Poulsen et al 2010) Institute of Cellular and Molecular Medicine, Copenhagen University, Denmark	Case series Level IV interventional evidence Medium quality (NHS CRD = 4/6)	N = 54 subjects from 22 unrelated families who agreed to participate in this study	*Inclusion:* All subjects with pathogenic VHL mutations spanning 1971–2008, who were alive and resident in Denmark on 1 June 2008 *Exclusion:* Not stated	*Intervention*: Details of genetic testing not reported *Comparator:* Clinical diagnosis included full medical histories obtained through detailed interviews, and evaluation of clinical, radiographic and histological general practitioners' records	Prevalence	N/A
(Priesemann et al 2006) Barts and The London NHS Trust, London, UK	Level IV diagnostic evidence CX P1 Q3	N = 7 first-degree relatives (children) of 3 probands (2 were siblings) who had a clinical and genetic diagnosis of VHL syndrome	*Inclusion:* First-degree relatives of 3 VHL patients from 2 families *Exclusion:* Not stated	*Intervention*: Details of genetic testing not reported	Diagnostic yield	N/A
(Rasmussen et al 2006) Instituto Nacional de Neurología y Neurocirugía (INNN) Manuel Velasco Suárez, Mexico City, Mexico	Comparative study Level III-2 diagnostic evidence CX P1 Q1	N = 93 kindred from 16 families n = 23 symptomatic; 14 patients diagnosed with VHL, 4 with possible VHL, 5 with sporadic disease n = 14 asymptomatic relatives with VHL mutation n = 6 with signs of disease identified by screening	*Inclusion:* All the patients with CNS HB admitted to the INNN, 2002–04 *Exclusion:* Not stated	*Intervention*: DNA sequencing *Comparator:* Clinical diagnosis and screening involved MRI of craniospinal axis and internal auditory canal, ophthalmological examination, abdominal CT scanning, and 24-hour urinary catecholamine determination	Diagnostic accuracy	N/A
	Case series	N = 70 asymptomatic	Not reported	*Intervention*:	Diagnostic	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
	Level IV diagnostic evidence CX P1 Q3	relatives		DNA sequencing	yield	
(Rasmussen et al 2010) University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 17 patients from 17 suspected VHL families n = 10 patients diagnosed with VHL n = 7 patients with possible VHL N = 92 first- and second-degree relatives n = 85 asymptomatic n = 7 symptomatic	*Inclusion:* The proband was ascertained at the National Institute of Neurology and Neurosurgery in Mexico City in 2002, with a diagnosis of VHL disease or possible VHL disease *Exclusion:* Not stated	*Intervention*: DNA sequencing *Comparator:* Clinical criteria according to Neumann (1987)	Diagnostic accuracy	N/A
	Case series Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 157 first- and second-degree relatives of 12 GT positive probands n = 36 patients that receive annual screening n = 12 VHL patients n = 24 GT positive relatives	*Inclusion:* first- and second-degree relatives of 12 GT positive probands *Exclusion:* Not stated	*Intervention*: Counselling, genetic testing Annual screening	Effective-ness Change in manage-ment	5 years
(Ritter et al 1996) University of Munich, Munich, Germany	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 7 first-degree family members with familial PHs n = 6 with clinical signs of PHs n = 1 with no clinical signs of disease	*Inclusion:* Members of a family with PHs, followed since 1988 *Exclusion:* Not stated	*Intervention*: SSCP and DNA sequencing *Comparator:* Clinical screening or ultrasonography	Diagnostic accuracy	N/A
(Rocha et al 2003)	Comparative study	N = 20 patients with VHL	*Inclusion:*	*Intervention*:	Diagnostic	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
Hospital do Câncer A C Camargo, Sao Paulo, Brazil	Level III-2 diagnostic evidence CX P1 Q1	syndrome	Patients that were clinically evaluated at the Hospital do Câncer, or were referred by other centres *Exclusion:* Not stated	DNA sequencing, Southern blotting *Comparator:* Clinical status was determined by physical examinations (including ophthalmoscopy), radiological evaluations and laboratory testing according to methods described by Choyke et al (1995)	accuracy	
(Ronning et al 2010) Oslo University Hospital, Oslo, Norway	Comparative study Level III-2 diagnostic evidence CX P2 Q2	N = 31 patients diagnosed with HB	*Inclusion:* Patients successively diagnosed with CNS HB with no family history of HB or any other VHL-associated tumours, January 2000 – December 2007 *Exclusion:* Not stated	*Intervention*: DHPLC, DNA sequencing, MLPA *Comparator:* Clinical screening for VHL (Note: clinical diagnosis of VHL made on basis of HB plus renal cysts)	Diagnostic accuracy	N/A
(Ruiz-Llorente et al 2004) Centro Nacional de Investigaciones Oncolo´gicas, Madrid, Spain	Comparative study Level III-2 diagnostic evidence CX P1 Q1	N = 35 unrelated patients suspected of having VHL disease n = 24 with a family history	*Inclusion:* Patients were initially selected based upon their medical and familial histories *Exclusion:* Not stated	*Intervention*: DNA sequencing, Southern blotting *Comparator:* Clinical screening by CT or MRI of the CNS and the internal auditory canal, CT of the abdomen, exploration of the optical fundus, measurement of catecholamines and metanephrines	Diagnostic accuracy	N/A
	Case series Level IV diagnostic evidence	N = 103 relatives from 20 families n = 25 presented with some clinical symptoms	Not reported	*Intervention*: DNA sequencing, Southern blotting	Diagnostic yield	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
	CX P1 Q3					
(Shah et al 2000) Rush Presbyterian St. Luke's Medical Centre, Chicago, Illinois, USA	Comparative study Level III-2 diagnostic evidence CX P1 Q3	N = 5 family members (4 first- and 1 second-degree) of an index patient with RCC n = 1 with clinical signs of disease n = 4 with no clinical signs of disease	*Inclusion:* Not stated *Exclusion:* Not stated	*Intervention*: DNA sequencing *Comparator:* Clinical screening (criteria not reported)	Diagnostic accuracy	N/A
(Shuin et al 1999) Kochi Medical School, Yokohama City University, Kochi, Japan	Comparative study Level III-2 diagnostic evidence CX P1 Q3	N = 69 unrelated VHL patients	*Inclusion:* not stated *Exclusion:* Not stated	*Intervention*: SSCP, DNA sequencing, Southern blotting *Comparator:* clinical diagnosis (criteria not reported)	Diagnostic accuracy	N/A
(Singh et al 2002) Wills Eye Hospital, Thomas Jefferson University, Philadelphia, PA, USA	Case series Level IV diagnostic evidence CX P2 Q3	N = 10 patients with solitary RCH and no other signs of VHL disease	*Inclusion:* The computerised database of the Oncology Service was reviewed and all patients with the diagnosis of solitary RCH were retrieved (1975–2000) *Exclusion:* diagnosis of VHL	*Intervention*: Southern blotting, conformation-sensitive gel electrophoresis, DNA sequencing *Comparator:* Clinically diagnosed with VHL disease if they had any one of the following features: family history of VHL disease, systemic features of VHL disease or more than one RCH	Diagnostic yield	N/A
(Siu et al 2011) Princess Margaret Hospital, Hong	Comparative study Level III-2 diagnostic evidence	N = 9 probands with suspected VHL n = 7 patients with VHL	*Inclusion:* Probands with clinical features of VHL referred from clinical departments for	*Intervention*: DNA sequencing and MLPA *Comparator:*	Diagnostic accuracy	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
Kong, China	CX P1 Q2	n = 2 patients with bilateral PHs	the analysis of *VHL* genes *Exclusion:* Not stated	Clinical diagnosis (criteria not reported)		
	Case series Level IV diagnostic evidence CX P1 Q3	N = 10 first-degree relatives n = 2 symptomatic n = 8 asymptomatic	*Inclusion:* First-degree relatives of the probands *Exclusion:* Not stated	*Intervention*: DNA sequencing, MLPA	Diagnostic yield	N/A
(Sovinz et al 2010) Medical University of Graz, Graz, Austria	Comparative study Level III-2 diagnostic evidence CX P1 Q3	N = 5 (3 first- and 2 second-degree) relatives of an index case n = 1 with clinical signs of disease n = 4 with no clinical signs of disease	*Inclusion:* Not stated *Exclusion:* Not stated	*Intervention*: Mutation analysis *Comparator:* Clinical screening by MRI imaging of abdomen, head and spine, as well as fundus examination	Diagnostic accuracy	N/A
(Stanojevic et al 2007) Institute for Nuclear Sciences Vinca, Belgrade, Serbia	Case series Level IV diagnostic evidence CX P1 Q3	N = 18 first- and second-degree relatives from 3 families of hospitalised VHL patients n = 5 symptomatic n = 13 asymptomatic	*Inclusion:* Patients hospitalised for VHL syndrome in the Military Medical Academy or the Clinical Centre of Serbia *Exclusion:* Not stated	*Intervention*: SSCP and DNA sequencing	Diagnostic yield	N/A
(Stolle et al 1998) University of Pennsylvania, Philadelphia, PA; and the Clinical Center of the National Institutes	Comparative study Level III-2 diagnostic evidence CX P1 Q1	N = 93 patients diagnosed with VHL	*Inclusion:* Patients from consecutive VHL families *Exclusion:* Not stated	*Intervention*: DNA sequencing, Southern blotting *Comparator:* Clinical examination at the Clinical Center of the National Institutes of Health; diagnosis of	Diagnostic accuracy	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
of Health, Bethesda, MD; USA				VHL as reported in Glenn et al (1990)		
(Tong et al 2006) Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China	Case series Level IV diagnostic evidence CX P2 Q3	N = 5 unrelated families with non-syndromic familial PHs	*Inclusion:* Not stated *Exclusion:* Not stated	*Intervention*: DNA sequencing	Diagnostic yield	N/A
(Tong et al 2009) Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China	Case series Level IV diagnostic evidence CX P1 Q3	N = 8 family members n = 3 patients from 1 family that initially presented with PHs n = 5 asymptomatic relatives	*Inclusion:* Not stated *Exclusion:* Not stated	*Intervention*: DNA sequencing	Diagnostic yield	N/A
(Van der Harst et al 1998) Erasmus University Rotterdam Medical School and Dijkzigt University Hospital, Rotterdam, The Netherlands	Case series Level IV diagnostic evidence CX P2 Q3	N = 68 patients with sporadic PHs	*Inclusion:* Patients identified from the hospital database who had undergone surgery for PH during the past 20 years and had no personal or family history indicative of VHL disease, MEN 2 or NF1 *Exclusion:* Not stated	*Intervention*: SSCP and DNA sequencing	Diagnostic yield	N/A
(Waldmann et al 2009)	Case series Level IV diagnostic	N = 26 patients with sporadic PHs (no family history of	*Inclusion:* Patients with sporadic PHs who had	*Intervention*: DHPLC and DNA sequencing	Diagnostic yield	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
University Hospital Giessen and Marburg, Marburg, Germany	evidence CX P2 Q3	disease)	undergone surgery since 1993 *Exclusion:* Not stated			
(Webster et al 1999b) Addenbrooke's Hospital, Cambridge University, Cambridge, UK	Case series Level IV diagnostic evidence CX P1 Q3	N = 183 identified VHL mutation carriers from 81 families	*Inclusion:* Recruited patients and families with VHL disease from all UK ophthalmic and clinical genetics departments *Exclusion:* Not stated	*Intervention*: SSCP and DNA sequencing, Southern blotting	Diagnostic yield	N/A
	Case series Level IV interventional evidence High quality (NHS CRD = 4.5/6)	N = 183 VHL mutation carriers from 81 families r	*Inclusion:* Recruited patients and families with VHL disease from all UK ophthalmic and clinical genetics departments *Exclusion:* Not stated	*Intervention*: Examination included corrected Snellen visual acuity, slitlamp examination, funduscopy, and fluorescein angioscopy or angiography	Prevalence Effective-ness	N/A
(Webster et al 1999a) Addenbrooke's Hospital, Cambridge University, Cambridge, UK	Case series Level IV diagnostic evidence CX P2 Q3	N = 17 patients had a VHL-like ocular angioma in the absence of any other VHL complications in the patients or their relatives	*Inclusion:* Recruited patients diagnosed with a solitary ocular angioma in the absence of any other VHL complications in the patients or their relatives by contacting all UK ophthalmic and clinical genetics departments *Exclusion:* Not stated	*Intervention*: SSCP and DNA sequencing, Southern blotting	Diagnostic yield	N/A
(Weil et al 2003) National Institutes of Health, Bethesda,	Comparative study Level III-2 diagnostic evidence CX	N = 12 patients that were clinically diagnosed with VHL and brainstem HBs	*Inclusion:* Consecutive patients with VHL disease who underwent surgery to remove symptomatic or rapidly enlarging	*Intervention*: SSCP and DNA sequencing, Southern blotting *Comparator:*	Diagnostic accuracy	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
Maryland, USA	P1 Q3		brainstem HBs, 1987–98 *Exclusion:* Not stated	Standard diagnostic criteria for VHL disease		
(Wong et al 2008) National Eye Institute, National Institutes of Health, Bethesda, Maryland, USA	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 11 patients with atypical ocular lesions and VHL disease who had genetic testing n = 10 with clinical VHL n = 1 family history of VHL	*Inclusion:* Patients with a consistent pattern of vascular proliferation that is variably associated with a fibrovascular component and epiretinal membrane, and is clearly distinct from the hallmark retinal HBs associated with ocular VHL disease, and who met clinical diagnostic criteria for VHL disease *Exclusion:* Not stated	*Intervention*: Genetic testing method not reported *Comparator:* These patients were evaluated for systemic manifestations of VHL disease as described by Melmon and Rosen (1964)	Diagnostic accuracy	N/A
(Woodward et al 1997) Department of Pathology, Cambridge, UK	Case series Level IV diagnostic evidence CX P2 Q3	N = 16 patients with PH n = 8 with familial PH	*Inclusion:* Patients with PH and no clinical evidence or family history of MEN 2, VHL or NF1 *Exclusion:* Not stated	*Intervention*: SSCP and DNA sequencing	Diagnostic yield	N/A
(Wu et al 2000) Kobe University School of Medicine, Himeji, Japan	Case series Level IV diagnostic evidence CX P1 Q3	N = 4 first-degree relatives of index patient	*Inclusion:* First-degree relatives of index patient *Exclusion:* Not stated	*Intervention*: Restriction-site polymorphism	Diagnostic yield	N/A
(Yoshida et al 2000) Yokohama City	Comparative study Level III-2 diagnostic evidence	N = 77 unrelated patients diagnosed with VHL syndrome, identified through	*Inclusion:* Unrelated VHL patients selected on the basis of availability and their willingness	*Intervention*: SSCP and DNA sequencing, Southern blotting	Diagnostic accuracy	N/A

Study and location	Study design Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
University School of Medicine, Yokohama, Japan	CX P1 Q2	referrals from hospital clinics associated with the diagnosis and treatment of VHL	to donate blood samples *Exclusion:* Not stated	*Comparator:* Medical records were reviewed to verify the clinical diagnosis of VHL (criteria not stated)		
(Zbar et al 1996) National Cancer Institute, Frederick, USA plus 7 international collaborating laboratories	Comparative study Level III-2 diagnostic evidence CX P1 Q1	N = 469 unrelated VHL families	*Inclusion:* VHL families with or without PH that were evaluated at one of the 8 participating laboratories *Exclusion:* The data were corrected for duplicate testing, the 18 families with the c.505 T/C founder mutation were treated as a single family	*Intervention*: SSCP or DGGE and DNA sequencing, Southern blotting *Comparator:* Clinical criteria for VHL as described by Neumann (1987) and Hosoe et al (1990)	Diagnostic accuracy	N/A
(Zhang et al 2008) Shanghai Jiaotong University School of Medicine, Shanghai, People's Republic of China	Comparative study Level III-2 diagnostic evidence CX P1 Q2	N = 27 index patients suspected of having VHL disease from unrelated families n = 23 with a family history n = 3 with *de novo* disease n = 1 did not fulfil the current clinical VHL diagnostic criteria	*Inclusion:* VHL patients recruited from medical centres in different regions of China *Exclusion:* Not stated	*Intervention*: DNA sequencing, UPQFM-PCR *Comparator:* Clinical data on all affected patients were collected and evaluated	Diagnostic accuracy	N/A

CNS = central nervous system; CSGE = conformation-sensitive gel electrophoresis; CT = Computed tomography; DGGE = denaturing gradient gel electrophoresis DHPLC = denaturing high-performance liquid chromatography, DNA = deoxyribonucleic acid; FISH = fluorescence in-situ hybridisation; GT = genetic test; HB = haemangioblastoma; HNP = head and neck paragangliomas; ICT = islet cell tumour; MEN 2 = Multiple endocrine neoplasia type 2 ; MLPA = multiplex ligation-dependent probe amplification; MRI = magnetic resonance imaging; NF1 = *neurofibromatosis* type 1; PCR = polymerase chain reaction, Q-PCR= quantitative PCR; PGL = paraganglioma; PH = phaeochromocytoma; PNET = pancreatic neuro-endocrine tumour; RCC = renal cell carcinoma; RCH = retinal capillary haemangioma; SSCP = single-strand conformational polymorphism; UPQFM-PCR = universal primer quantitative fluorescent multiplex PCR; VHL = von Hippel-Lindau

Appendix H Excluded studies

Incorrect outcomes

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Appendix I Supplementary data for economic evaluation

Table 58 Studies used to calculate genetic test sensitivity for the economic analysis

Study	Clinically positive	Genetically positive	Sensitivity
Maher (1996)	138	109	0.790
Stolle (1998)	93	93	1.000
Libutti (2000)	44	44	1.000
Ruiz-Llorente (2004)	35	32	0.914
Rocha (2003)	20	20	1.000
Gergics (2009)	11	11	1.000
Corcos (2008)	35	35	1.000
Cybulski (2002)	34	30	0.882
Li (1998)	10	7	0.700
Hoebeeck (2005)	16	16	1.000
Hes (2007)	55	40	0.727
Zhang (2008)	26	26	1.000
Cho (2009)	15	15	1.000
Kang (2005)	11	10	0.909
Gomy (2010)	9	7	0.778
Siu (2011)	7	7	1.000
Fisher (2002)	2	2	1.000
Total	**561**	**504**	**0.898**

Table 59 Studies used to calculate genetic test specificity for the economic analysis

Study	Clinically negative	Genetically negative	Specificity
Hoebeeck (2005)	1	1	1.000
Hes (2007)	91	88	0.967
Zhang (2008)	1	1	1.000
Cho (2009)	11	11	1.000
Kang (2005)	4	2	0.500
Gomy (2010)	1	1	1.000
Fisher (2002)	4	4	1.000
Hering (2006)	5	5	1.000
Total	**118**	**113**	**0.958**

Table 60 Studies used to calculate prevalence of VHL syndrome among patients suspected of having VHL syndrome

	Total number	Clinically positive	Prevalence
PATIENTS SUSPECTED OF HAVING VHL			
Klein (2001)	79	68	0.861
Rasmussen (2006)	23	16	0.696
Hoebeeck (2005)	17	16	0.941
Hes (2007)	146	55	0.377
Olschwang (1998)	110	92	0.836
Ciotti (2009)	43	30	0.698
Hattori (2006)	31	27	0.871
Zhang (2008)	27	26	0.963
Cho (2009)	26	15	0.577
Magnani (2001)	18	9	0.500
Rasmussen (2010)	17	10	0.588
Martin (1998)	16	14	0.875
Kang (2005)	15	11	0.733
Gomy (2010)	10	9	0.900
Siu (2011)	9	7	0.778
Total	**587**	**405**	**0.690**
PATIENTS WITH CNS HAEMANGIOBLASTOMA			
Glasker (1999)	141	94	0.667
Glasker (2001)	31	18	0.581
Ronning (2010)	20	7	0.350
Fisher (2002)	6	2	0.333
Glasker (2005)	6	4	0.667
Total	**204**	**125**	**0.613**
PATIENTS WITH RETINAL HAEMANGIOBLASTOMA			
Kreusel (2000)	37	29	0.784
Niemela (2000)	29	8	0.276
Kreusel (2007)	11	6	0.545
Total	**77**	**43**	**0.558**
GRAND TOTAL	***868***	***573***	***0.660***

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