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CLINICAL UTILITY GENE CARD

Clinical utility gene card for: von Hippel–Lindau (*VHL*)

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1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

von Hippel–Lindau Syndrome (VHL).

1.2 OMIM# of the disease

193300.

1.3 Name of the analyzed genes or DNA/chromosome segments

VHL (3p25.3).[1]

1.4 OMIM# of the gene(s)

(i) 608537 von Hippel–Lindau (*VHL*) and (ii) 168461 cyclin D1 gene (CCND1), a potential modifier of *VHL*.

1.5 Mutational spectrum

VHL mutations have been identified in all the three exons. About 30–60% are missense mutations, 20–40% large intragenic deletions (0.5–250 kb), 12–20% microdeletions or insertions and 7–11% nonsense mutations.[2–5] Genotype–phenotype correlations (see section 2.5 Positive clinical predictive value) have been described. Some hotspot/founder mutation have been reported.[6] No mutations have been reported in the first 53 amino acids of pVHL.[3,5]

For the standard reference sequence in relation to the variants reported, a RefSeqGene record, for example, NCBI Reference Sequence: NM_000551.3, should be applied.

VHL gene variants can be found in the Human Gene Mutation Database. It is important for DNA diagnostics to share all new findings through this or similar databases.

1.6 Analytical methods

Most mutations can be identified by bi-directional sequencing analysis of all exons and short adjacent intronic sequences. Large genomic and intragenic deletions may be identified by Southern blotting, including quantitative Southern blotting, pulsed field gel electrophoresis (PFGE) or/and fluorescence *in situ* hybridization (FISH), or more recently by Q-RT-PCR (quantitative real-time PCR), MLPA (multiplex ligation-dependent probe amplification) or CGH (comparative genomic hybridization). Today, most laboratories will apply sequencing first, followed by MLPA.

1.7 Analytical validation

(i) Verification by analyzing a second independent specimen, (ii) certification of the laboratory in accordance to established quality standards (DIN, CAP, etc) and (iii) external validation by exchange of DNA control samples with other diagnostic institutions.

In some cases, the interpretation of MLPA results might be difficult and a validation with another semiquantitative method should be considered, such as long-range PCR sequencing, RNA analysis or CGH (comparative genomic hybridization) and segregation analysis.

1.8 Estimated frequency of the disease (incidence at birth ('birth prevalence') or population prevalence)

Birth incidence has been estimated as 1/39 000 (Germany) and 1/36 000 (East Anglia, UK). Prevalence is 1/85 000–1/31 000. Median age at diagnosis for the first time is 22–26 years.[5] Due to increasing awareness, and applying molecular diagnostics, the age at diagnosis is decreasing. Incidence of *de novo* mutations may be up to 20%. All ethnic groups are involved, there is no sex bias.[3]

1.9 If applicable, prevalence in the ethnic group of investigated person

Not applicable.

1.10 Diagnostic setting:

	Yes	No
A. (Differential) diagnostics	☒	☐
B. Predictive testing	☒	☐
C. Risk assessment in relatives	☒	☐
D. Prenatal	☒	☐

Comment:

Prenatal and pre-implantation genetic diagnostics (PID/PGD) are rarely requested for VHL. In any case, both should be performed in accordance to each country's legal regulations, always accompanied by an approriate, comprehensive and non-directive genetic counselling, before and following to genetic testing.

The cyclin D1 gene (CCND1, OMIM 168461) has been described as a potential modifier of VHL.[7] So far, in 2013 this gene is not part of regular testing for VHL.

In a small subset of familial erythrocytosis (type 2, Chuvash OMIM 263400), *VHL* has been found to be involved (see last paragraph in section 2.5. para 'Genotype–phenotype correlation').[8]

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2. TEST CHARACTERISTICS

	Genotype or disease		A: True positives B: False positives	C: False negative D: True negative
	Present	Absent		
Test				
Positive	A	B	Sensitivity:	A/(A + C)
			Specificity:	D/(D + B)
Negative	C	D	Positive predictive value:	A/(A + B)
			Negative predictive value:	D/(C + D)

2.1 Analytical sensitivity
(proportion of positive tests if the genotype is present)

Applying both sequencing and large deletion/rearrangement diagnostic tests such as MLPA, the analytical sensitivity will be >98%.

2.2 Analytical specificity
(proportion of negative tests if the genotype is not present)

Nearly 100%.

2.3 Clinical sensitivity
(proportion of positive tests if the disease is present)

The clinical sensitivity can be dependent on variable factors, such as age or family history. In such cases, a general statement should be given, even if a quantification can only be made case by case.

100%, in case a typical VHL symptom is present either in the person tested or in the family when the index person presents with the identical mutation. Mosaicism has been reported infrequently but may complicate interpretation of the results of the analyses. In case of an atypical VHL disease family, the possibility of a mosaicism should be carefully considered.

2.4 Clinical specificity
(proportion of negative tests if the disease is not present)

The clinical specificity can be dependent on variable factors, such as age or family history. In such cases, a general statement should be given, even if a quantification can only be made case by case.

Nearly 100%, if *VHL* polymorphisms can be excluded.

2.5 Positive clinical predictive value
(life-time risk of developing the disease if the test is positive)

Penetrance by age of 65 years is >90%, depending upon the specific VHL phenotypic manifestation. VHL patients may present with a variety of tumors affecting eye, central nervous system, inner ear, adrenal gland, kidney, pancreas and epididymis. Most frequent tumors include hemangioblastomas (HB) of the eye (=retinal angioma, overall life-time risk is 50–73%), HB of the cerebellum (55–59%) and HB of the spinal cord (13–25%), which are usually benign. However, due to their location, the size of the accompanying cysts and the resulting functional consequences, these clinical features will result in severe VHL complications. Other benign lesions include pheochromocytoma (7–20%), renal and pancreatic cysts (22–76%). Renal cell carcinomas (RCC, 24–52%) are malignant and histologically almost exclusively of the clear cell subtype (CCRCC), which comprises also the majority (>70%) of all sporadic RCCs.[9] Tumors and cysts are frequently bilateral and/or multiple in origin.

The mean age at diagnosis (i) of pheochromocytomas is 20–24 years, (ii) of HBs of the retina 29–30 years, (iii) of HBs of the cerebellum 33–34 years and (iv) of HBs of the spinal cord 33–34 years. In the recent 17 years, the mean age at diagnosis of (v) renal cancer did decrease from 44 (±10,9) years to 39.7 years (±10,7) years. Among other reasons, this is likely due to improved efficiency of surveillance of persons at risk following the widespread application of molecular diagnostics.[3,5]

Genotype–phenotype correlation:[3,5,6,10–18] There is a difference of frequencies of the types of germline mutations between VHL type 1 and VHL type 2 families (see classification details below), reflecting the occurrence of renal carcinomas and/or pheochromocytomas in combination with or without other VHL typical features.

Based on the presence or absence of pheochromocytoma, VHL disease is phenotypically subclassified into VHL type 1 (without pheochromocytoma, applies to the majority of all families) and VHL type 2 (with pheochromocytoma, about 7–20% of all families). The VHL type 2 is further subdivided into type 2A (without CCRCC), type 2B (with CCRCC) and type 2C (pheochromocytomas as the sole manifestation). In VHL type 2C disease, it is especially important to carefully establish the molecular diagnosis in affected patients, as pheochromocytoma is also a manifestation of other inherited syndromes, such as multiple endocrine neoplasia type 2, neurofibromatosis type 1 and others.

With a few exceptions, most VHL type 2-associated germline mutations are of the missense type. The predicted consequence of the majority of missense mutations is a single amino-acid change in an otherwise full-length protein. The molecular basis for the genotype–phenotype correlations has not been precisely defined, but pheochromocytoma-associated *VHL* mutations may compromise programmed developmental apotosis in the fetal adrenal medulla. Other genetic causes of pheochromocytoma may also impinge on this PHD3/EGLN3-related pathway.[11] The spectrum of VHL type 1-associated germline mutations is much more diverse and includes large deletions, microdeletions, insertions, nonsense, frameshift and missense as well as splice site mutations. Most of these mutations cause severe damage and most likely loss of *VHL* function due to the destruction of the domain required for one of the major functions of the VHL protein (pVHL).

There is evidence that variation in the cyclin D1 gene (CCND1, OMIM 168461) on chromosome 11q13 may modify the VHL phenotype.[7] Other genes, such as FANCD2, and IRAK2 could also be involved in modifying the VHL phenotype. The various functions of pVHL are not subject of this report and are described in many recent reports and reviews.[15,16,18]

A high prevalence of renal cell cancers has been seen in patients with partial germline *VHL* deletions relative to patients with complete gene deletions. Deletion mapping revealed that development of RCC had an even greater correlation with retention of HSPC300 (C3orf10), located within the 30-kb region of chromosome 3p, immediately telomeric to *VHL* (52.3 *vs* 18.9%, $P<0.001$).[10,12]

Interestingly, homozygous or compound heterozygous germline mutations in the *VHL* gene have been found to cause familial erythrocytosis type 2, Chuvash (OMIM 263400).[8]

2.6 Negative clinical predictive value
(probability of not developing the disease if the test is negative)

Assume an increased risk based on family history for a non-affected person. Allelic and locus heterogeneity may need to be considered.

Index case in the family had been tested:
Almost 100%.
Index case in the family had not been tested:
Depending on age, phenotypes and numbers of family members being investigated. As a general rule, for the ease of interpretation, this situation should be avoided.

3. CLINICAL UTILITY

3.1 (Differential) diagnostics: the tested person is clinically affected
(To be answered if in 1.10 'A' was marked)

3.1.1 Can a diagnosis be made other than through a genetic test?

No	☐ (continue with 3.1.4)	
Yes	☒	
	Clinically	☒
	Imaging	☒
	Endoscopy	☐
	Biochemistry	☐
	Electrophysiology	☐
	Other (please describe)	

—A simplex case (ie, an individual with no known family history of VHL syndrome) presenting with two or more characteristic lesions (eg, two or more hemangioblastomas of the retina or brain or a single hemangioblastoma in association with a visceral manifestation, such as renal cell carcinoma; adrenal or extra-adrenal pheochromocytomas; and, less common, endolymphatic sac tumors, neuroendocrine tumors of the pancreas) often indicates a *de novo VHL* mutation. Renal or epididymal cysts may occur in normal population, so cannot be relied on for reliable diagnosis.
—An individual with a positive family history of VHL syndrome in whom one or more of the following disease manifestations is present: retinal angioma, spinal or cerebellar hemangioblastoma, pheochromocytoma, renal cell carcinoma before age 60 years. Multiple pancreatic cysts, epididymal or broad ligament cystadenomas or multiple renal cysts at a young age highly suggestive.

3.1.2 Describe the burden of alternative diagnostic methods to the patient
Though a diagnosis of VHL based exclusively on clinical data is possible,[3] a molecular analysis is highly recommended as it is readily available, affordable, safe and clear in its interpretation. In the first place, however, molecular-established/verified diagnosis in as yet clinically unaffected persons will prevent unnecessary and potentially dangerous diagnostic procedures, such as computed tomography and many others (see Surveillance, 3.2.1.) for unaffected members of the family who would have been classified at a 50% risk before the era of molecular testing.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?
Besides unneeded exposure to diagnostic procedures with potential harm to the proband, the costs are much higher for conventional clinical procedures, such as MRI combined with other imaging methods.

3.1.4 Will disease management be influenced by the result of a genetic test?

No	☐	
Yes	☒	
	Therapy (please describe)	Malignant renal tumors can be diagnosed earlier, improving the effect of early surgery, resulting in an increase of the percentage of nephron sparing renal surgery[19]. Since the introduction of molecular analysis, the increased frequency of presymptomatic diagnosis of eye complication resulted in more effective laser therapy.
	Prognosis (please describe)	For renal cancers, the likelihood of potential cure and of the prevention of metastases has improved the prognosis of VHL. Prevention of blindness could be improved substantially by increasing the presymptomatic detection of HBs of the retina from $<40\%$ to $>60\%$.
	Management (please describe)	Since the introduction of molecular diagnostics, the management of VHL has changed accordingly, that is, presymptomatic detection of renal cancer and eye complication have improved the prognosis. Family members at risk can be included or excluded from surveillance program (see below, 3.2.1.) So far, CNS complications have been treated only when causing symptoms. With the advent of antiangiogenic drugs and other targeted agents, new therapeutic options are becoming available beyond conventional treatment; some may prove to have potential for presymptomatic therapy for these CNS complications.

3.2 Predictive setting: the tested person is clinically unaffected but carries an increased risk based on family history
(To be answered if in 1.10 'B' was marked)

3.2.1 Will the result of a genetic test influence lifestyle and prevention?
If the test result is positive (please describe)
Yes. Affected or at-risk individuals should be undergo a comprehensive multidisciplinary surveillance: (1) careful ophthalmic examination every 12 months beginning in infancy or early childhood, (2) MRI scans of the head and spine every 12–36 months beginning in adolescence, (3) MRI scan and/or ultrasound of the abdomen every 12 months from age 16 years. Though more sensitive, computer tomography should only be applied in particular situations as on regular follow-up, the cumulative radiation load would be too high, and (4) yearly screening for pheochromocytoma should be started in early childhood: measurement of normetanephrine plasma level and MRI of the abdomen.
If the test result is negative (please describe)
Yes: as a consequence of a negative result, there will be no need for an intensified screening program, concurrently also resulting in a psychological relief.

3.2.2 Which options in view of lifestyle and prevention does a person at risk have if no genetic test has been done (please describe)?
Basically, the very same as in a proven carrier of a VHL causing mutation.

3.3 Genetic risk assessment in family members of a diseased person
(To be answered if in 1.10 'C' was marked)

3.3.1 Does the result of a genetic test resolve the genetic situation in that family?

Yes, in case the causative *VHL* mutation could be established.

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?

Yes: Provided the *VHL*-associated mutation detected in the index patient could not be detected in a family member, this person can be excluded from the demanding surveillance program.

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?

Yes.

3.4 Prenatal diagnosis

(To be answered if in 1.10 'D' was marked)

3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?

Yes.

4. IF APPLICABLE, FURTHER CONSEQUENCES OF TESTING

Please assume that the result of a genetic test has no immediate medical consequences. Is there any evidence that a genetic test is nevertheless useful for the patient or his/her relatives? (Please describe).

Not applicable, as the result of *VHL* mutation testing is of medical consequences.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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