Von Hippel-Lindau Disease: Genetics and Role of Genetic Counseling in a Multiple Neoplasia Syndrome

Sarah M. Nielsen[1], Lindsay Rhodes[2], Ignacio Guillermo Blanco[3], Wendy K. Chung[4], Charis Eng[5,6], Eamonn R. Maher[7], Stéphane Richard[8], and Rachel H. Giles[10, 11]

[1]Center for Clinical Cancer Genetics and Global Health, Department of Medicine, The University of Chicago, Chicago, IL, 60637 USA

[2]Department of Pediatrics, The University of Chicago, Chicago, IL 60637 USA

[3]Spanish Association of Human Genetics, Hospital Universitari Germans Trias i Pujol, UAB - Universitat Autònoma de Barcelona**,** Barcelona, Spain

[4]Departments of Pediatrics and Medicine, Columbia University, New York, NY 10032 USA

[5]Genomic Medicine Institute, Lerner Research Institute and Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH 44195 USA

[6]Department of Genetics and Genome Sciences and Germline High Risk Focus Group, CASE Comprehensive Cancer Center, Case Western Reserve University School of Medicine, Cleveland, OH 44106 USA

[7]Department of Medical Genetics, University of Cambridge and Cambridge NIHR Biomedical Research Centre, Cambridge CB2 0QQ, UK

[8]Centre Expert National Cancers Rares PREDIR, INCa/AP-HP, Hôpital Bicêtre, 94275 Le Kremlin Bicêtre ; Génétique Oncologique EPHE, INSERM U1186, Gustave Roussy Cancer Campus, 94800 Villejuif-France

[10]Department of Nephrology, University Medical Center Utrecht, Regenerative Medicine Center Utrecht, Uppsalalaan 6, 3584CT Utrecht, The Netherlands

[11]Chair, Dutch VHL Patient Organization, www.vonhippellindau.nl

Von Hippel-Lindau Disease: Genetics and Role of Genetic Counseling in a Multiple Neoplasia Syndrome

Running Head: Genetics of VHL Disease and Genetic Counseling Considerations

Key Words: VHL, genetics, genetic counseling, multiple neoplasia syndromes, genotype-phenotype correlation

Word Count: 5,219

Corresponding Author:

Sarah Nielsen, MS, CGC
The University of Chicago Medicine & Biological Sciences
5841 S. Maryland Ave., MC 2115, Chicago, IL, 60637
Office: 773-702-4749
Fax: 773-834-3834
Email: snielsen@medicine.bsd.uchicago.edu

Von Hippel-Lindau Disease: Genetics and Role of Genetic Counseling in a Multiple Neoplasia Syndrome

Abstract

Von Hippel-Lindau disease (VHL) is one of the most common inherited neoplasia syndromes characterized by highly vascular tumors of the eyes, brain and spine, as well as benign and malignant tumors and/or cysts of the kidneys, adrenal medullae/sympathetic paraganglia, endolymphatic sac, epididymis, and broad ligament. Since the discovery of the *VHL* gene in 1993, >900 families with VHL have been identified and examined. Genetic testing for *VHL* is widely available and will detect a disease-causing mutation in rate 95-100% of individuals who have a clinical diagnosis of VHL, making it the standard of care for diagnosis of VHL. Furthermore, genetic testing for *VHL* is indicated in some individuals with apparently sporadic VHL-related tumor types since ≤10% of pheochromocytoma or early-onset renal cell carcinoma (personal communication, Dr. Brian Shuch) and up to 40% of CNS hemangioblastoma harbor germline *VHL* mutations without a family history or additional features of VHL disease.[1,2] The majority of *VHL* mutations are private, but there are also well-characterized founder mutations. VHL is a complex, multi-organ disease which spans the breadth of oncology subspecialties, and as such, providers in these subspecialties should be aware of 1) when to consider a diagnosis of VHL, 2) when to refer to a genetics specialist for consideration of gene testing, and perhaps most importantly, 3) how to communicate this sensitive information in an age-appropriate manner to at-risk families. This manuscript will provide state of the art information on the genetics of VHL and will serve as a key reference for non-genetics professionals who encounter VHL patients.

Von Hippel-Lindau Disease: Genetics and Role of Genetic Counseling in a Multiple Neoplasia Syndrome

Von Hippel-Lindau disease (VHL) is an inherited multiple-neoplasia syndrome characterized by highly vascular tumors of the eyes, brain and spine (retinal and CNS hemangioblastomas; HB), as well as benign and malignant tumors and/or cysts of the kidneys (clear cell renal cell carcinoma; RCC), adrenal glands/sympathetic paraganglia (pheochromocytoma; PCC, paraganglioma; PGL), pancreas (cysts/cystadenomas or pancreatic neuroendocrine tumors; PNETs), endolymphatic sac (endolymphatic sac tumors; ELSTs), epididymis (epididymal cysts and cystadenomas), and broad ligament (broad ligament/mesosalpinx cystadenoma).[3-6] Since the first description of the disease in 1926 [7] and the discovery of the *VHL* gene over 60 years later [8], >900 families worldwide with VHL have been identified and examined.[9,10]

A timely review of the genetics of VHL is warranted because genetics is becoming increasingly integrated into healthcare in the name of "personalized" or "precision" medicine; as such, health care providers must now more than ever understand the sensitive and unique nature of communicating genetic information to patients. This is especially true in the field of oncology, where both somatic and germline genetic testing is becoming a standard part of oncologic work-up and clinical care. In the context of VHL, it is important to consider the sensitive nature of pre-symptomatic testing of children, as well as "unexpected" (incidental) VHL diagnoses as a result of the incorporation of multi-gene next-generation sequencing panels into clinical practice for sporadic VHL-associated tumors, namely RCC and PCC/PGL. Other special considerations in VHL include preconception counseling (including pregnancy-related risks), and the burden of extensive clinical screening.

Clinical Diagnosis and *VHL* Gene

A VHL diagnosis is established in an individual with a family history of VHL when they present with a single characteristic VHL-related tumor (e.g. retinal or cerebellar HB, RCC etc.). In the absence of a family history of VHL, a diagnosis requires two or more retinal or cerebellar HB, or one HB and a visceral tumor (excluding epididymal and renal cysts which are common in the general population).[3,11-13]

The VHL tumor suppressor gene (*VHL)* was mapped to 3p25-26 in 1993.[8] Individuals with the hereditary form of these tumors inherit a single mutant *VHL* allele, and tumor development occurs when the second wild-type copy is spontaneously lost or inactivated. This "second hit" can occur through a variety of mechanisms, including point mutations, deletions, or promoter hypermethylation.[14] As with many predisposition genes causing rare inherited cancer syndromes, somatic loss-of-function of *VHL* occurs in sporadic cancers.[15-17] Indeed, inactivation of *VHL* is a critical driver of nearly all clear-cell RCC, [18,19] approximately 40% of sporadic CNS HB and 10% of sporadic PCC.[20-23]

Molecular Biology of VHL

Over two decades of research has implicated VHL protein (pVHL) in transcriptional regulation, apoptosis, extracellular matrix formation, and ubiquitinylation of specific targets.[23] In particular, the role of pVHL in the adaptive cellular response to hypoxia has been robustly investigated; pVHL regulates hypoxia-inducible genes through the targeted ubiquitinylation and degradation of the alpha-subunits of the hypoxia-inducible factor transcription factors (HIF-1α, -2α, -3α).

pVHL binds to elongin C, which forms a complex with elongin B, cullin-2 and Rbx1. This complex catalyzes the polyubiquitinylation of specific proteins and targets them for proteosomal degradation. Under normoxic conditions, HIFα subunits are hydroxylated by prolyl hydroxylases, a reaction requiring oxygen. The VHL protein then binds to hydroxylated HIFα, targeting it for degradation by its attached destruction complex. In the absence of oxygen or functional pVHL, HIFα subunits are stabilized, accumulate, and translocate to the nucleus where HIFα forms heterodimers with HIFβ to activate the transcription of dozens of hypoxia-inducible genes (i.e., *VEGF*, *EPO*, *TGFα*, *PDGFβ*).[23] *VHL*-mutant cells experience pseudo-hypoxia and shift metabolism to glycolysis even in the presence of oxygen, a process referred to as the Warburg effect. In fact, our ability to better manage patients with non-familial advanced renal cell carcinoma as well as surgical management guidelines of small renal masses has been largely driven by a better understanding of the VHL and consequent biochemical alterations underlying these tumors, in particular first-line VEGF-targeting agents.[24]

Pathogenic variants in *VHL* either reduce expression (i.e., deletions, frameshifts, nonsense variants, and splice site variants) or lead to the expression of an abnormal protein (i.e., pathogenic missense variants). The type of VHL that results from a pathogenic missense variant depends on its effect on the three-dimensional structure of the protein.[25] Pathogenic variants in *VHL* cause misfolding and subsequent chaperonin-mediated breakdown.[26] Pathogenic missense variants that destabilize packing of the alpha-helical domains, decrease the stability of the alpha-beta domain interface, interfere with binding of elongin C and HIFα, or disrupt hydrophobic core residues result in loss of HIF regulation. Furthermore, mutant pVHL may predispose to

pheochromocytoma by altering the molecular regulation of apoptosis of sympatho-adrenal precursor cells during development.[27]

HIF-independent pVHL functions have added greater breadth to the understanding of the pathophysiology of VHL. For example, cyst formation in VHL patients has been linked microtubule-based organelles called primary cilia. pVHL directs microtubule orientation and subsequent stability.[28-30] pVHL also regulates primary cilia, through both HIF- and microtubule-independent functions.[31-33] Furthermore, genetic instability in tumors is driven by VHL loss.[34]

pVHL has been shown to mediate in the transcriptional regulation of the nuclear factor NF-κB[35], the Rbp1 large subunit of the RNA polymerase complex II[36,37], the p53 tumor suppressor[38], the p400 chromatin remodeling factor[39]and the JunB transcription factor via aPKC.[27,40] However pVHL also plays an important transcription-independent role in the regulation of the extracellular matrix and the microtubule cytoskeleton. pVHL is involved in the correct formation and turnover of the extracellular matrix[41] by interacting with collagen IV[42] and fibronectin.[43] *C. elegans vhl-1* knock out worms also displayed genetic evidence for defects in ECM formation.[44] Furthermore, the activity of enzymes involved in degradation and remodeling of the ECM, matrix metalloproteinases MMP-2 and MMP-9[45], is increased in VHL-mutant cells, and HIF-2α induces the expression of membrane type 1-MMP. Since endothelial cells require pVHL for correct vascular patterning and maintenance of vascular integrity during development[46], loss of pVHL function causes both HIF-independent and HIF-dependent defects in the ECM that may promote angiogenesis, invasion and metastasis of tumor cells.[41]

The development of malignant disease after biallelic inactivation of *VHL* has only been adequately addressed in the context of renal cell carcinoma. Several RCCs sequenced in two

VHL patients revealed clonally independent and distinct secondary events all converging on the PI3K-AKT-mTOR signalling pathway, and not characterized by inactivating mutations in p53, similar to sporadic renal cell carcinomas in the general public.[47] Overall there was limited evidence of intra-tumor heterogeneity in VHL patients, although the number was very small and requires independent validation.

Role of Genetic Testing, Mutation Frequency and Spectra

The role of genetic testing in VHL is to confirm or exclude a diagnosis in: 1) at-risk relatives from established VHL kindreds, 2) individuals with suspected clinical diagnoses, or 3) individuals with atypical presentation or moderate suspicion, keeping in mind that failure to find a disease-causing mutation does not rule out a clinical diagnosis in scenario 2.

Approximately 200 distinct mutations have been identified in >900 VHL kindreds.[9,10,48] The mutation spectrum includes missense (52% of patients), frameshift (13%), nonsense (11%), large/complete deletions (11%), in-frame deletions or insertions (6%), and splice-site (7%).[9] Almost 100% individuals meeting classic VHL criteria with multi-organ involvement carry identifiable germline *VHL* mutations; 24% in those meeting criteria with limited VHL manifestations; and 3.3% in those with VHL-associated tumors but do not meet diagnostic criteria.[49] Unexpected germline *VHL* mutations can be found in patients with apparently sporadic VHL-type tumors. *VHL* germline mutations occur in 30-50% of patients with retinal HB, 4-40% of patients with CNS HB, 20% of patients with ELST, 3-11% of patients with PCC and 1-2% of patients with RCC.[1-3,50-55] These observations underlie guidelines suggesting genetics evaluation when certain tumors or clinical features are present. Referral to genetics professionals for consideration of testing (Table 1) has been suggested for individuals with simplex cases of

retinal or CNS HB, PCC, or ELST, as well as clear-cell RCC with any of the following features: 1) diagnosed ≤ age 46, 2) bilateral or multifocal tumors, 3) ≥ 1 close relative with clear-cell RCC.[56] Whereas other groups have suggested that, in addition to the above, genetic evaluation is warranted for individuals with >1 of the following: pancreatic cystadenoma, pancreatic neuroendocrine tumor, epididymal/adnexal cystadenoma.[57]

***VHL* Gene Testing**

VHL genetic testing is available at multiple laboratories worldwide (Table 2). Sanger sequencing of the coding region, along with deletion/duplication analysis, is the gold standard for analyzing the *VHL* gene and results in 95-100% detection rate (though mosaicism may cause false negative test results).[58,59] Multiplex ligation-dependent probe amplification (MLPA) is used for detecting partial and complete gene deletions/duplications.[49,60]

When there is a high prior-probability of *VHL* mutation, single gene testing is appropriate. However, when the genetic differential diagnosis is large, next-generation sequencing multi-gene panels should be considered in the setting of genetic counseling. This technology is often utilized for testing apparently sporadic PCC/PGL or familial/early-onset RCC to determine if there is an inherited component. Thus, referral for genetics evaluation should be considered for RCC diagnosed ≤ 46 years old[61] (though for clinical purposes we often cast a wider net and consider referral for diagnoses ≤ 50 years old) or with the presence of a family history or other syndromic features.[62] Over 40% PCC/PGL are associated with germline mutation in any of 12 genes supporting multi-gene panel testing for all cases of PCC/PGL.[54]

Many laboratories worldwide now offer "multi-gene" panels targeted towards PCC/PGL and RCC. It is critical to include genetics professionals in this testing process as they have the skills to ensure that the appropriate testing method is followed through their astute analysis of the personal and family history. If more than one syndrome is in the differential diagnosis, they will review the implications of each syndrome so that the patient is providing true informed consent, and there are no "surprise" diagnoses. Additionally, genetics teams have expertise in the interpretation of genetic testing results, which is particularly crucial in the realm of next-generation sequencing as results are often not straightforward and misinterpretation can have devastating effects on the patient and family. While positive genetic test results seem relatively straightforward, there are many issues to consider once a diagnosis has been made. Genetics professionals can help individuals cope with a diagnosis and develop strategies to share this information with at-risk family members. A negative genetic test result must be interpreted in the context of the individual's personal and family history; very often it DOES NOT rule out a clinical or a hereditary cause for the cancer/disease in the family. In this instance, an individualized approach must be taken to further surveillance recommendations, taking into account personal/family history and patient preferences. Lastly, variants of uncertain significance (VUS) identified through genetic testing need to be carefully considered. A VUS means a genetic change is identified, but the significance of that change is unknown. The patient and their medical team need to understand that these results do not immediately affect medical management recommendations, which are always based on personal/family history until that VUS is reclassified, a process that could take several months or years.

Clinical and Genetic Features

The incidence of VHL is approximately 1 in 36,000.[63] It has a penetrance of over 90% by age 65, with mean age at tumor diagnosis of 26 years (range 1-70).[5,64] The breakdown of frequency and mean age at diagnosis of specific VHL tumor types is outlined in Table 3. Long-term outcomes in individuals with VHL continue to improve due to improvements in surveillance and treatment of RCC and CNS HB, the leading causes of morbidity and mortality in VHL.[64] Phenotypic heterogeneity (both inter- and intrafamilial) is a hallmark of VHL.[65,66] Clinically, VHL is classified into type 1 or type 2 disease based on the frequency of RCC and PCC (see Table 4).[67-69] Although this classification facilitates genotype-phenotype studies (reviewed elsewhere[9]), it has limited clinical utility as kindreds move between subtypes as additional tumors are discovered.[6,23] Briefly, kindreds with truncating mutations or exon deletions infrequently manifest PCC, and thus usually have type 1 disease characterized by increased incidence of RCC and retinal/CNS HBs (but not PCC), whereas Type 2 VHL is characterized by missense mutations predisposing to PCC, some with PCC alone (type 2C) and other families with additional manifestations.[70] As the relationship between genotype and phenotype is still evolving, it is recommended that all individuals with a diagnosis of VHL follow the same surveillance protocol, which screens for *all* possible manifestations of the disease.

As with most tumor suppressors, the majority of *VHL* mutations are private. However, recurrent founder mutations are well-documented. The most well-characterized founder mutations are those originating from Germany: c.T292C (previously c.T505; p.Y98H) in families from the Black Forest region, and c.T334C (previously c.T547C; p.Y112H) in families from east central Germany (Leipzig).[67,71-75] These families have migrated across Europe and America,

especially Western Pennsylvania. Both mutations predispose to Type 2A VHL, with a high risk of PCC, moderate risk of retinal/CNS HB, and low risk of RCC.[74,76]

A mutation hotspot is at codon 167, due to the presence of CpG dinucleotide with attendant risks of deamination.[69] There have been more than 82 families identified with mutations at this location, representing ~43% of mutations in American and Canadian families with VHL type 2.[9,59,69] Individuals with mutations at this location have a high risk of developing PCC (~62%) and RCC.[69]

VHL genotype-phenotype correlation is further complicated by a unique congenital polycythemia syndrome caused by biallelic (homozygous/compound heterozygous) mutations of the *VHL* gene without any manifestations of VHL disease.[6] The most frequent mutation in this syndrome is c.C598T (p.R200W).[77] Individuals homozygous for this mutation have polycythemia, pulmonary hypertension, varicose veins, elevated serum VEGF concentrations, and occasional vertebral hemangiomas.[6,78]

VHL is inherited in an autosomal dominant manner, with the majority (80%) of cases being inherited from an affected parent, and up to 20% *de novo*.[79,80] Once a VHL mutation is identified in a family, it is recommended to offer genetic testing to that individual's parents (if they are available) even in the absence of an apparent family history, as family history may appear to be negative due to reduced penetrance or later age-of-onset, variable expressivity among family members, or death of an affected parent before onset of symptoms.[81] Mosaicism occurs when a new mutation arises in some but not all tissues, which can result in negative genetic testing if an unaffected cell or tissue type is sampled.[80] There are data suggesting that

mosaicism is an under-recognized phenomenon in VHL, which could result in an overestimation of true cases of *de novo* mutations in probands. Generally, mosaic individuals tend to be more mildly affected or asymptomatic[81], though this is not always the case and mosaicism has been confirmed in individuals with classic VHL disease as well.[82,83] It is now recommended that additional testing methods be employed to rule out mosaicism in parents of a proband with an apparent *de novo* mutation, as well as cases moderately or highly suspicious of VHL in whom standard testing methods have failed to detect a disease-causing mutation.[80-83] Various techniques have been successful in identifying mosaic mutations.[80,82] Alternatively, different tissue types can be sampled, including skin fibroblasts and oral epithelial cells.[81,84] Detection of mosaicism is critical in terms of confirming a diagnosis and estimating the risk to siblings and offspring.[82,83]As next-generation sequencing replaces Sanger sequencing, mosaic cases may be more easily detected which could provide the data needed to elucidate the true frequency of mosaicism in VHL.

Genetic Counseling & Surveillance Considerations

Genotype-phenotype issues are only of scientific value at this point and play no role in clinical or counseling care to the VHL patient. Given the complexity of the disease, a multidisciplinary approach with coordination of care amongst multiple medical specialists, including genetics professionals, is essential for individuals with VHL. Although preventative treatment cannot yet be offered for VHL, it is generally accepted that comprehensive surveillance programs do improve the outcomes by preventing avoidable morbidity and mortality.[6,85,86] Therefore, genetics professionals may take a more directive approach to their counseling because of the importance of initiating or continuing screening for gene carriers (and

discontinuing it for non-carriers).[86] At-risk family members who received genetic counseling were more likely to pursue *VHL* testing compared to those who were only informed of the possibility of testing through a relative or written material.[87] Genetics professionals can aid families in the risk communication process and help identify and work through barriers to genetic testing and follow-up.

Pre-symptomatic testing of children

Since children of an individual with VHL each have a 50% chance of being affected, the topic of genetic testing for VHL in children is one that arises often in clinical care. For healthy children, many professional statements and guidelines support the recommendation that genetic testing only be pursued if the condition is associated with childhood onset and if a positive result leads to effective and safe screening and/or interventional options, thereby reducing morbidity and mortality.[88,89] Although the average age of onset of VHL tumors is in the third decade of life, some patients do develop tumors under the age of 10 and as early as infancy and therefore pre-symptomatic genetic testing in VHL is justified.[90] Pre-symptomatic genetic testing also identifies those children who did not inherit the familial *VHL* mutation, thus sparing them from a lifetime of clinical screening.

There are many factors to be considered before pre-symptomatic genetic testing is pursued. Genetic testing may be offered to a family with a child who is too young to give their informed assent, leading to potential loss of autonomy for that child who may grow up with genetic information that they were not able to elect for themselves.[91] Testing can also be associated with many complex emotions, including anxiety, denial, and guilt on the part of the

parent who is affected with VHL.[87,89] Many families may have lived through the experience of loved ones developing tumors or other VHL-related complications at young ages that may impact their coping when their children are found to carry the VHL mutation.[87,91] Additionally, at-risk or affected children may be treated differently than their non-carrier siblings.[87,91-93] Surveillance fatigue/burnout should also be considered in families considering testing for VHL in their children, since the surveillance is initiated in childhood for mutation-positive children and is lifelong.[94] This topic is discussed more extensively in a subsequent section. It is strongly recommended that genetic counseling for pre-symptomatic genetic testing be conducted by a genetics professional in a comfortable environment, with the option of having multiple genetic counseling sessions, as necessary.[87,89,91,95]

Genetic communication needs of children with VHL

A major question that arises from parents and health professionals is how and when to inform children of their genetic status. Research specific to communication among families with VHL is limited, but studies on the communication needs of children with other genetic condition have found that the majority of patients would have preferred receiving information before the age of 12 years, ideally between 6 and 10 years of age. The majority parents with VHL want their children tested either at birth or at least before the age of 10 years.[96,97] Adolescents preferred that the focus of a genetic counseling session be on understanding and managing their health condition rather than on their reproductive risks. They also identified their parents as their primary source of genetic information (with doctors or other health professionals coming second to this) and many wanted to be seen by their health care provider with their parents present.[98]

Parents are often unsure of the best way to discuss information regarding a genetic diagnosis with their children and common concerns include how the information may impact their child's self-esteem, coping, and anxiety level.[99] Parents may feel overwhelmed by the information, not knowing how, when, or what information to provide.[93] Additionally, they may feel insecure in their own understanding of that information.[98] Studies show that parents feel guidance and support from health care professionals on how to deliver this information is very important though often not available.[93] An important part of the genetic counseling process is to discuss with families how they plan to present the information to their children throughout different stages of their lives.[100]

Children report their preference of learning about their genetic condition gradually through open and continuous communication throughout childhood.[92] In this way children are provided with developmentally appropriate information that they can ask questions about and understand at their own pace, helping them come to terms with their genetic risk in a natural and self-driven way.[93,100] This genetic information then becomes part of the family narrative or culture; talking about those in the family who are also affected helps normalize the information by making it part of a shared family identity.[92,93,100]

In contrast, not acknowledging the genetic condition in the family dissuades children from asking questions, as they do not want to upset their parents.[92,93,99,100] Genetics professionals can help facilitate the communication process, elicit any perceived barriers to communication and provide a safe place for parents to practice these discussions. Resources that provide parents with techniques, diagrams, and appropriate language to use while communicating with their children can be developed, and support groups or additional professional psychosocial support

should be encouraged. [93,100] One such example available to the VHL community is the VHLA Handbook Kids' Edition (accessible at http://www.vhl.org/wordpress/patients-caregivers/resources/vhl-handbooks/).

Pre-conception counseling (including pregnancy-related risks)

The availability of genetic testing for VHL introduces options for couples at-risk of having a child with VHL, including prenatal diagnosis and pre-implantation genetic diagnosis (PGD). Although perceptions may vary across countries, the French VHL study group interviewed 18 women and 13 men with VHL and found that a significant proportion of them (11 women and 11 men; ~70%) intended to use prenatal diagnosis in the case of a pregnancy, although half of the group would not terminate an affected pregnancy (or were undecided) because they hoped for better outcomes and treatments in the future for their children compared with their own experience of VHL.[96] PGD uses in-vitro fertilization techniques to identify genetic mutations within embryos prior to implantation.[101] This is a technology that VHL patients are interested in exploring: 71% of Australian patients (10/14 respondents) viewed it as a favorable option to avoid having a child with VHL compared to 33% (26/79 respondents) in the Dutch population.[87,102] At least eleven children have already been born using this method from the French VHL study group, which now comprises 802 individuals living with VHL, 28 of whom have attempted to have children using PGD (Coupier et al., unpublished data). It is important that patients understand the practical limitations of PGD, namely cost as many insurances currently will not cover the service. Additionally, there are limitations to the technology that must be communicated, including 1) the germline mutation in the family must be known; 2) there are no data about the effect of IVF-related hormone treatment for egg harvest on

tumor development; and 3) the process often takes over a year and if a tumor develops in that period, application of PGD may be delayed or abandoned.

Another factor that complicates reproductive decision making in VHL are the pregnancy-related risks associated with the disease. While generally pregnancy is not considered a contraindication in VHL patients, certain precautions must be considered. Available studies on the subject show conflicting results regarding development or progression of tumors and disease-related complications.[103-105] It is critical to evaluate for potentially life-threatening complications, namely PCC or hydrocephalus due to cerebellar HB, before attempting to become pregnant.[103] Many groups also advocate for additional screening and monitoring during pregnancy, including routine evaluation of retinal angiomas, non-contrast MRI during fourth month for CNS lesions, plasma metanaphrine testing during early, mid and late pregnancy as well as consideration of delivery via caesarian section to lower the chance of increased intracranial pressure.[103,106] As long as patients are followed closely by a multidisciplinary team, pregnancy is typically a safe option for women with VHL, but other options such as surrogacy and adoption can also be explored.

Psychosocial impact of VHL, including burden of lifelong surveillance

There are little data on the psychosocial impact of complex tumor predisposition syndromes with limited prevention options. However, the data that do exist suggest that a significant proportion of those affected by VHL and those who care for them experience clinically important levels of distress.[107] Those that experienced the death of a close relative due to VHL during adolescence were particularly vulnerable. About one-third of participants had

401 received professional psychosocial support, and the rest seemed amenable to it.[107] Many patients
402 struggle with the complex medical, social and psychological aspects of VHL, for example,
403 uncertainty about future tumor development, frustration regarding lifelong screening, strained
404 relationships with family and partners, difficulty communicating with others about VHL, and
405 complex decisions regarding childbearing. [87,108]

406 A highly sensitive concern for VHL patients is the burden of lifelong surveillance.
407 Suggested surveillance guidelines are summarized in Table 5. While surveillance can pick up
408 early-stage lesions that are amenable to treatment i.e. retinal angiomas, it also identifies lesions
409 for which there is no immediate clinical benefit i.e. pancreatic cysts, and even findings unrelated
410 to VHL that require further follow-up.[86] For these reasons, most individuals with VHL view
411 surveillance regimens as a "necessary yet anxiety-provoking burden" that incite a variety of
412 responses including denial, anger, fear, sadness and anxiety.[87] While the uptake of genetic testing
413 is generally high in VHL families, as many as 60% of identified mutation carriers will be lost to
414 follow-up 5 years after testing, suggesting that patients' concerns regarding surveillance are not
415 being appropriately addressed.[90] As current VHL surveillance guidelines are largely based on
416 expert medical opinion and limited evidence, healthcare providers also struggle with determining
417 "optimal" surveillance recommendations while also minimizing the patient's psychological
418 distress ("scanxiety") and expenses to the healthcare system.[109]

419 Recently, nationwide efforts by VHL care teams in Denmark and the Netherlands have
420 sought to address these issues by providing additional evidence regarding the optimal initiation
421 and frequency of surveillance regimes.[109-113] Kruizinga et al. calculated organ-specific age to
422 initiate surveillance (and surveillance intervals), which were 0 years/at birth for the adrenal

glands (4 year interval between screenings), 7 years for the retina (2 year interval), 14 for the cerebellum (1 year interval), 15 for the spinal cord (1 year interval), 16 for the pancreas (2 year interval), and 18 for the kidneys (1 year interval).[111] These findings represent significant deviation from current surveillance guidelines particularly in terms of adrenal and retinal surveillance, with a 6 year later initiation of retinal surveillance and one year longer interval follow-up, and 5 year earlier initiation of adrenal surveillance with 3 year longer interval follow-up.[111] They were also able to calculate the age, 34 years old, at which the probability of developing a *first* manifestation of VHL is <5%, representing a reasonable age to stop surveillance, for instance in at-risk family members of a proband whose disease-causing mutation cannot be identified.[111]

Other groups have suggested possible modifications in the current guidelines for other organs, for example, Poulson et al. found that biennial CNS examinations led to relatively high rate (7.2%) of interval lesions with clinical consequences, whereas annual screening reduced the risk to an acceptable rate of 2.7%.[113] Binderup et al. pointed out that these and other previous studies have assumed the risk of new tumor development is constant throughout a VHL patient's lifetime, however, risk can vary significantly with age and genotype and depends on the organ involved.[109] They found that tumor development was highest at 30-34 years, and when broken down by the most common organs affected in their cohort, the risk of retinal tumors was highest during teenage years (15-19 years) and the risk of cerebellar tumors was highest during the 30s. Therefore, adherence to surveillance of these organs during those times should be particularly encouraged. Additionally, they stratified by genotype, and found that carriers of truncating mutations had significantly higher rates of manifestations compared to missense mutation

carriers, with the exception of retinal tumors which were significantly less frequent in carriers of truncating mutations.[109] While some argue that surveillance recommendations should not be influenced by specific mutations as the second hit that results in tumor development is random, the goal in any hereditary cancer predisposition syndrome is to be able to tailor management guidelines based on individual factors, such as genotype, environmental stimuli, or other genetic alterations.

As all of the above mentioned studies represent significant deviations from the current surveillance guidelines, they would need to be confirmed by larger prospective studies in other geographic populations before being incorporated into clinical practice.

In terms of practical strategies for increasing adherence to surveillance protocols, it is crucial for health care providers to set expectations before screening regarding benefits and limitations, and logistical matters including how and when results will be relayed. It is important to engage the whole family in this discussion if possible, as studies have shown that family members tend to take the same stance towards long-term surveillance.[90] Families also need to understand that absence of symptoms is not a reason to delay screening; the variable nature of the disease within families can create the misconception that only the members who are the most symptomatic require close follow-up.[90,112] It is also important that health care providers adhere to national guidelines for surveillance because patients given variable advice at different institutions tend not to fully adhere to the given advice.[112] If feasible, a "case manager" (specially trained nurse practitioner or genetic counselor) for VHL families is advised in order to serve as a primary contact and help coordinate multidisciplinary care, including medical follow-up and psychosocial needs.[112]

The role of patient associations is crucial in disseminating up-to-date information about rare diseases to patients and physicians. The VHL Alliance, established in 1994, has a worldwide presence and actively contributes to supporting VHL research and works with experts in the field to establish current surveillance recommendations (www.vhl.org). There are now National VHL networks and/or specialized clinical care centers in over 30 countries that offer specialized medical and psychosocial support and opportunity to connect with VHL patients from around the world.[48,106] Table 4 provides a list of VHL and genetics resources for patients, their caregivers, and managing physicians.

In summary, VHL is a complex and intriguing disease from a genetic, clinical, and psychosocial standpoint. It is a disease that spans a breadth of pediatric and adult oncologic subspecialties and as such providers should be aware of when consider the diagnosis and the special considerations involved in genetic workup and familial testing. There have been many advances in the understanding of VHL over the years, and continued discoveries will lend insight into the treatment of variety of hereditary and sporadic cancers and help optimize all aspects of care for VHL patients and their families. Strategies may need to adjust as new therapies are expected to become available, likely on an organ-by-organ basis. For example, for the treatment of ccRCC, both VHL patients and sporadic patients have enormously benefited from VHL-driven molecular biology, in particular VEGF pathway inhibitors like sunitinib and pazopanib which currently form the only two first-line therapies recommended for treatment of metastasized RCC. However, recent FDA approval of immuno-oncological agents like nivolumab for second-line treatment of advanced renal cell carcinoma[114] should be carefully followed in VHL patients, since presumably a single VHL patient may have hundreds or more

subclinical lesions throughout their body. Exciting developments in gene therapy offer future promise for VHL patients,[115] but for now surveillance remains the best approach for disease management.

Acknowledgements

This review is dedicated to all our patients and their families, and their multidisciplinary caregivers and researchers. We would also like to thank the VHL Alliance, particularly Ilene Sussman, and Eric Jonasch, MD for suggesting this collaboration and supporting our efforts. C.E. is the Sondra J. and Stephen R. Hardis Endowed Chair of Cancer Genomic Medicine at the Cleveland Clinic, and an American Cancer Society Clinical Research Professor. RHG acknowledges the Dutch Kidney Foundation "KOUNCIL" consortium (CP11.13).

References

1. Hes FJ, McKee S, Taphoorn MJ, et al. Cryptic von Hippel-Lindau disease: germline mutations in patients with haemangioblastoma only. *Journal of medical genetics.* 2000;37(12):939-943.
2. Neumann HP, Bausch B, McWhinney SR, et al. Germ-line mutations in nonsyndromic pheochromocytoma. *The New England journal of medicine.* 2002;346(19):1459-1466.
3. Maher ER, Yates JR, Harries R, et al. Clinical features and natural history of von Hippel-Lindau disease. *Q J Med.* 1990;77(283):1151-1163.
4. Friedrich CA. Von Hippel-Lindau syndrome. A pleomorphic condition. *Cancer.* 1999;86(11 Suppl):2478-2482.
5. Lonser RR, Glenn GM, Walther M, et al. von Hippel-Lindau disease. *Lancet.* 2003;361(9374):2059-2067.
6. Maher ER, Neumann HP, Richard S. von Hippel-Lindau disease: a clinical and scientific review. *Eur J Hum Genet.* 2011;19(6):617-623.
7. Lindau A. Studien uber Kleinhirncysten: Bau, Pathologenese und Beziehungen zur Angiomatosis Retinae. *Acta Pathol Microbiol Scand Supp.* 1926;1:1-128.
8. Latif F, Tory K, Gnarra J, et al. Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science (New York, N.Y.).* 1993;260(5112):1317-1320.
9. Nordstrom-O'Brien M, van der Luijt RB, van Rooijen E, et al. Genetic analysis of von Hippel-Lindau disease. *Human mutation.* 2010;31(5):521-537.
10. Beroud C, Joly D, Gallou C, Staroz F, Orfanelli MT, Junien C. Software and database for the analysis of mutations in the VHL gene. *Nucleic Acids Res.* 1998;26(1):256-258.
11. Melmon KL, Rosen SW. LINDAU'S DISEASE. REVIEW OF THE LITERATURE AND STUDY OF A LARGE KINDRED. *Am J Med.* 1964;36:595-617.

12. Neumann HP. Basic criteria for clinical diagnosis and genetic counselling in von Hippel-Lindau syndrome. *Vasa.* 1987;16(3):220-226.

13. Choyke PL, Glenn GM, Walther MM, Patronas NJ, Linehan WM, Zbar B. von Hippel-Lindau disease: genetic, clinical, and imaging features. *Radiology.* 1995;194(3):629-642.

14. Prowse AH, Webster AR, Richards FM, et al. Somatic inactivation of the VHL gene in Von Hippel-Lindau disease tumors. *Am J Hum Genet.* 1997;60(4):765-771.

15. Crossey PA, Foster K, Richards FM, et al. Molecular genetic investigations of the mechanism of tumourigenesis in von Hippel-Lindau disease: analysis of allele loss in VHL tumours. *Hum Genet.* 1994;93(1):53-58.

16. Tory K, Brauch H, Linehan M, et al. Specific genetic change in tumors associated with von Hippel-Lindau disease. *Journal of the National Cancer Institute.* 1989;81(14):1097-1101.

17. Zbar B, Brauch H, Talmadge C, Linehan M. Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma. *Nature.* 1987;327(6124):721-724.

18. Kaelin WG, Jr. The von Hippel-Lindau tumor suppressor gene and kidney cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2004;10(18 Pt 2):6290S-6295S.

19. Gerlinger M, Horswell S, Larkin J, et al. Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing. *Nat Genet.* 2014;46(3):225-233.

20. Glasker S, Bender BU, Apel TW, et al. Reconsideration of biallelic inactivation of the VHL tumour suppressor gene in hemangioblastomas of the central nervous system. *J Neurol Neurosurg Psychiatry.* 2001;70(5):644-648.

21. Oberstrass J, Reifenberger G, Reifenberger J, Wechsler W, Collins VP. Mutation of the Von Hippel-Lindau tumour suppressor gene in capillary haemangioblastomas of the central nervous system. *J Pathol.* 1996;179(2):151-156.

22. Eng C, Crossey PA, Mulligan LM, et al. Mutations in the RET proto-oncogene and the von Hippel-Lindau disease tumour suppressor gene in sporadic and syndromic phaeochromocytomas. *Journal of medical genetics.* 1995;32(12):934-937.

23. Gossage L, Eisen T, Maher ER. VHL, the story of a tumour suppressor gene. *Nat Rev Cancer.* 2015;15(1):55-64.

24. Frantzen C, Klasson TD, Links TP, Giles RH. Von Hippel-Lindau Syndrome. In: Pagon RA, Adam MP, Ardinger HH, et al., eds. *GeneReviews(R).* Seattle WA: University of Washington, Seattle; 1993.

25. Stebbins CE, Kaelin WG, Jr., Pavletich NP. Structure of the VHL-ElonginC-ElonginB complex: implications for VHL tumor suppressor function. *Science (New York, N.Y.).* 1999;284(5413):455-461.

26. McClellan AJ, Scott MD, Frydman J. Folding and quality control of the VHL tumor suppressor proceed through distinct chaperone pathways. *Cell.* 2005;121(5):739-748.

27. Lee S, Nakamura E, Yang H, et al. Neuronal apoptosis linked to EglN3 prolyl hydroxylase and familial pheochromocytoma genes: developmental culling and cancer. *Cancer cell.* 2005;8(2):155-167.

28. Hergovich A, Lisztwan J, Thoma CR, Wirbelauer C, Barry RE, Krek W. Priming-dependent phosphorylation and regulation of the tumor suppressor pVHL by glycogen synthase kinase 3. *Mol Cell Biol.* 2006;26(15):5784-5796.

29. Lolkema MP, Mehra N, Jorna AS, van Beest M, Giles RH, Voest EE. The von Hippel-Lindau tumor suppressor protein influences microtubule dynamics at the cell periphery. *Exp Cell Res.* 2004;301(2):139-146.

30. Schermer B, Ghenoiu C, Bartram M, et al. The von Hippel-Lindau tumor suppressor protein controls ciliogenesis by orienting microtubule growth. *J Cell Biol.* 2006;175(4):547-554.

31. Dere R, Perkins AL, Bawa-Khalfe T, Jonasch D, Walker CL. beta-catenin links von Hippel-Lindau to aurora kinase A and loss of primary cilia in renal cell carcinoma. *J Am Soc Nephrol.* 2015;26(3):553-564.

32. Ding XF, Zhou J, Hu QY, Liu SC, Chen G. The tumor suppressor pVHL down-regulates never-in-mitosis A-related kinase 8 via hypoxia-inducible factors to maintain cilia in human renal cancer cells. *J Biol Chem.* 2015;290(3):1389-1394.

33. Troilo A, Alexander I, Muehl S, Jaramillo D, Knobeloch KP, Krek W. HIF1alpha deubiquitination by USP8 is essential for ciliogenesis in normoxia. *EMBO Rep.* 2014;15(1):77-85.

34. Hell MP, Duda M, Weber TC, Moch H, Krek W. Tumor suppressor VHL functions in the control of mitotic fidelity. *Cancer Res.* 2014;74(9):2422-2431.

35. Yang H, Minamishima YA, Yan Q, et al. pVHL acts as an adaptor to promote the inhibitory phosphorylation of the NF-kappaB agonist Card9 by CK2. *Mol Cell.* 2007;28(1):15-27.

36. Kuznetsova AV, Meller J, Schnell PO, et al. von Hippel-Lindau protein binds hyperphosphorylated large subunit of RNA polymerase II through a proline hydroxylation motif and targets it for ubiquitination. *Proc Natl Acad Sci U S A.* 2003;100(5):2706-2711.

37. Yi Y, Mikhaylova O, Mamedova A, et al. von Hippel-Lindau-dependent patterns of RNA polymerase II hydroxylation in human renal clear cell carcinomas. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2010;16(21):5142-5152.

600 38. Roe JS, Kim HR, Hwang IY, et al. Phosphorylation of von Hippel-Lindau protein by checkpoint
601 kinase 2 regulates p53 transactivation. *Cell Cycle.* 2011;10(22):3920-3928.
602 39. Young AP, Schlisio S, Minamishima YA, et al. VHL loss actuates a HIF-independent senescence
603 programme mediated by Rb and p400. *Nat Cell Biol.* 2008;10(3):361-369.
604 40. Okuda H, Saitoh K, Hirai S, et al. The von Hippel-Lindau tumor suppressor protein mediates
605 ubiquitination of activated atypical protein kinase C. *J Biol Chem.* 2001;276(47):43611-43617.
606 41. Kurban G, Hudon V, Duplan E, Ohh M, Pause A. Characterization of a von Hippel Lindau pathway
607 involved in extracellular matrix remodeling, cell invasion, and angiogenesis. *Cancer Res.*
608 2006;66(3):1313-1319.
609 42. Kurban G, Duplan E, Ramlal N, et al. Collagen matrix assembly is driven by the interaction of von
610 Hippel-Lindau tumor suppressor protein with hydroxylated collagen IV alpha 2. *Oncogene.*
611 2008;27(7):1004-1012.
612 43. Stickle NH, Chung J, Klco JM, Hill RP, Kaelin WG, Jr., Ohh M. pVHL modification by NEDD8 is
613 required for fibronectin matrix assembly and suppression of tumor development. *Mol Cell Biol.*
614 2004;24(8):3251-3261.
615 44. Bishop T, Lau KW, Epstein AC, et al. Genetic analysis of pathways regulated by the von Hippel-
616 Lindau tumor suppressor in Caenorhabditis elegans. *PLoS Biol.* 2004;2(10):e289.
617 45. Koochekpour S, Jeffers M, Wang PH, et al. The von Hippel-Lindau tumor suppressor gene inhibits
618 hepatocyte growth factor/scatter factor-induced invasion and branching morphogenesis in renal
619 carcinoma cells. *Mol Cell Biol.* 1999;19(9):5902-5912.
620 46. Tang N, Mack F, Haase VH, Simon MC, Johnson RS. pVHL function is essential for endothelial
621 extracellular matrix deposition. *Mol Cell Biol.* 2006;26(7):2519-2530.

622 47. Fisher R, Horswell S, Rowan A, et al. Development of synchronous VHL syndrome tumors reveals
623 contingencies and constraints to tumor evolution. *Genome Biol.* 2014;15(8):433.
624 48. Richard S, Gardie B, Couve S, Gad S. Von Hippel-Lindau: how a rare disease illuminates cancer
625 biology. *Semin Cancer Biol.* 2013;23(1):26-37.
626 49. Hes FJ, van der Luijt RB, Janssen AL, et al. Frequency of Von Hippel-Lindau germline mutations in
627 classic and non-classic Von Hippel-Lindau disease identified by DNA sequencing, Southern blot
628 analysis and multiplex ligation-dependent probe amplification. *Clin Genet.* 2007;72(2):122-129.
629 50. Richard S, David P, Marsot-Dupuch K, Giraud S, Beroud C, Resche F. Central nervous system
630 hemangioblastomas, endolymphatic sac tumors, and von Hippel-Lindau disease. *Neurosurg Rev.*
631 2000;23(1):1-22; discussion 23-24.
632 51. Woodward ER, Wall K, Forsyth J, Macdonald F, Maher ER. VHL mutation analysis in patients with
633 isolated central nervous system haemangioblastoma. *Brain.* 2007;130(Pt 3):836-842.
634 52. Singh A, Shields J, Shields C. Solitary retinal capillary hemangioma: hereditary (von Hippel-Lindau
635 disease) or nonhereditary? *Arch Ophthalmol.* 2001;119(2):232-234.
636 53. Brauch H, Hoeppner W, Jahnig H, et al. Sporadic pheochromocytomas are rarely associated with
637 germline mutations in the vhl tumor suppressor gene or the ret protooncogene. *J Clin Endocrinol*
638 *Metab.* 1997;82(12):4101-4104.
639 54. Fishbein L, Merrill S, Fraker DL, Cohen DL, Nathanson KL. Inherited mutations in
640 pheochromocytoma and paraganglioma: why all patients should be offered genetic testing. *Ann*
641 *Surg Oncol.* 2013;20(5):1444-1450.
642 55. Neumann HP, Bender BU, Berger DP, et al. Prevalence, morphology and biology of renal cell
643 carcinoma in von Hippel-Lindau disease compared to sporadic renal cell carcinoma. *J Urol.*
644 1998;160(4):1248-1254.

56. Hampel H, Bennett RL, Buchanan A, Pearlman R, Wiesner GL. A practice guideline from the American College of Medical Genetics and Genomics and the National Society of Genetic Counselors: referral indications for cancer predisposition assessment. *Genet Med.* 2015;17(1):70-87.

57. FJ OB, Danapal M, Jairam S, et al. Manifestations of Von Hippel Lindau syndrome: a retrospective national review. *QJM.* 2014;107(4):291-296.

58. Maher ER, Kaelin WG, Jr. von Hippel-Lindau disease. *Medicine (Baltimore).* 1997;76(6):381-391.

59. Stolle C, Glenn G, Zbar B, et al. Improved detection of germline mutations in the von Hippel-Lindau disease tumor suppressor gene. *Human mutation.* 1998;12(6):417-423.

60. Cho HJ, Ki CS, Kim JW. Improved detection of germline mutations in Korean VHL patients by multiple ligation-dependent probe amplification analysis. *J Korean Med Sci.* 2009;24(1):77-83.

61. Shuch B, Vourganti S, Ricketts CJ, et al. Defining early-onset kidney cancer: implications for germline and somatic mutation testing and clinical management. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2014;32(5):431-437.

62. Reaume MN, Graham GE, Tomiak E, et al. Canadian guideline on genetic screening for hereditary renal cell cancers. *Can Urol Assoc J.* 2013;7(9-10):319-323.

63. Maher ER, Iselius L, Yates JR, et al. Von Hippel-Lindau disease: a genetic study. *Journal of medical genetics.* 1991;28(7):443-447.

64. Wilding A, Ingham SL, Lalloo F, et al. Life expectancy in hereditary cancer predisposing diseases: an observational study. *Journal of medical genetics.* 2012;49(4):264-269.

65. Glenn GM, Daniel LN, Choyke P, et al. Von Hippel-Lindau (VHL) disease: distinct phenotypes suggest more than one mutant allele at the VHL locus. *Hum Genet.* 1991;87(2):207-210.

667 66. Neumann HP, Wiestler OD. Clustering of features of von Hippel-Lindau syndrome: evidence for a
668 complex genetic locus. *Lancet.* 1991;337(8749):1052-1054.
669 67. Chen F, Kishida T, Yao M, et al. Germline mutations in the von Hippel-Lindau disease tumor
670 suppressor gene: correlations with phenotype. *Human mutation.* 1995;5(1):66-75.
671 68. Zbar B, Kishida T, Chen F, et al. Germline mutations in the Von Hippel-Lindau disease (VHL) gene
672 in families from North America, Europe, and Japan. *Human mutation.* 1996;8(4):348-357.
673 69. Crossey PA, Richards FM, Foster K, et al. Identification of intragenic mutations in the von Hippel-
674 Lindau disease tumour suppressor gene and correlation with disease phenotype. *Human*
675 *molecular genetics.* 1994;3(8):1303-1308.
676 70. !!! INVALID CITATION !!! 4,44-46,57.
677 71. Mulvihill JJ, Ferrell RE, Carty SE, Tisherman SE, Zbar B. Familial pheochromocytoma due to
678 mutant von Hippel-Lindau disease gene. *Arch Intern Med.* 1997;157(12):1390-1391.
679 72. Tisherman SE, Gregg FJ, Danowski TS. Familial pheochromocytoma. *JAMA.* 1962;182:152-156.
680 73. Tisherman SE, Tisherman BG, Tisherman SA, Dunmire S, Levey GS, Mulvihill JJ. Three-decade
681 investigation of familial pheochromocytoma. An allele of von Hippel-Lindau disease? *Arch Intern*
682 *Med.* 1993;153(22):2550-2556.
683 74. Brauch H, Kishida T, Glavac D, et al. Von Hippel-Lindau (VHL) disease with pheochromocytoma in
684 the Black Forest region of Germany: evidence for a founder effect. *Hum Genet.* 1995;95(5):551-
685 556.
686 75. Crossey PA, Eng C, Ginalska-Malinowska M, et al. Molecular genetic diagnosis of von Hippel-
687 Lindau disease in familial phaeochromocytoma. *Journal of medical genetics.* 1995;32(11):885-
688 886.

689 76. Chen F, Slife L, Kishida T, Mulvihill J, Tisherman SE, Zbar B. Genotype-phenotype correlation in
690 von Hippel-Lindau disease: identification of a mutation associated with VHL type 2A. *Journal of*
691 *medical genetics.* 1996;33(8):716-717.
692 77. Ang SO, Chen H, Gordeuk VR, et al. Endemic polycythemia in Russia: mutation in the VHL gene.
693 *Blood Cells Mol Dis.* 2002;28(1):57-62.
694 78. Gordeuk VR, Sergueeva AI, Miasnikova GY, et al. Congenital disorder of oxygen sensing:
695 association of the homozygous Chuvash polycythemia VHL mutation with thrombosis and
696 vascular abnormalities but not tumors. *Blood.* 2004;103(10):3924-3932.
697 79. Glenn G, Choyke P, Zbar B, Linehan W. von Hippel-Lindau disease: clinical review and molecular
698 genetics *Probl Urol.* 1990;4:312-330.
699 80. Sgambati MT, Stolle C, Choyke PL, et al. Mosaicism in von Hippel-Lindau disease: lessons from
700 kindreds with germline mutations identified in offspring with mosaic parents. *Am J Hum Genet.*
701 2000;66(1):84-91.
702 81. Santarpia L, Sarlis NJ, Santarpia M, Sherman SI, Trimarchi F, Benvenga S. Mosaicism in von
703 Hippel-Lindau disease: an event important to recognize. *J Cell Mol Med.* 2007;11(6):1408-1415.
704 82. Coppin L, Grutzmacher C, Crepin M, et al. VHL mosaicism can be detected by clinical next-
705 generation sequencing and is not restricted to patients with a mild phenotype. *Eur J Hum Genet.*
706 2014;22(9):1149-1152.
707 83. Wu P, Zhang N, Wang X, et al. Mosaicism in von Hippel-Lindau disease with severe renal
708 manifestations. *Clin Genet.* 2013;84(6):581-584.
709 84. Ajzenberg H, Slaats GG, Stokman MF, et al. Non-invasive sources of cells with primary cilia from
710 pediatric and adult patients. *Cilia.* 2015;4:8.

85. Priesemann M, Davies KM, Perry LA, et al. Benefits of screening in von Hippel-Lindau disease--comparison of morbidity associated with initial tumours in affected parents and children. *Horm Res.* 2006;66(1):1-5.

86. Evans DG, Maher ER, Macleod R, Davies DR, Craufurd D. Uptake of genetic testing for cancer predisposition. *Journal of medical genetics.* 1997;34(9):746-748.

87. Kasparian NA, Rutstein A, Sansom-Daly UM, et al. Through the looking glass: an exploratory study of the lived experiences and unmet needs of families affected by Von Hippel-Lindau disease. *Eur J Hum Genet.* 2015;23(1):34-40.

88. Ross LF, Saal HM, David KL, Anderson RR. Technical report: Ethical and policy issues in genetic testing and screening of children. *Genet Med.* 2013;15(3):234-245.

89. Schiffman JD, Geller JI, Mundt E, Means A, Means L, Means V. Update on pediatric cancer predisposition syndromes. *Pediatr Blood Cancer.* 2013;60(8):1247-1252.

90. Rasmussen A, Alonso E, Ochoa A, et al. Uptake of genetic testing and long-term tumor surveillance in von Hippel-Lindau disease. *BMC Med Genet.* 2010;11:4.

91. Tischkowitz M, Rosser E. Inherited cancer in children: practical/ethical problems and challenges. *Eur J Cancer.* 2004;40(16):2459-2470.

92. Metcalfe A, Plumridge G, Coad J, Shanks A, Gill P. Parents' and children's communication about genetic risk: a qualitative study, learning from families' experiences. *Eur J Hum Genet.* 2011;19(6):640-646.

93. Rowland E, Metcalfe A. Communicating inherited genetic risk between parent and child: a meta-thematic synthesis. *Int J Nurs Stud.* 2013;50(6):870-880.

94. Wolfe Schneider K, Jasperson K. Unique Genetic Counseling Considerations in the Pediatric Oncology Setting. *Current Genetic Medicine Reports.* 2015.

95. Knapke S, Zelley K, Nichols KE, Kohlmann W, Schiffman JD. Identification, management, and evaluation of children with cancer-predisposition syndromes. *Am Soc Clin Oncol Educ Book.* 2012:576-584.

96. Levy M, Richard S. Attitudes of von Hippel-Lindau disease patients towards presymptomatic genetic diagnosis in children and prenatal diagnosis. *Journal of medical genetics.* 2000;37(6):476-478.

97. Lips CJ. Clinical management of the multiple endocrine neoplasia syndromes: results of a computerized opinion poll at the Sixth International Workshop on Multiple Endocrine Neoplasia and von Hippel-Lindau disease. *J Intern Med.* 1998;243(6):589-594.

98. Szybowska M, Hewson S, Antle BJ, Babul-Hirji R. Assessing the informational needs of adolescents with a genetic condition: what do they want to know? *J Genet Couns.* 2007;16(2):201-210.

99. Metcalfe A, Coad J, Plumridge GM, Gill P, Farndon P. Family communication between children and their parents about inherited genetic conditions: a meta-synthesis of the research. *Eur J Hum Genet.* 2008;16(10):1193-1200.

100. McConkie-Rosell A, Heise EM, Spiridigliozzi GA. Genetic risk communication: experiences of adolescent girls and young women from families with fragile X syndrome. *J Genet Couns.* 2009;18(4):313-325.

101. Rechitsky S, Verlinsky O, Chistokhina A, et al. Preimplantation genetic diagnosis for cancer predisposition. *Reprod Biomed Online.* 2002;5(2):148-155.

102. Lammens C, Bleiker E, Aaronson N, et al. Attitude towards pre-implantation genetic diagnosis for hereditary cancer. *Fam Cancer.* 2009;8(4):457-464.

103. Frantzen C, Kruizinga RC, van Asselt SJ, et al. Pregnancy-related hemangioblastoma progression and complications in von Hippel-Lindau disease. *Neurology.* 2012;79(8):793-796.

104. Frantzen C, van Asselt SJ, Kruizinga RC, et al. Letter to the editor: Pregnancy and von Hippel-Lindau disease. *J Neurosurg.* 2013;118(6):1380.

105. Ye DY, Bakhtian KD, Asthagiri AR, Lonser RR. Effect of pregnancy on hemangioblastoma development and progression in von Hippel-Lindau disease. *J Neurosurg.* 2012;117(5):818-824.

106. Alliance V. The VHL handbook: what you need to know about VHL: a reference handbook for people with von Hippel-Lindau disease, their families, and support personnel. . 2014.

107. Lammens CR, Bleiker EM, Verhoef S, et al. Psychosocial impact of Von Hippel-Lindau disease: levels and sources of distress. *Clin Genet.* 2010;77(5):483-491.

108. Lammens CR, Bleiker EM, Verhoef S, et al. Distress in partners of individuals diagnosed with or at high risk of developing tumors due to rare hereditary cancer syndromes. *Psychooncology.* 2011;20(6):631-638.

109. Binderup ML, Budtz-Jorgensen E, Bisgaard ML. Risk of new tumors in von Hippel-Lindau patients depends on age and genotype. *Genet Med.* 2016;18(1):89-97.

110. Binderup ML, Bisgaard ML, Harbud V, et al. Von Hippel-Lindau disease (vHL). National clinical guideline for diagnosis and surveillance in Denmark. 3rd edition. *Dan Med J.* 2013;60(12):B4763.

111. Kruizinga RC, Sluiter WJ, de Vries EG, et al. Calculating optimal surveillance for detection of von Hippel-Lindau-related manifestations. *Endocr Relat Cancer.* 2014;21(1):63-71.

112. Lammens CR, Aaronson NK, Hes FJ, et al. Compliance with periodic surveillance for Von-Hippel-Lindau disease. *Genet Med.* 2011;13(6):519-527.

113. Poulsen ML, Budtz-Jorgensen E, Bisgaard ML. Surveillance in von Hippel-Lindau disease (vHL). *Clin Genet.* 2010;77(1):49-59.

779 114. Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus Everolimus in Advanced Renal-
780 Cell Carcinoma. *The New England journal of medicine.* 2015;373(19):1803-1813.
781 115. Ashtari M, Zhang H, Cook PA, et al. Plasticity of the human visual system after retinal gene
782 therapy in patients with Leber's congenital amaurosis. *Sci Transl Med.* 2015;7(296):296ra110.
783 116. Maher ER, Webster AR, Richards FM, et al. Phenotypic expression in von Hippel-Lindau disease:
784 correlations with germline VHL gene mutations. *Journal of medical genetics.* 1996;33(4):328-
785 332.
786 117. Hes F, Zewald R, Peeters T, et al. Genotype-phenotype correlations in families with deletions in
787 the von Hippel-Lindau (VHL) gene. *Hum Genet.* 2000;106(4):425-431.

788

789

790

791

792

793

794

795

796

797

798 **Table 1.** Indications for consideration of genetic counseling/testing for VHL[56,57,61]

Simplex Case is Sufficient for Referral	Presence of >1 Tumor is Suggested for Referral
Retinal/CNS HB	Pancreatic cystadenoma
PCC/PGL	PNET
ELST	Epididymal/adnexal cystadenoma**
Clear-cell RCC*	Clear-cell RCC***

799

800 ***If diagnosed <50 years old or ≥ 1 close relative with clear-cell RCC

801 ** Bilateral papillary cystadenomas of adnexal/broad ligament are pathognomonic for VHL

802 *** If diagnosed >50 years old or no close relatives clear-cell RCC

803

804 **Table 2.** VHL and Genetics Testing Resources

Organization	Website
VHL Family Alliance	www.vhl.org
GeneReviews® -VHL	http://www.ncbi.nlm.nih.gov/books/NBK1463/
PDQ® Summaries- VHL	http://www.cancer.gov/types/kidney/hp/kidney-genetics-pdq
National Society of Genetic Counselors	www.nsgc.org
Genetic Testing Registry	http://www.ncbi.nlm.nih.gov/gtr/
GeneTests™	https://www.genetests.org/

805

806

807

808

809

810

811

812

Table 3. Frequency and Age of Onset of VHL-associated Tumors[5,6,48,58]

	Mean (range) age of onset (years)	Frequency in patients
CNS		
Retinal HB	25 (1-68)	25-60%
ELST	22 (12-50)	10-15%
Craniospinal HB (overall)	30 (9-70)	60-80%
Cerebellum	33 (9-78)	44-72%
Brainstem	32 (12-46)	10-25%
Spinal cord	33 (11-66)	13-50%
Visceral		
RCC or cysts	39 (13-70)	25-75%
PCC	27 (5-58)	10-25%
PNET or cyst	36 (5-70)	35-75%*
Epididymal cystadenoma	Unknown	25-60%
Broad ligament cystadenoma	Unknown (16-46)	Unknown

* Frequency of PNET is 11-17% whereas that of pancreatic cysts is up to 75%[48]

Adapted from Lonser et al. 2003[5] and Maher & Kaelin, 1997[58]

827 **Table 4.** VHL Subtypes[76,116,117]

828

VHL Subtype	VHL Mutation Type	High Risk	Low Risk
Type 1	Deletions, insertions, truncations, missense	CNS/retinal HB, RCC	PCC
Type 1B	Contiguous gene deletions encompassing *VHL*	CNS/retinal HB	PCC, RCC (risk may increased if *C3orf10* remains increased)
Type 2A	Missense; e.g. p.Y98H, p.Y112H, p.V116F	CNS/retinal HB, PCC	RCC
Type 2B	Missense; e.g. p.R167Q, p.R167W	CNS/retinal HB, RCC, PCC	
Type 2C	Missense; e.g. p.V84L p.L188V	PCC	CNS/retinal HB, RCC absent

829

830

831

832

833

834

835

836

837

838

839

840 **Table 5.** Suggested VHL Surveillance Guidelines[106,109,113]

Age	Screening	Frequency
0-4	• Eye/retinal exam with indirect ophthalmoscope • Physical exam with blood pressure check & neurological assessment	Annually Annually
5-15	Above plus: • Test for plasma free metanephrines, or urinary metanephrines using 24-hour urine test • Abdominal u/s from 8yrs or earlier if indicated; abdominal MRI or functional imaging scan only if biochemical abnormalities found • Audiology assessment; in the case of repeated ear infections, MRI with contrast of the internal auditory canal	 Annually Annually 2-3 yrs (1 yr if tinnitus, hearing loss or vertigo)
16+	Above plus: • Quality ultrasound of abdomen • MRI of abdomen with and without contrast • MRI of brain and cervical spine	 Annually Every 2 yrs Every 1-2 yrs

841

842

843