# Von Hippel-Lindau Disease

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Catherine A. Stolle

**Abstract**

Von Hippel-Lindau disease (VHLD) is an autosomal dominant cancer predisposition syndrome characterized by tumors of the brain and spine, retina, kidney, adrenal glands, and several other organs. Point mutations and deletions in the *VHL* tumor suppressor gene have been identified in virtually 100 % of patients who meet strict clinical criteria. Molecular diagnostic testing consists of DNA sequence analysis of the coding region (~72 % of mutations) as well as deletion analysis (~28 % of mutations). Most cases are inherited; however about 20 % of cases are the result of de novo mutations; individuals mosaic for a VHL gene mutation have been reported. Molecular testing may be used to confirm a clinical diagnosis in an affected patient or rule out the disease in individuals with a single typical tumor. In families with a known mutation, molecular testing may be used for predictive testing of at-risk members or for prenatal testing.

**Keywords**

Von Hippel-Lindau disease • *VHL* gene • Tumor suppressor • De novo mutations • Molecular testing • Diagnostic testing • Predictive testing • Prenatal testing

## Introduction

Von Hippel-Lindau disease (VHLD; OMIM#19330) is an autosomal dominant cancer predisposition syndrome that gives rise to hemangioblastomas of the brain and spine, retinal angiomas, clear cell renal cell carcinoma, pheochromocytoma, endolymphatic sac tumors, tumors of the epididymis or broad ligament, and pancreatic tumors or cysts [1]. The incidence of VHLD is approximately 1 in 36,000 live births [2]. Onset is typically between the second and fourth decades of life, with penetrance for the disease nearly complete by the age of 65 years. In most cases, a family history of the disorder is apparent. In about 20 % of cases, however, the proband appears to have acquired a new mutation [3].

C.A. Stolle, Ph.D. (✉)
Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia, ARC 714G, 34th and Civic Center Boulevard, Philadelphia, PA 19104, USA
e-mail: stolle@email.chop.edu

## Molecular Basis of Disease

The *VHL* tumor suppressor gene was isolated by positional cloning in 1993 [4]. The gene, which consists of three exons spanning about 10 kilobases (kb) of genomic DNA on the short arm of chromosome 3 (3p25.3), is highly conserved among worms, flies, frogs, fish, chickens, humans, and other mammals (reviewed in Refs. 5 and 6). Two transcripts, 6.0 and 6.5 kb in size, are almost ubiquitously expressed and encode proteins of 213 and 160 amino acid residues, respectively. The latter isoform is the major product in most tissues and results from initiation of translation from an internal methionine codon at position 54. Both protein isoforms appear to be functional.

The VHL protein has been implicated in a variety of functions including transcriptional regulation, posttranscriptional gene expression, apoptosis, extracellular matrix formation, and ubiquitylation (reviewed in Refs. 5 and 6). The role of VHL in the regulation of hypoxia-inducible genes through the targeted ubiquitylation and degradation of hypoxia-inducible factor 1 alpha subunit (HIF1A) has been elucidated, leading



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to a model of how disruption of the *VHL* gene results in the production of highly vascularized tumors.

Normal VHL binds to the protein elongin C, which forms a complex with elongin B, cullin-2 (CUL2), and Rbx1. This complex resembles the SKP1-CUL1-F-box protein (SCF) ubiquitin ligase or E3 complex in yeast that catalyzes the polyubiquitylation of specific proteins and targets them for degradation by proteosomes. Under normoxic conditions, HIF1A is hydroxylated at a specific asparagine residue by a member of the egg-laying deficiency protein nine-like (EGLN) protein family of prolyl hydroxylase enzymes. VHL binds to hydroxylated HIF1A and targets it for degradation. Under hypoxic conditions, HIF1A is not hydroxylated, VHL does not bind, and HIF1A subunits accumulate. HIF1A forms heterodimers with HIF1B and activates transcription of a variety of hypoxia-inducible messenger RNAs (i.e., *VEGF*, *EPO*, *TGFA*, *PDGFB*). Likewise, when VHL is absent or mutated, HIF1A subunits accumulate, resulting in cell proliferation and the neovascularization of tumors characteristic of VHLD [5].

Predisposition to VHLD is inherited in an autosomal dominant manner. However, tumor formation requires inactivation of the second allele (i.e., through loss of heterozygosity, methylation, or point mutation), and so the disease is recessive at the level of the cell. Mutations known to result in predisposition to VHLD include partial or complete deletions of the gene and point mutations (missense, nonsense, frameshift, and splice site). Point mutations are predicted either to truncate the protein, alter protein folding, or interfere with the binding of VHL to elongin C, HIF1A, or other target proteins [5, 7]. Although a handful of mutations and one mutation "hotspot" in exon 3 are common, point mutations are distributed over all three exons of the gene from codon 54 (internal initiator methionine) to the stop codon.

Four VHLD phenotypes have been described based on the likelihood of pheochromocytoma or renal cell carcinoma [8]. Type 1 is characterized by a low risk for pheochromocytoma. Truncating mutations or missense mutations that are predicted to grossly disrupt the folding of the VHL protein [7] are associated with VHLD type 1. VHLD type 2 is characterized by a high risk for pheochromocytoma. Most patients with VHLD type 2 have missense mutations of *VHL*. VHLD type 2 is further subdivided into those with a low risk (type 2A) and those with a high risk (type 2B) of renal cell carcinoma, as well as individuals at risk for pheochromocytoma only (type 2C). Some missense mutations correlate with a specific type 2 VHLD phenotype [6, 8].

A novel genotype-phenotype correlation has been reported, but is not yet considered to be a separate VHL type [9]. Individuals with a complete deletion of the *VHL* gene are more likely to present with multiple hemangioblastomas of the brain or spine or both as the first or only symptom of disease. Pheochromocytoma and renal cell carcinoma are less likely or may present at a later stage in life. At present, it is not clear why a complete deletion of the *VHL* gene would result in a phenotype distinctly different from that caused by a partial deletion or truncating mutation (i.e., VHL type 1). However, deletion of a neighboring gene(s) at the same locus may contribute to this phenotype [6].

## Clinical Utility

Clinical molecular testing for VHLD has proven to be virtually 100 % effective at detecting germline mutations in patients with pathology-proven disease [10]. For this reason, molecular testing may be used to confirm a clinical diagnosis in an affected patient, screen for a mutation in an unaffected individual with a family history of VHLD, or rule out the disease in individuals with one VHLD-like tumor but no family history. When the mutation in the family is known, molecular testing may be used for diagnostic or presymptomatic testing of at-risk family members or for prenatal testing.

A clinical diagnosis of VHLD may be made in an individual with at least two typical VHLD tumors with or without a family history of VHLD or in an individual with at least one typical tumor and a significant family history [1]. In such cases, a *VHL* gene mutation is almost invariably found. Identification of a *VHL* mutation confirms the clinical diagnosis, establishes the need for periodic clinical screening, and facilitates presymptomatic testing of at-risk relatives.

Presymptomatic testing of at-risk family members for a known *VHL* gene mutation permits identification of presymptomatic mutation carriers and leads to early detection of tumors, timely intervention, and improved outcome. Identification of mutation-negative individuals eliminates the need for costly annual clinical screening. Genetic testing for a known *VHL* gene mutation is definitive.

Since the detection rate for germline mutations is high, and since approximately 20 % of patients have VHLD due to a de novo mutation, testing is indicated in individuals with a single VHLD-type tumor and no family history of the disease. A negative-mutation screen greatly reduces the risk of VHLD in this circumstance. Since tumors have been reported in children as young as 4 years of age, and since the mutation status of at-risk individuals affects clinical care, presymptomatic testing of asymptomatic children is appropriate. Prenatal testing for a *VHL* gene mutation is possible when the disease-causing mutation in an affected parent is known. However, prior to testing, consultation with a genetic counselor knowledgeable about the natural history of the disease and available treatment options is strongly recommended.

## Available Assays

A 100 % mutation detection rate has been reported in individuals with a germline mutation in the *VHL* gene using a combination of deletion analysis and DNA sequence analysis [10]. A variety of methods, including quantitative Southern blot analysis [10], fluorescence in situ hybridization [11], real-time quantitative PCR [12], multiplex ligation-dependent probe amplification (MLPA; [13]), and array comparative genomic hybridization [14], have been used for the analysis of deletions in the *VHL* gene. MLPA is commonly used in clinical molecular laboratories for deletion analysis of the *VHL* gene since it is a relatively simple and rapid technique. Partial and complete gene deletions account for approximately 28 % of all cases of VHLD, with complete deletions occurring in 3–5 % of patients. Duplications have not as yet been reported to cause VHL disease.

PCR amplification and DNA sequence analysis of exons 1–3 of the *VHL* gene (including the adjacent splice donor and acceptor sequences) will detect all disease-causing point mutations. The *VHL* gene has few benign polymorphisms. DNA sequence analysis is typically performed by Sanger sequencing using fluorescently labeled dideoxy terminator nucleotides. Analysis for known point mutations in the *VHL* gene using next-generation sequencing technologies is available from several clinical molecular laboratories as part of a panel of cancer-related genes.

## Interpretation of Results

Using a combination of deletion analysis and DNA sequence analysis, germline mutations in the *VHL* gene have been identified in over 300 consecutive patients with well-documented (pathology-proven) VHL disease seen at the National Cancer Institute since 1995. The sensitivity and specificity of the assays used to detect *VHL* gene mutations, therefore, are very high.

If a disease-causing mutation is identified in an affected or at-risk individual, that individual will be predisposed to developing tumors characteristic of VHLD. Periodic screening of target organ systems is recommended for early detection and treatment of tumors. Molecular testing of offspring would be appropriate, as they are at 50 % risk of inheriting the disease-causing mutation. The parents of the proband should be offered molecular testing, as they may have unappreciated disease. The risk to siblings depends on the mutation status of the parents, since the mutations in approximately 20 % of patients are de novo.

The interpretation of a negative-mutation screen depends on the circumstances surrounding the testing and the strength of the clinical diagnosis in the affected family member. A negative test in an individual at risk for a known *VHL* gene mutation is definitive, and the individual is not at risk for developing VHLD and need not undergo clinical screening for VHLD-associated tumors.

In screening of an unaffected at-risk individual for an unknown mutation in the *VHL* gene (i.e., first-degree relative of a patient with a clinical diagnosis of VHLD but no identified *VHL* mutation), a negative-mutation screen indicates only that the individual does not have a germline *VHL* gene mutation. This may be because (1) the individual did not inherit the mutant allele in the affected relative (i.e., a parent), (2) the affected relative does not have VHLD (i.e., as a result of an incorrect or inconclusive diagnosis), or (3) if the unaffected individual is the parent, he or she may be mosaic for a mutation that was transmitted to the affected offspring. Whenever possible, careful clinical evaluation or molecular analysis of an affected family member is suggested to improve the accuracy of the test interpretation for at-risk family members.

For an individual with a clinical diagnosis of VHLD but no family history, a negative-mutation screen indicates that either the patient is a phenocopy (coincidental occurrence of tumors typical of VHLD but without a gene mutation) or a mosaic (with a mutation in some, but not all, cells of the body). Mosaicism has been documented in the affected but mutation-negative parents of patients with a germline *VHL* gene mutation [3]. The frequency of mosaicism is not known but is believed to be low (i.e., <5 %). If the diagnosis of VHLD is supported by pathologic findings, i.e., magnetic resonance imaging of brain or spine, eye examination, abdominal ultrasound or computed tomography scan, urinary catecholamines, etc., then the patient is more likely to be mosaic and should be periodically screened for additional tumors characteristic of VHLD. Offspring (if any) should be considered at risk and should be considered for periodic clinical screening for tumors or molecular testing. The degree of risk (0–50 %) depends on the extent of mosaicism and the potential for transmitting the mutant allele, neither of which can be determined by clinical molecular testing at this time.

For patients who do not fulfill the clinical criteria for VHLD diagnosis because they have a single type of tumor and no family history, a negative-mutation screen would indicate that they are very unlikely to have VHLD; however, mosaicism cannot be entirely ruled out. Although routine clinical surveillance for characteristic VHLD tumors would not appear warranted, regular follow-up visits to a physician regarding the initial tumor would be expected. Likewise, routine screening of offspring would not be indicated unless the proband develops additional tumors (especially of another typical organ system) or if the offspring begin to develop symptoms of VHLD.

Occasionally, a base change in the *VHL* gene will be detected that is neither a known disease-causing mutation nor a known polymorphism. These "variants of unknown significance" with regard to VHLD are generally missense mutations or mutations in introns outside the consensus splice sequences. Novel mutations may be evaluated as disease-causing mutations or polymorphisms using criteria described by Cotton and Scriver [15] and in silico programs such as SIFT and PolyPhen. In such cases, it is helpful to test other affected or unaffected family members or both to determine whether the mutation segregates with the disease in the family.

## Laboratory Issues

Point mutation analysis of the *VHL* gene is performed as a laboratory-developed test. PCR primers and conditions have been published [10]. A kit for deletion analysis of the *VHL* gene by MLPA is available from MRC Holland (Amsterdam, the Netherlands). No organized proficiency testing program is available for VHLD; therefore, proficiency testing may be performed either by interlaboratory exchange or by repeat testing of previously analyzed samples.

Various sources of DNA are suitable for use in DNA sequencing and MLPA assays. Ethylenediaminetetraacetic acid anticoagulated peripheral blood is the most commonly submitted specimen; however, DNA, frozen tissue, cultured cells, saliva, paraffin-embedded tissue, cheek epithelial cells (buccal swab), unspun amniocytes, or direct chorionic villus sampling specimens may be used as well. Mutation screening by MLPA and DNA sequence analysis can be completed in approximately 1 week. Interpretation of the assays is fairly straightforward (described above). Point mutations may be compared to an online database of human gene mutations, either at the Human Gene Mutation Database at http://www.hgmd.org/hosted by the Institute of Medical Genetics in Cardiff or a list of *VHL* gene mutations in the Universal VHL-Mutation Database (http://www.umd.be/) [16].

The mutation detection rate quoted by laboratories in the USA varies from 95 % to greater than 99 %. Since all laboratories are using the same basic methodologies, this variability is likely due to uncertainty regarding the patient's diagnosis or inadequate clinical information rather than the inability to detect germline mutations in the *VHL* gene.

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