

OPHTHALMOLOGIC DIAGNOSIS AND FOLLOW-UP IN VON HIPPEL-LINDAU DISEASE

A close look at a small group of patients helped devise a new approach to managing patients with this rare syndrome.

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In memory of Silvana Penco, MD

Von Hippel-Lindau (VHL) disease is a relatively uncommon (1 in 36,000 live births), multisystem, dominantly inherited syndrome. The disease predisposes individuals to the development of benign and malignant tumors in the retina and central nervous system, kidney, pancreas, adrenal gland, epididymis, broad ligament, and endolymphatic sac.¹ The most common signs are hemangioblastoma (HB) of the central nervous system (CNS) and the retina, renal cell carcinoma, pancreatic carcinoma, pheochromocytoma, and visceral cyst.²⁻⁸

The disease is caused by a germline alteration of the *VHL* gene, a tumor suppressor gene located on chromosome 3p25-p26.^{9,10} The gene encodes a 213–amino acid protein ubiquitously expressed in fetal and adult human tissues. The protein degrades hypoxia-inducible factors,¹¹ which are produced in response to hypoxia. Loss of VHL function by mutation of the *VHL* gene results in unregulated high levels of hypoxia-inducible factors, which in turn produce

high levels of downstream gene products such as VEGF and platelet-derived growth factor (PDGF).¹² It is thought that constitutively elevated levels of proteins such as VEGF and PDGF stimulate angiogenesis, growth, and cell division, contributing to tumor formation.¹³

Individuals with a *VHL* gene defect develop clinical manifestations, and their children have a 50% risk of inheriting the gene and the disease.⁶ Half of all cases are familial, and half are caused by de novo mutations.⁵ The leading cause of death is renal cell carcinoma, followed by HB of the CNS,¹¹ at a mean age ranging from 40 to 50 years.¹⁴ The eye lesion is the retinal capillary hemangioblastoma (RCH, also called retinal HB or hemangioma), which appears as a round, circumscribed, orange-red vascular lesion, found in either the peripheral or the juxtapapillary retina.¹⁵ RCH is the first manifestation in approximately half of patients, and it is usually bilateral and multifocal or becomes so over the years.^{6,16}

In this article we report the ophthalmologic assessment of a group of Italian patients with a *VHL* gene mutation and some of their family members who also have the mutation.



AT A GLANCE

- VHL disease is a rare syndrome, the ophthalmic sign of which is retinal hemangioblastoma.
- Not all lesions are easily identifiable by biomicroscopic evaluation; however, infrared and FA imaging can be used to detect even small and peripheral lesions.
- The authors developed an algorithm for diagnosis and follow-up of patients with VHL disease.

STUDY SPECIFICS

Patients with a clinical diagnosis of VHL disease were referred to the Unit of Medical Genetics - Niguarda Ca' Granda Hospital in Milan, Italy, for VHL genetic testing. All probands were positive at the genetic test; molecular evaluation of at-risk family members was also performed. Genomic DNA from each proband and all consenting relatives was extracted from peripheral blood leukocytes using the salting-out method. All coding exons and the corresponding intron/exon boundaries of the *VHL* gene were amplified by polymerase chain reaction with a specific subset of primers. Sequencing reactions were analyzed using a 3730 DNA

TABLE 1. OPHTHALMOLOGIC ASSESSMENT OF PATIENTS WITH VON HIPPEL-LINDAU DISEASE

Patient	Age at Evaluation (years)	VA	RE	Mean IOP (mm Hg)	RCH	Leakage	Treatment
Patient 1	54	20/20 OU	E	18	N	N	N
Patient 2	37	20/20 OU	OD: E; OS: +0.25 -0.75 x80	16	N	N	N
Patient 3	36	20/20 OU	E	14	OD: 3 RCHs (periphery); OS: 1 RCH (periphery)	N	N
Patient 4 (index case)	62	20/20 OU	OU: +1.50 sphere	13	N	N	N
Patient 5 (relative of Patient 4)	31	20/20 OU	OU: -5.50 sphere	15	OS: 1 RCH (periphery)	N	N
Patient 6 (relative of Patient 4)	55	20/20 OU	E	17	OS: 1 RCH (periphery)	OD: 1 area (periphery)	Y (argon laser OS)
Patient 7 (index case)	70	20/20 OU	E	16	OD: 1 RCH (periphery)	N	N
Patient 8 (relative of Patient 7)	43	20/20 OU	OD: E; OS: -0.50 x180	13	N	OS: 1 area (posterior pole)	N

Abbreviations: E, emmetropia; IOP, intraocular pressure; N, none; OD, right eye; OS, left eye; OU, each eye; RE, refractive error; RCH, retinal capillary hemangioblastoma; VA, visual acuity; Y, yes

Analyzer (Applied Biosystems by ThermoFisher Scientific). The nucleotide position of variants present in the coding regions refers to the complementary DNA (cDNA) sequence. We used two web-based bioinformatic tools to rank novel mutations: Mutalyzer 2.0 beta-26 (LUMC) to support checks of sequence variant nomenclature according to the guidelines of the Human Genome Variation Society, and Mutation Taster (Mutation Taster) to evaluate the disease-causing potential of the sequence alterations identified.

At the San Paolo Hospital ophthalmology unit, we examined 16 eyes of eight patients with a positive genetic test to evaluate for ocular signs of VHL disease. Two independent ophthalmologists, medical retina specialists, performed complete ocular examinations consisting of visual acuity, biomicroscopic fundus exam using three-mirror contact lenses (Volk Optical), and color retinography (3D OCT-2000; Topcon). Infrared (IR), fluorescein angiography (FA) and optical coherence tomography (OCT) examinations were conducted with the Heidelberg Retina Angiograph (Heidelberg Engineering).

Ocular lesions were observed during eye examinations previously performed at other centers in both eyes of Patient 3 and in the left eye of Patient 6. In view of their characteristics, a VHL genetic test was requested. Patient 6 had received argon laser treatment for RCH.

STUDY FINDINGS

Among the eight patients analyzed for the study, five had the familial form of VHL disease and three were apparently de novo cases (Patients 1-3). Mean age was 48.5 years (range

31-70 years); all were men. In all patients, BCVA was 20/20. There were no significant alterations of the cornea and anterior segment (Table 1).

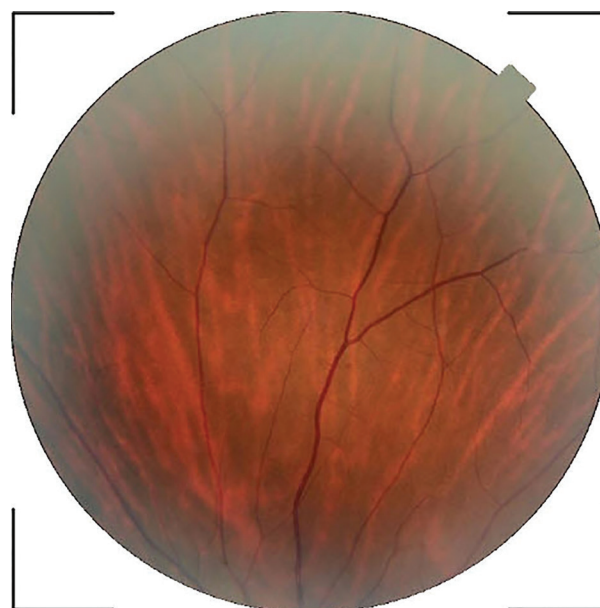


Figure 1. Patient 5: RCH is not clearly identifiable in the extreme superior periphery with retinography. RCH was identified on reevaluation of retinography images after it was seen in IR examination.

TABLE 2. OPHTHALMOLOGIC DIAGNOSTIC EXAMS IN PATIENTS WITH VON HIPPEL-LINDAU DISEASE				
Patient	Fundus (biomicroscopy and retinography)	IR	FA	OCT on RCHs
Patient 1	N	N	N	NP
Patient 2	N	N	N	NP
Patient 3	OD: 3 RCHs (periphery); OS: 1 RCH (periphery)	same as fundus exam	RCHs leakage	perilesional edema; vitreoretinal traction
Patient 4 (index case)	N	N	N	NP
Patient 5 (relative of Patient 4)	N	OS: 1 RCH (periphery)	same as IR, no leakage	N
Patient 6 (relative of Patient 4)	OS: 1 RCH (periphery)	same as fundus exam	OD: leakage (periphery)	N
Patient 7 (index case)	N	OD: 1 RCH (periphery)	same as IR, no leakage	N
Patient 8 (relative of Patient 7)	N	N	OS: leakage (posterior pole)	NP

Abbreviations: E, emmetropia; FA, fluorescein angiography; IOP, intraocular pressure; IR, infrared; N, negative; NP, not performed; OCT, optical coherence tomography; OD, right eye; OS, left eye; OU, each eye; RCH, retinal capillary hemangioblastoma; RE, refractive error; VA, visual acuity; Y, yes

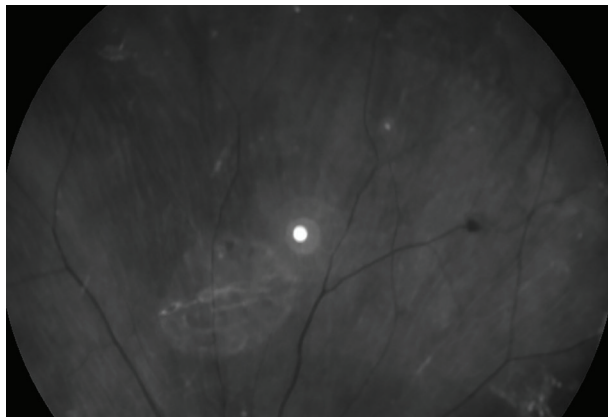


Figure 2. Patient 5: RCH appears as a small dark lesion in the extreme superior periphery on IR examination.



Figure 3. Patient 5: RCH appears on FA as a small area of leakage in the superior periphery.

Nine of the 16 eyes were normal. Seven eyes of five patients presented retinal signs attributable to VHL disease. RCH was detected in five of seven eyes with retinal changes, in one case bilaterally. Angiographic vascular leakage, a sign of early formation of retinal HB, was detected in the other two of seven eyes, one eye unilateral and the other with RCH in the contralateral eye. Only Patient 6 had received previous treatment (Table 1).

Biomicroscopic fundus examination and retinography revealed RCH in three eyes of two patients, which appeared as a round, circumscribed, orange-red vascular lesion in the peripheral retina (Figure 1, Table 2). IR examination showed RCH in five eyes, three unilateral and one bilateral case; the RCH appeared as a small, dark, circular lesion in the periphery. In two eyes (Patients 5 and 7), the RCH had not been recognized on biomicroscopy or retinography. In Patient 5, the retinal HB was the first lesion detected attributable to VHL disease (Figure 2, Table 2).

FA confirmed the RCHs already identified by IR examination, observable as circular hyperfluorescent lesions (Figure 3). In two eyes (Patients 6 and 8), which did not show any retinal changes at biomicroscopy, retinography, or IR examinations, FA detected an area of vascular leakage: in the superior periphery in Patient 6 and below the macula in Patient 8. Patient 8 was unilateral, and Patient 6 had RCH in the contralateral eye. Only Patient 3 demonstrated slight leakage of the RCH (Table 2).

OCT showed no macular edema in any eye. Volume OCT scans, localized on the averaged periphery of the RCH, showed ectatic vessels. In Patient 3, slight perilesional edema and limited vitreous retinal traction were noted (Figure 4). Patient 6 showed the outcomes of argon laser treatment on the RCH (Table 2).

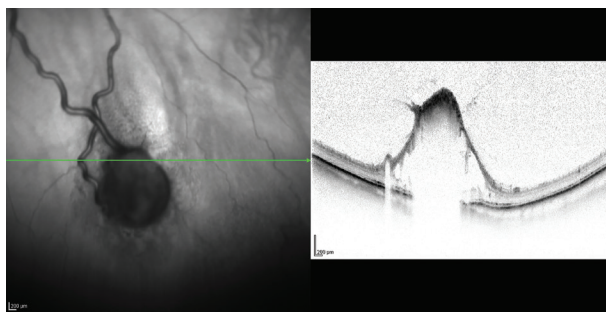


Figure 4. Patient 3: Vitreoretinal traction on RCH is shown on OCT.

DISCUSSION

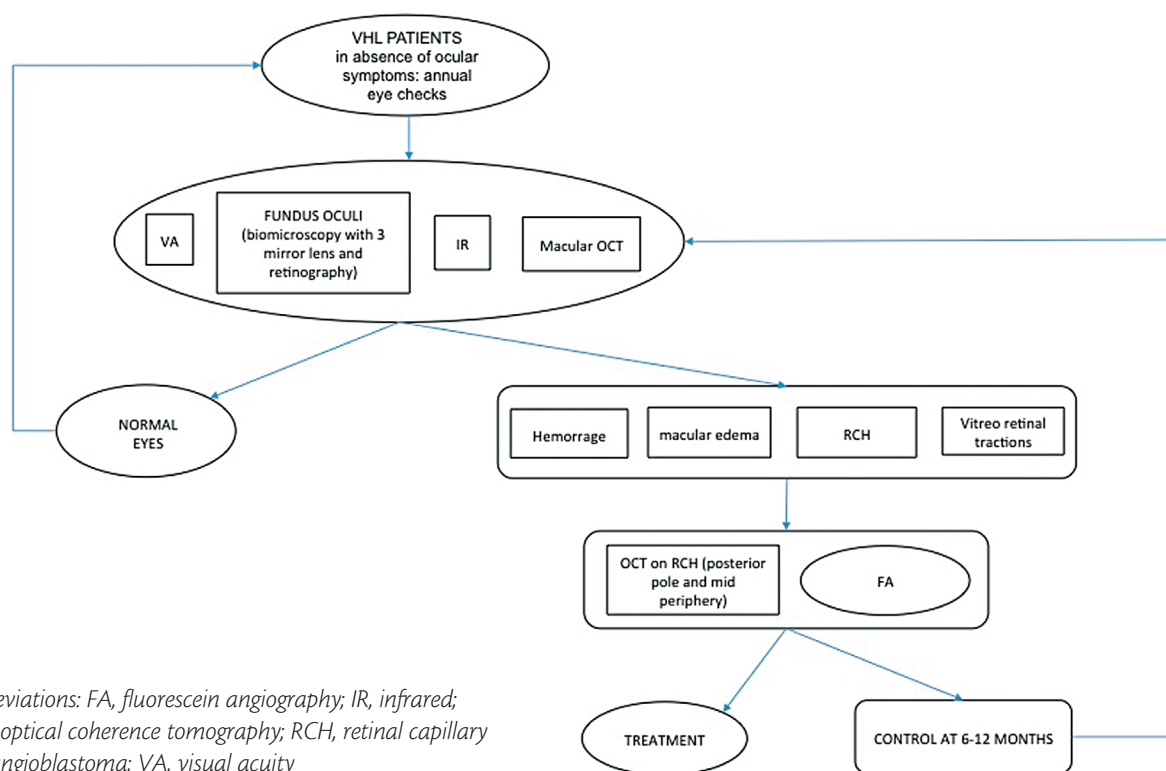
There are no indications in the literature to guide ophthalmic follow-up of patients with VHL disease. Although atypical incipient HBs without draining vessels have been described,¹⁷ retinal HBs are distinctive enough to permit ophthalmoscopic diagnosis.¹⁸⁻²⁰ However, our examinations in this study showed that small peripheral retinal lesions are not easily found with biomicroscopy or retinography during fundus examination (Figure 1). In Patients 5 and 7, we detected small, peripheral RCHs only on IR examination (Figure 2), subsequently confirmed on FA (Figure 3). Vascular

leakage, in the absence of retinal HBs, as in Patients 6 and 8, was detected only by FA (Table 2).

Although small, peripheral RCHs and areas of leakage do not cause symptoms, it is important to locate them and to follow any changes over time. Therefore, if fundus examination (biomicroscopy with a three-mirror lens and retinography) and/or IR examination indicates a new finding of retinal HB, FA is advisable to confirm the diagnosis and to measure the lesions (Figure 5).

The mean age at initial manifestation of VHL disease is 25 years.^{6,16,20} The condition can be asymptomatic for years²⁰ and may even regress spontaneously.²¹ Typically, the lesions grow and cause visual impairment due to leakage, leading to secondary changes in the vitreous and retina. The most frequent causes of vision loss are intraretinal exudation, exudative retinal detachment, hemorrhage, and epiretinal fibrosis.^{19,22-24} With early treatment, however, visual prognosis is good,²⁰ and thus follow-up is important.

In the absence of ocular symptoms, we recommend that patients with VHL disease receive an eye examination once a year, during which it is advisable to perform visual acuity, fundus (biomicroscopy with 3-mirror lens and retinography), macular OCT, and IR evaluations. In patients with VHL disease with no ocular signs, we recommend an eye examination every year. In patients with retinal HB,



Abbreviations: FA, fluorescein angiography; IR, infrared; OCT, optical coherence tomography; RCH, retinal capillary hemangioblastoma; VA, visual acuity

Figure 5. Ophthalmologic follow-up in VHL disease.

intraretinal hemorrhage, macular edema, or vitreoretinal traction FA may be useful (Figure 5).

When RCH is localized at the posterior pole or the midperipheral retina, volume OCT scans can assess the size of HBs and identify perilesional edema and vitreoretinal traction. If the RCH is in the extreme periphery, vascular leakage and lesion size can be assessed with FA (Figures 4 and 5). Retinal HBs that are small and peripheral normally will not cause significant visual symptoms; therefore, eye examinations can be repeated every 6 to 12 months. In patients with larger HB (such as Patient 6 in our study), or with macular edema or vitreoretinal traction the need for medical or surgical treatment should be assessed.

AN ALGORITHM IS BORN

Although based on a small number of patients, our study sought to establish what might be the least invasive and most effective approach to the diagnosis of RCH and the ophthalmologic monitoring of patients with VHL disease. We underline the importance of a diagnostic eye algorithm in VHL disease because, although RCH may remain asymptomatic for years, the visual prognosis is good with early treatment.

We recommend repeating IR at each visit, in addition to annual fundus examination. IR can detect small RCHs that may not be identified at fundus examination, and it can be used to follow lesions over time. Furthermore, volume OCT scans of retinal HB can be used to assess perilesional edema and vitreoretinal traction. Additionally, FA is fundamental, both to confirm the diagnosis of retinal HB and to monitor RCH. For monitoring RCH, FA should be repeated at each visit. ■

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