Clinical Characteristics of Ocular Angiomatosis in von Hippel-Lindau Disease and Correlation With Germline Mutation

Andrew R. Webster, FRCOphth; Eamonn R. Maher, MD, FRCP; Anthony T. Moore, FRCOphth

Objectives: To examine the epidemiologic and clinical characteristics of the ocular manifestations of von Hippel-Lindau (VHL) disease and to detect phenotype-genotype relationships of disease severity.

Design: A cross-sectional clinical and molecular genetic study.

Patients and Methods: One hundred eighty-three affected VHL gene carriers from 81 unrelated pedigrees were interviewed and examined; clinical data were also obtained from 12 living and 39 deceased affected relatives. DNA extracted from venous blood was used to identify mutations in the VHL gene.

Results: The prevalence of ocular angiomatosis (hemangioblastomas) in von Hippel-Lindau disease was 67.8% (124/183), and the mean number of angiomas in gene carriers was 1.85 (range, 0-15). Neither prevalence nor angioma count increased with age. Severe vision loss in 1 or both eyes was associated with presentation at a young age. The cumulative probability of incurring vision loss by age 50 years was 35% in all gene carriers, 55% in those with angiomatosis, and significantly worse in those coming to us with symptoms. Angiomas were nonrandomly distributed in the fundus, occurring rarely at the posterior pole (1% of retinal tumors) and commonly on the optic disc (8% of eyes) and supratemporal retina. Complications of ocular angiomatosis included disc and retinal neovascularization; secondary angioma formation; retinal detachment, exudation, and membrane; and retinal and vitreous hemorrhage. Germ-line VHL mutations were detected in 161 of 183 patients and 69 (85%) of 81 pedigrees and included deletions (n = 16), missense (mutations causing amino acid substitutions; n = 24), nonsense (premature stop codons; n = 15), frameshift (n = 13), and splice-site (n = 1) mutations. There was no association between the type or position of mutation and the severity of ocular angiomatosis.

Conclusions: A systematic clinical description of a large cohort of VHL gene carriers further defines the ocular phenotype. There is no general influence of germline mutation on severity of ocular disease in VHL.

Clinical Relevance: The ophthalmic and molecular genetic description of patients with VHL disease.


On hippel-lindau (VHL) disease is a dominantly inherited cancer syndrome with an estimated birth incidence of heterozygotes of 1 in 36 000. The clinical manifestations include benign mixed-cell vascular tumors (hemangioblastoma or angioma) of the eye and central nervous system, renal carcinoma, and pheochromocytoma. Ocular tumors characteristic of VHL disease are hyperfluorescent vascular lesions on the optic disc or retina. To identify gene carriers among family members and to detect lesions at a presymptomatic stage so that morbidity and mortality can be minimized, clinical screening protocols have been recommended.

The VHL gene on chromosome 3p was characterized in 1993 through a positional cloning approach and encodes a novel protein of 213 amino acids. Germ-line VHL mutations have been identified in most kindreds with the disease and include complete or partial gene deletions and intragenic mutations causing amino acid substitutions and protein truncation. Although the disease is highly penetrant by age 60 years, there is a large degree of variability in tumor susceptibility between and within families. Interfamilial variability in the susceptibility to pheochromocytoma appears to correlate with the underlying germline mutation so that missense mutations confer a higher susceptibility than mutations predicted to cause an absent or truncated protein.
PATIENTS AND METHODS

ASCERTAINMENT OF CASES

A letter was sent to all ophthalmic and clinical genetics departments in the United Kingdom requesting the recruitment of patients and families with VHL disease. Consent was given by each person after the intended research was fully explained. The study was approved by the ethics committee at Addenbrooke’s Hospital, Cambridge, England.

PATIENT INTERVIEW AND EXAMINATION

Patients were interviewed by 2 of us (A.R.W. and A.T.M.), and 1 of us (A.R.W.) performed all of the examinations. Interview and medical records review determined each patient’s ocular and family history, including the presentation, diagnosis, and treatment of ocular angiomas and the age at which vision loss due to angiomas occurred (best corrected visual acuity of 20/40 or worse). Examination included corrected Snellen visual acuity, slitlamp examination of the anterior segment, indirect funduscopy (20 diopter lens), and slitlamp biomicroscopic funduscopy (90 diopter lens). Fluorescein angiography or angiography was performed when the diagnosis of an angiomatic lesion was uncertain. The fundal position of angiomas was assessed by the position of untreated or partly treated lesions or of the retinal scars from completely treated lesions. Complications of angiomas were evident at examination or from medical records. The ocular history of eyes with no fundal view was determined from medical records as far as possible. A 10-ml specimen of venous blood was extracted from each patient into EDTA for DNA analysis. Medical and family records were used to collect clinical details on deceased family members and affected alive relatives unable to be examined. Standard methods were used to extract DNA for molecular genetic analysis. Southern blot analysis was performed to detect or exclude large deletions in the VHL gene. Single-strand conformation polymorphism analysis was performed to detect intragenic mutations. Direct sequencing of the whole coding region was performed in pedigrees in whom the mutation remained unidentified. This protocol identifies mutations in at least 80% of the kindred with VHL disease. Gene-carrier status was determined by confirming the presence of the family mutation, if identified, or, alternatively, using the informative, closely linked, microsatellite polymorphisms D3S1317.

GENOTYPE-PHENOTYPE CORRELATION

Subjects were divided into 4 groups on the basis of DNA analysis: undetermined mutations, deletions, protein-truncating mutations, including frameshift and nonsense mutations (premature stop codons), and missense mutations (mutations causing amino acid substitutions). The hypothesis that all groups had a similar prevalence of ocular angiomas was tested using the χ² test for independence. Nonparametric analysis of variance (Kruskall-Wallis test) was used to test the hypothesis that all mutation groups caused the same mean number of ocular angiomas per person. Approximate power calculations were performed to estimate the likely detectable difference in sample means or sample proportions, with a power of 80% or more and an α of .05 (2-tailed) given the sample sizes. These calculations were based on normal distribution theory. To identify gene regions having substantial influence on disease severity, each intragenic mutation was entered onto a plot of codon number vs the number of angiomas per patient (Figure 1).

RESULTS

PATIENT DETAILS

One hundred eighty-three gene carriers were interviewed and examined (mean age, 33.8 years; age range, 7-74 years; 101 males; P = .15). Patients were derived from 81 unrelated pedigrees (mean number of affected examined members per pedigree, 2.3; range, 1-10). Twenty-nine persons (mean age, 31.0 years; range, 7-74 years; 14 pedigrees) were classified asymptomatic gene carriers on the basis of being an obligate carrier (n = 4), harboring the family VHL germline mutation (n = 15), and carrying the high-risk haplotype (n = 10). Given the estimated recombination rate between the markers used and the VHL gene (<1%), the probability that 1 or more of this last group being mistakenly labeled as gene carriers...
is less than 10%. There were 17 patients who had no known alive or deceased relatives affected by VHL disease. Clinical data concerning the age and degree of vision loss were collected in 12 unavailable living affected relatives (5 men; mean age, 41.3 years; range, 19-76 years) and 39 deceased affected relatives (26 males; mean age at death, 43.0 years; range, 13-66 years) from the same pedigrees. These data were used only in analyzing vision loss (Figure 2, Figure 3, and Figure 4).

Epidemiologic Features of Ocular Angiomatosis and Vision Loss in VHL Syndrome

Ocular Angiomatosis

Of the 183 patients systematically examined, 124 (67.8%) had 1 or more retinal angiomas. The median age of patients with angiomatosis (31 years) was significantly lower than that of patients without angiomatosis (n = 59, 38 years) (P < .05, 2-tailed, Mann-Whitney U test). The prevalence of retinal angiomatosis was calculated for age groups: 0 to 19 years (n = 34), 20 to 29 years (n = 35), 30 to 39 years (n = 53), 40 to 49 years (n = 37), and 50 years or older (n = 24) (Figure 5). The prevalence of angiomatosis did not increase with age.

In 156 of 183 examined affected persons, an accurate count of bilateral ocular angiomas was possible (the other persons having no useful fundal view in 1 or both eyes). The frequency distribution of the number of angiomas (mean, 1.85; range, 0-15) is shown in Figure 6. This distribution did not change significantly when estimates from medical records of angioma number in 22 unilaterally blind patients were included (n = 178; data not shown). The number of angiomas per person did not increase with age and, in patients younger than 30 years at examination, was significantly higher than in those 30 years or older (mean, 2.5 vs 1.4; P < .05, 2-tailed, Mann-Whitney U test).

Vision Impairment

Of the 183 examined affected persons, 48 (26.2%) had some degree of permanent vision loss (best corrected visual acuity of ≤ 20/40 in 1 or both eyes) due to ocular angiomatosis, its treatment, or both. Of these, 35 (19.1%) had visual acuity of less than 20/200 in 1 or both eyes. This group generally was seen by ophthalmologists at an...
Of the 29 eyes with disc angiomas, 12 eyes had 1 or more angiomas elsewhere in the retina. Of the 17 eyes that did not have angiomas, 9 eyes from 9 patients had a single disc angioma as the only VHL disease ocular manifestation, 2 eyes from 1 person had disc angiomatosis without any retinal angiomas, and 6 eyes were from patients who had angiomatosis in the contralateral eye. Hence, optic disc angiomatosis was the sole ocular sign of VHL disease in 17 (4.8%) of 350 eyes or 10 (5.7%) of 175 gene carriers.

Because the probability of vision loss in persons with VHL disease is age dependent, Kaplan-Meier survival analysis was used to determine the cumulative probability of suffering irreversible vision deficit (visual acuity of ≤20/40 in 1 or both eyes) and is shown in Figure 2 for the whole cohort (N = 234) of affected and deceased persons in the study. The probability of incurring permanent angioma-related vision deficit approaches 35% if a person lives long enough. Furthermore, this deficit is most likely to occur before age 20 years (cumulative probability, 20%), and the risk is less thereafter (lifelong probability of vision loss if no vision loss at 20 years, 19%). Figure 3 illustrates a similar analysis of subjects with ocular angiomatosis showing a lifelong cumulative probability of permanent vision deficit of 60%. Most of this risk is borne at a young age (cumulative probability by age 30 years, 43%; lifelong probability of vision loss if no vision loss at 30 years, 20%). When persons affected by angiomatosis are divided into 2 groups depending on the mode of presentation (with vs without symptoms), the symptomatic group is significantly more likely to have vision deficit at any given age (P<.001; log-rank test), the cumulative probability of vision deficit by age 40 years being 82% for symptomatic persons vs 35% for those who are presymptomatic (Figure 4).

**CLINICAL FEATURES OF OCULAR ANGIOMATOSIS**

**Optic Disc Angiomatosis**

Optic disc angiomas (Figure 7) occurred in 26 (14.8%) of 175 examined gene carriers, or 29 (8.3%) of 350 eyes (the presence or absence of optic disc angiomatosis in 1 or both eyes could not be determined in 8 examined persons). Three patients had disc angiomatosis in both eyes. Of the 29 eyes with disc angiomas, 12 eyes had 1 or more angiomas elsewhere in the retina. Of the 17 eyes that did not have angiomas, 9 eyes from 9 patients had a single disc angioma as the only VHL disease ocular manifestation, 2 eyes from 1 person had disc angiomatosis without any retinal angiomas, and 6 eyes were from patients who had angiomatosis in the contralateral eye. Hence, optic disc angiomatosis was the sole ocular sign of VHL disease in 17 (4.8%) of 350 eyes or 10 (5.7%) of 175 gene carriers.

Of 29 optic disc angiomas, 15 occurred on the temporal side of the disc, 6 were nasal, 4 were inferior, 2 were superior, and 2 involved most of the disc surface. Eight eyes (8/29, 28%) with disc angiomatosis had lost vision due to complications of the disc lesions. Seven of these had been treated with laser therapy, and, in 5, vision had worsened after treatment. Of those in whom vision had been lost, 5 optic disc angiomas were on the temporal disc, 1 was on the superior disc, and 2 obscured most of the disc surface. Associated findings in patients with disc angiomas included intraretinal exudation (8 eyes); retinal traction, pucker, or striae (5 eyes); exudative retinal detachment (4 eyes); and vitreous hemorrhage (1 eye).

**Fundal Location of Retinal Angiomas**

Within the 183 examined gene carriers, it was possible to determine the position of 347 retinal angiomas relative to the optic disc with the following distribution: supranasal (n = 50), infranasal (n = 68), infratemporal (n = 95), and supratemporal (n = 134). Assuming that the disc and ora serrata subtend angles of 15° and 100° to fixation, respectively, these observations differ significantly from those expected with a null hypothesis of an even distribution throughout the fundus (χ² test for independence with Yates correction [3 df], 9.41; P = .02). Of 314 retinal angiomas that could be positioned in an anteroposterior axis, only 4 (1.3%) occurred at the posterior pole (within the temporal arcades and between the optic disc and twice the fovea-disc distance). These occurred in 4 eyes of 4 patients, each of whom showed angiomatosis occurring elsewhere in the retina of the same eye. Of 314 angiomas, 44 (14.0%) occurred anterior to the ocular equator. Of the remainder, 266 (84.7%) occurred in the middle of the periphery.
Ocular Complications of Retinal Angiomatosis

The complications of retinal angiomatosis occurring in 193 eyes with 1 or more retinal angiomas (data available for 370 primary retinal angiomas) are summarized in Table 1, and medical records were used to determine complications in 28 blind eyes. Details of the ocular complications in 6 blind eyes of 6 gene carriers were not available. Exudative retinal detachment occurred in 30 eyes. In at least 10 eyes, detachment occurred immediately after cryotherapy for retinal angioma. Small secondary angiomas in or adjacent to areas of treatment or detachment (Figure 8) developed in 27 eyes. These were not recorded before treatment and had presumably occurred in response to detachment or treatment (or both) of adjacent primary retinal angiomas. New vessel complexes not part of angiomatous tumors occurred in 9 eyes either on the disc (n = 5) (Figure 9) or at the periphery (n = 4). Intraretinal exudation was a common complication of retinal angioma and was sometimes transient. It was seen in 19 eyes with angiomatosis (5 of which also had disc angiomas) at the time of the study. The regression of peripheral angiomatous lesions that had not been previously treated was seen in 5 eyes of 5 patients. These appeared as white lesions with attenuated feeding vessels (Figure 10) and remained hyperfluorescent on fluorescein angioscopy.

The size of retinal angiomas varied from microscopic to as much as 3 disc diameters wide. Tumor size was difficult to assess because many angiomas had been completely or partially treated. Generally, larger angiomas tended to be more commonly associated with potentially sight-threatening complications, such as exudation, retinal traction, or hemorrhage. Of 65 primary angiomas that were estimated to be less than a third of a disc diameter (<0.5 mm) in size and seen by us (partially or not treated), none were directly causing sight-threatening complications.

<table>
<thead>
<tr>
<th>Complication</th>
<th>No. of Eyes†</th>
</tr>
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<tbody>
<tr>
<td>Exudative retinal detachment‡</td>
<td>30</td>
</tr>
<tr>
<td>Secondary angiomatosis</td>
<td>27</td>
</tr>
<tr>
<td>Intraretinal exudation</td>
<td>19</td>
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<tr>
<td>Epiretinal membrane</td>
<td>18</td>
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<tr>
<td>Vitreous hemorrhage</td>
<td>5</td>
</tr>
<tr>
<td>Retinal hole and/or rhegmatogenous detachment</td>
<td>5</td>
</tr>
<tr>
<td>Neovascularization Disc</td>
<td>5</td>
</tr>
<tr>
<td>Neovascularization Periphery</td>
<td>4</td>
</tr>
</tbody>
</table>

*VHL indicates von Hippel-Lindau.
†Of 193 eyes with retinal angiomatosis, no data were available on the complications occurring in 6 of 199 eyes with angiomatosis.
‡Ten cases occurred directly following treatment.

Figure 8. Small secondary angiomas noncontiguous with treated retinal lesions.

Figure 9. Disc neovascularization and dilated feeder vessels supplying nearby retinal angiomas.

Figure 10. An autoregressed peripheral retinal angioma showing regressed feeder vessels.
Morphologic Features of Retinal Angiomas and Other Ocular Findings

Although variable in size and color, retinal angiomas were always associated with 1 or more feeder vessels from an adjacent retinal artery and vein. Otherwise, the retinal vasculature in VHL disease eyes appeared normal. Other than optic disc and retinal angiomatosis, no other ocular signs were more common in gene carriers than in normal controls. Neither “twin vessels” nor nonangiomatous retinal lesions were seen in any persons. Of 25 eyes blind through previous ocular angiomatosis, 4 showed apparent rubeosis iridis. No specific angiomatous lesions were seen in the anterior segment, however.

**GENOTYPE-PHENOTYPE CORRELATION**

Of 183 gene carriers examined, the underlying germ-line mutation was identified in 161 (69 pedigrees). The mutation detection rate did not differ significantly in 17
patients with no known affected relatives, compared with that in VHL pedigrees (13/17 vs 56/64; $\chi^2 = 0.30; P = .26$). The germline mutations determined in this study are listed in Table 2 and have been published previously. They include deletions of 1 or more of the 3 exons (51 patients), missense mutations (39 patients), nonsense mutations (40 patients), small insertions or deletions causing frameshifts (30 patients), and 1 patient with a splice-site mutation. The prevalence of angiomas in VHL disease is thought to occur after the VHL gene inherited in the germline, and a further somatic mutation of the normal allele needs to occur in both VHL alleles in a susceptible cell have been inactivated, as proposed by Knudson for retinoblastoma. In patients with VHL disease, all cells have 1 mutated copy of the VHL gene inherited in the germline, and a further somatic mutation of the normal allele needs to occur in a cell before tumorigenesis. The finding of somatic mutation of the wild-type allele in DNA extracted from tumors from patients with VHL disease supports this hypothesis. If there were a lifelong risk of retinal somatic mutation and subsequent ocular angioma formation, then the prevalence of ocular angiomas is significantly lower than the mean age of those not affected. One explanation is that susceptibility to ocular complications may be highest in persons who are also more susceptible to the life-threatening complications of the syndrome. Subsequently, the surviving older persons included in the study represent those with generally mild disease. There is no evidence for the view that severe ocular angiomas generally coexist with severe systemic manifestations.

In this study, affected persons with severe vision loss (visual acuity of 20/200 in 1 or both eyes) came to us at a significantly younger age than those with less severe retinal angiomas. Survival analysis of the whole cohort showed that the risk of incurring a permanent vision deficit occurred mostly before the age of 30 years. Furthermore, patients with angiomas did better when they were diagnosed before the onset of visual symptoms. These data confirm the importance of ophthalmic screening of affected and at-risk persons, particularly at a young age, to minimize vision loss.

A common observation in angiomatous eyes was that of small secondary angiomas in or near areas of detached or treated retina (Figure 8). They occurred in at least 27 angiomaticus eyes and were associated with areas of laser and cryosurgical treatment, emphasizing the need to keep treated areas under observation. We hypothesize that new retinal lesions are most likely to occur when retinal endothelial or other vascular cells are mitotically active and susceptible to mutation, such as in the developing retina or areas of adult retina rendered ischemic through detachment or ablative treatment.

Ocular angiomas were nonrandomly distributed throughout the retina, occurring rarely at the posterior pole (1% of tumors) and commonly in the supratemporal quadrant. Furthermore, the optic disc was 30 times more susceptible to angiomas than the retina with respect to its surface area. The cause of these differences in angioma susceptibility is not known. The disc relative to the retina is also more susceptible to neovascularization in diabetic retinopathy and retinal vein occlusion.

Disc and peripheral neovascularization was apparent in 9 angiomaticus eyes, despite an otherwise healthy retinal vasculature (Figure 9). The expression of vascular endothelial growth factor has been shown to be upregulated in cultured VHL null cells and in cerebellar hemangioblastomas. The secretion of this growth factor, given the increasing evidence for its role in retinal neovascularization, might explain this association.

Most lesions were treated by ablation with cryotherapy or laser. Because the study included patients from many centers with differing treatment protocols, the rela-
tive efficacy of these 2 main modes of treatment cannot be compared. The treatment of optic disc angiomas is hazardous because of the possibility of concomitant damage to the optic nerve head, and, in this study, such treatment was often followed by further vision loss. Treatment of this condition is clearly challenging. Novel therapies based on the knowledge of tumor pathogenesis are a worthwhile aim for further research.

The type and position of the underlying germ line VHL gene mutation generally does not affect the prevalence or severity of retinal angiomatosis. However, we cannot exclude the possibility that 1 or more specific mutations are significantly different because the numbers for each specific mutation are small (Table 2). Variability of ocular angiomatosis must be due to factors other than allelic variation. The identification of these factors, given the significant visual morbidity of this disease, is an important goal for future investigation.

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Reprints: Anthony T. Moore, FRCPht, Consultant Ophthalmic Surgeon, PO Box 41 (Clinic 3), Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, England (e-mail: atm22@hermes.cam.ac.uk).

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