Genotype-Phenotype Correlation in Ocular von Hippel-Lindau (VHL) Disease: The Effect of Missense Mutation Position on Ocular VHL Phenotype

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PURPOSE. von Hippel-Lindau (VHL) disease is a dominantly inherited, multisystemic tumor syndrome caused by mutations in the *VHL* gene. This study was conducted to establish genotype-phenotype correlations between the positions of disease-causing missense mutations and the ocular phenotypes of VHL disease.

METHODS. Participants with clinically defined VHL disease and documented germline missense mutations in the *VHL* gene were identified in a cross-sectional study ($n = 412$). Statistical analysis was used to correlate the position of the missense mutation in either the α - or β -domain of the VHL protein with the ocular disease phenotype.

RESULTS. Missense mutations among study participants were located in 47 of the 213 possible codons in the *VHL* gene. Almost all mutations (98.5%) were located in one of two structural domains of the VHL protein: the α - and β -domains. α -Domain mutations were significantly associated with a higher prevalence of retinal capillary hemangioblastomas (RCHs) compared with the β -domain mutations ($P = 0.016$). Among patients with RCHs, the prevalence of the lesions in the juxtapapillary position was also significantly higher in patients with α -domain mutations ($P = 0.0017$). Conversely, β -domain mutations correlated with a higher prevalence of peripherally located RCHs ($P = 0.0104$).

CONCLUSIONS. The location of missense mutations in the *VHL* gene correlates significantly with the prevalence and phenotype of ocular disease, and as such, influences the risk of visual loss in affected patients. These genotype-phenotype correlations can assist in the prognostic counseling and follow-up of VHL patients and may provide a basis for molecular inferences on ocular VHL disease pathogenesis. (*Invest Ophthalmol Vis Sci.* 2010;51:4464–4470) DOI:10.1167/iovs.10-5223

Von Hippel-Lindau (VHL) disease (Online Mendelian Inheritance in Man 193300; <http://www.ncbi.nlm.nih.gov/Omim/> provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD) is a dominantly inherited cancer syndrome with multisystemic involvement,

caused by mutations in the VHL tumor-suppressor gene.¹ In the eye, VHL disease manifests as benign retinal capillary hemangioblastomas (RCHs), a pink, globular, vascular lesion, located typically in the peripheral retina or in the juxtapapillary area.² RCH lesions may, through exudative and/or tractional effects, result in vision loss and disruption of ocular structures,^{2–4} constituting a significant part of overall VHL disease-associated morbidity.⁵

As described by the two-hit hypothesis of Knudson,⁶ affected patients inherit one mutated copy of the *VHL* gene from an affected parent through the germline. Later in life, the other normal copy of the *VHL* gene undergoes somatic mutation in susceptible tissues (i.e., the second hit), initiating local tumorigenesis. The VHL protein is ubiquitously expressed,⁷ and its molecular function is primarily associated with the formation of a ubiquitin ligase complex with other participating proteins (elongin B, elongin C, cullin 2, and Rbx1) that subsequently bind and direct the degradation of the transcription factor hypoxia inducible factor (HIF).⁸ The loss of *VHL* function in mutated cells is thus thought to result in the dysregulated accumulation of HIF, which then directs the excessive transcription of downstream genes, including angiogenic growth factors such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF).^{7,9,10} In the retina, elevations of these angiogenic growth factors are thought to promote the RCH formation and growth observed in ocular VHL disease.^{11,12}

With the identification of the *VHL* gene,¹ genetic diagnostic tests that detect germline mutations in the gene have been made available,¹³ enabling early disease diagnosis, improved tumor surveillance, and a reduction of disease morbidity.^{14,15} With this technology, a variety of mutation types have been since been characterized¹⁶ and curated in databases (<http://www.umd.be/>).¹⁷ With this information, genotype-phenotype correlations have also been established,¹⁸ aiding patient counseling concerning disease prognosis, as well as furthering a molecular understanding of the function of the VHL protein.¹⁹

With respect to ocular VHL disease, in a large group of genotyped participants ($n = 873$) with clinically defined VHL disease, in earlier work we established genotype-phenotype correlations between the nature of the mutation (amino acid substitutions, protein-truncating mutations, and complete deletions of VHL protein) and the prevalence of ocular disease and visual function.²⁰ However, the impact of missense mutations in different parts of the *VHL* gene on ocular phenotype has not been analyzed. In the present study, we examined a subset of 412 participants with clinical VHL disease, in whom the germline *VHL* mutation consisted of a missense mutation resulting in a single amino acid substitution in the VHL protein. In this study, we established correlations between the position of the missense mutation with the ocular phenotype, to examine how point mutations arising from different VHL protein domains may influence the nature of retinal angiomas. We

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analyzed the position of missense mutations according to their location in two main functional domains: the α -domain, which organizes the ubiquitin ligase complex by interacting with elongin B/C, Cul2 and Rbx1, and the β -domain, which binds the substrate HIF.^{21–24} We examined correlations between the domain position of missense mutations with the (1) prevalence of RCHs among VHL patients, (2) the ocular phenotype of RCHs, including RCH position, number, and severity, and (3) visual function. These correlations can assist in understanding how VHL gene mutations in particular affect the formation and development of ocular VHL disease and provide prognostic information for counseling and monitoring ocular disease in VHL patients.

METHODS

Participant Selection and Ascertainment

Participants in this study were enrolled in a protocol studying VHL disease from October 1988 to August 2005 at the National Cancer Institute (Bethesda, MD). The study adhered to the tenets of the Declaration of Helsinki and was approved by a local institutional review board (IRB). Informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study. The participants were screened for evidence of clinically defined systemic VHL disease at a single center. Evaluations included history and physical examinations by a multidisciplinary team, laboratory evaluations, and radiographic imaging (computed tomography or magnetic resonance imaging) of the abdomen, pelvis, brain, and spine. All participants also underwent ophthalmic examination with slit lamp biomicroscopy, indirect funduscopy, and, if indicated, fluorescein angiography. A total of 890 participants who met the clinical diagnostic criteria for VHL disease were examined and analyzed. The participants were followed up over time, and treatments for ocular VHL disease were provided as clinically indicated. Analysis of present participant information was performed in a cross-sectional design, with each participant's most recent visit designated as the study visit.

Genotype Analysis

Peripheral blood samples from at least one member of each pedigree were used for the analysis of VHL mutations.¹³ Germline mutations were characterized in 873 (98.1%) of all participants, and the mutations were classified according to the nature of the DNA change and their effect on VHL protein structure. Of these 873 participants, 412 with germline missense mutations (i.e., mutations resulting in the substitution of a single amino acid) were identified and constituted the group analyzed. These participants were further analyzed according to the position of the missense mutation within the *VHL* gene sequence and were subdivided into groups with missense mutations located in (1) the α -domain of the VHL protein, (2) the β -domain, and (3) outside of either domain.

Ocular Phenotype Analysis

Participants were evaluated with a full ophthalmic examination of both eyes, when available, including the measurement of best corrected visual acuity with the Early Treatment of Diabetic Retinopathy Study visual acuity chart, intraocular pressure measurement, slit lamp biomicroscopy, and indirect funduscopy. Each eye of every participant was evaluated in the study and scored for the presence of ocular involvement by VHL disease. Eyes with ocular VHL disease were scored for presence of severe tumor involvement, defined as causative of enucleation or phthisical and prephthisical changes that precluded an adequate view into the posterior pole. In less severely affected eyes in which the posterior pole could be visualized on fundus examination, the extent of retinal involvement (scored as the number of quadrants of the retina affected by ocular angiomas) was noted. In addition, the number and location (juxtapapillary or peripheral) of individual RCHs were also recorded. RCHs were classified as juxtapapillary RCHs if they were located on or adjacent to the optic nerve and as peripheral RCHs if they were located peripheral to the vascular arcades and twice the fovea-to-disc distance from the optic nerve.

Statistical Analysis

We determined how the position of missense mutations correlate with ocular phenotype and visual function by using age-adjusted logistic regression and analysis of variance. As the participants in this study were represented by pedigrees of various sizes, multiple outputation was applied to account for both family size and intrafamily correlation.²⁵ For the outputation technique, the optimal number of resamplings within a family for drawing inferences from the data was determined to be 2000. Logistic regression was used to analyze the following clinical phenotypic measures: presence of RCH, laterality of angiomas, presence of severe ocular involvement, location of RCHs, number of peripheral RCHs, and the extent of peripheral retinal involvement. Analysis of variance was performed to analyze the visual acuity.

RESULTS

Population of VHL Patients with Missense Mutations in the *VHL* Gene

Participant screening procedures involving systemic evaluations for VHL disease resulted in the identification and enrollment of 890 participants with clinically defined disease. Genotype information was obtained in 873 (98%). Of these, 412 (47.2%), originating from 135 separate family pedigrees, were found to have missense mutations that resulted in a single-residue substitution in the amino acid sequence of the *VHL* gene. The demographic features of these 412 participants with missense mutations were as follows: mean age $\pm$ SD, 37.1 $\pm$ 15.6 years (range, 4.2–84.3); sex, 186 male and 226 female

(male-female ratio 1:1.22); and self-reported race/ethnicity, 381 (93%) white, 18 (4%) Hispanic, 10 (2%) black, and 3 (1%) Asian.

Distribution of Missense Mutations in the *VHL* Gene

The distribution of VHL disease with single amino acid substitutions along the coding sequence of the *VHL* gene that were caused by missense mutations was analyzed for all 412 participants. As shown graphically by participant and by pedigree in Figure 1, missense mutations in this population were nonuniformly distributed along the length of the gene. In multiple participants and pedigrees, missense mutations were clustered in a few amino acid positions (in mutational hot spots), whereas many amino acid positions were not found to contain any mutations in any of the participants. Of the 213 codons that comprise the VHL coding sequence, only 47 (22.0%) were found to be involved in missense mutations (Table 1). Codons 98 and 167 were the most commonly mutated and were found in approximately 20% of the participants in each instance. No individual or pedigree was identified as carrying the mutation in more than one codon.

The structure of the VHL protein contains two primary functional domains: the α -domain, which is made up of amino acids 155-192 in the sequence of the VHL protein (total number of amino acids/codons, 38), and the β -domain, which comprises amino acids 63-154 and 193-204 (total number of amino acids/codons, 104).²⁴ In our study populations, almost all the missense mutations were located in either of these two structural domains. Of 412 participants, only 6 from two pedigrees (1.4% of all participants and 1.5% of all pedigrees) had mutations that were found outside of either structural domain (Fig. 1).

Of the remainder, a larger proportion of overall participants and pedigrees (252/406 [62.1%] and 76/133 [57.1%], respectively) were found to have missense mutations in the β -domain compared with those with mutations in the α -domain (154/406 [37.9%] and 57/133 [42.9%], respectively; Fig. 2). However, as the β -domain has 2.7 times as many codons as the α -domain, the overall proportional mutational rate (calculated as the percentage of participants/pedigrees per codon) is higher for codons in the α -domain than those in the β -domain (Fig. 3).

Relationship between Location of Missense Mutations in the α -Domain versus the β -Domain and the Prevalence of Ocular VHL Disease

We found that among 412 participants, 159 (38.6%) had ocular VHL disease, defined as a history or clinical evidence of VHL-related retinal angiomas in at least one eye. This prevalence of ocular involvement among patients with missense mutations is similar to that found for the overall population of patients with clinically defined VHL disease, across all mutational genotypes (37.6% [335/890]).^{4,20} This subset of patients with ocular VHL-disease had demographics similar to those of the overall population of participants with missense mutations: mean age $\pm$ SD, 39.2 $\pm$ 14.6 years (range, 8.8–84.3); sex, 70 male and 89 female (male-female ratio 1:1.27); and self-reported race/ethnicity, 141 (89%) white, 11 (7%) Hispanic, 5 (3%) black, and 2 (1%) Asian.

We hypothesized that the location of missense mutations in either the α -domain or the β -domain has an influence on the prevalence of ocular VHL disease among VHL patients. The overall distribution of missense mutations in the subset of participants ($n = 159$) and pedigrees ($n = 100$) with ocular VHL disease is shown in Figure 4. We found that the subset of participants with ocular VHL disease had mutations that were not clearly segregated to either the α - or the β -domain, but involved both domains. However, the prevalence of ocular VHL disease in all participants with missense mutations in the α -domain (46.1%, [71/154]) was significantly

TABLE 1. Distribution of Missense Mutations in the Coding Sequence of the VHL Protein in Participants and Pedigrees

Codon	Participants with Missense Mutation		Pedigrees with Missense Mutation	
	(n)	(%)	(n)	(%)
7	4	0.97	1	0.74
25	2	0.49	1	0.74
65	13	3.16	8	5.93
68	1	0.24	1	0.74
70	1	0.24	1	0.74
76	2	0.49	2	1.48
78*	23	5.58	13	9.63
86	13	3.16	6	4.44
88	12	2.91	2	1.48
90	1	0.24	1	0.74
93	7	1.70	2	1.48
98*	85	20.63	3	2.22
101	3	0.73	1	0.74
105	4	0.97	1	0.74
107	2	0.49	2	1.48
110	2	0.49	1	0.74
111	11	2.67	4	2.96
112	9	2.18	3	2.22
114	4	0.97	2	1.48
115	2	0.49	1	0.74
117*	22	5.34	6	4.44
119	3	0.73	1	0.74
121	1	0.24	1	0.74
124	3	0.73	1	0.74
125	4	0.97	1	0.74
128	6	1.46	1	0.74
129	1	0.24	1	0.74
131	2	0.49	2	1.48
133	1	0.24	1	0.74
136	3	0.73	2	1.48
143	1	0.24	1	0.74
149	7	1.70	1	0.74
151	1	0.24	1	0.74
153	1	0.24	1	0.74
156	2	0.49	1	0.74
158	10	2.43	3	2.22
161*	22	5.34	7	5.19
162	15	3.64	5	3.70
164	4	0.97	2	1.48
167*	82	19.90	29	21.48
175	5	1.21	2	1.48
178	2	0.49	1	0.74
180	1	0.24	1	0.74
184	5	1.21	3	2.22
186	2	0.49	1	0.74
188	4	0.97	2	1.48
200	1	0.24	1	0.74
Total	412	100	135	100

* Indicates mutation hot spots in the coding sequence of the *VHL* gene involving >5% of participants.

higher than that for participants with missense mutations in the β -domain (34.1% [86/252]; $P = 0.016$). This association indicated that participants with missense mutations in the α -domain may be more susceptible to ocular VHL involvement than those with missense mutations in the β -domain.

Relationship between Location of Missense Mutations in the α -Domain versus the β -Domain and VHL Ocular Phenotype

We hypothesized that the location of missense mutations in either the α - or the β -domain has an influence on phenotypic features of ocular VHL disease. The ocular phenotypes of each

FIGURE 2. Prevalence of missense mutations within the two functional domains of the VHL protein in the study population. (A) Percentage of participants with missense mutations located in the α -domain (gray), β -domain (white), and outside either domain (black); $n = 412$ participants. (B) Percentage of pedigrees with missense mutations located in the α -domain (gray), β -domain (white), and outside either domain (black); $n = 135$ pedigrees.

of the 159 participants with ocular VHL disease were characterized and categorized according to prospectively determined ocular phenotypic categories. Among the 159 participants in this subset, 59.7% [95/159] had evidence of VHL-related RCHs in both eyes. A total of 157 participants with RCH had missense mutations in either the α - or the β -domain. Among participants with α -domain mutations, the prevalence of bilateral involvement was 53.5% [38/71], whereas among participants with β -domain mutations, the prevalence was 64.0% [55/86]. No statistically significant correlation was found between the location of the missense mutation in the α -domain versus the β -domain and the laterality of ocular VHL disease ($P = 0.37$; Table 2).

In several participants, complications secondary to VHL-related retinal angiomas had resulted in severe structural disruptions of the globe (e.g., total retinal detachment, massive subretinal exudation, and phthisical changes) that had either precluded visualization of the posterior pole or had necessitated enucleation. These eyes were defined as having severe ocular involvement and were distinguished from less affected eyes in which individual RCHs could be visualized and counted. The prevalences of severe involvement in at least one

eye were tallied for participants with missense mutations in either the α - or the β -domain. The occurrence of such severe involvement in at least one eye was not associated with the position of the missense mutation ($P = 0.27$, Table 2).

The position of VHL-associated RCHs in each study eye was scored according to their presence in one or both susceptible fundus locations: the peripheral retina and the juxtapapillary area. The location of RCH in an affected eye is of clinical significance and influences the choice of treatment approaches. Juxtapapillary lesions, unlike the peripheral ones, are not often amenable to conventional ablative treatments, owing to their proximity to the optic nerve,²⁶ and are also associated with a worse visual outcome.⁴ Among participants with α -domain mutations, the prevalence of a juxtapapillary RCH in at least one eye was significantly higher than among participants with β -domain mutations ($P = 0.0017$; Table 2). Conversely, the prevalence of peripheral RCHs in at least one eye was higher in participants with β -domain mutations than in participants with α -domain mutations ($P = 0.0104$).

In participants who have RCHs in the peripheral retina, the individual RCHs in each eye were counted and the extent of peripheral retinal involvement scored. However, the tumor

FIGURE 3. Rate of missense mutations per codon in the two functional domains of the VHL protein for the study population. (A) Mean mutation rate expressed as a percentage of participants per codon for the α -domain (gray), β -domain (white), and outside either domain (black); $n = 412$ participants. (B) Mean mutation rate expressed as a percentage of pedigrees per codon for the α -domain (gray), β -domain (white), and outside either domain (black); $n = 135$ pedigrees.

FIGURE 4. The distribution of VHL missense mutations along the length of the VHL gene among participants with ocular VHL, as depicted by participant ($n = 159$; A) and by pedigree ($n = 100$; B).

burden in the peripheral retina, scored according to (1) the prevalence of those having three or more RCHs in at least one eye, (2) the prevalence of those having five or more RCHs in at least one eye, and (3) the prevalence of more than one quadrant of peripheral retinal involvement, did not correlate significantly with mutation position in the α -domain versus the β -domain (Table 2).

Relationship between Position of Missense Mutations in the α -Domain versus the β -Domain and Visual Acuity

We hypothesized that visual function also differs depending on the location of missense mutations in the α -domain versus the β -domain. The visual acuities of the better- and worse-seeing eyes in all participants were tabulated. Although mean visual acuities in the better- and worse-seeing eyes in participants with α -domain mutations were both slightly lower than those in participants with β -domain mutations, these differences were not statistically significant ($P > 0.05$; Table 3).

Effect of Location of Missense Mutations in Specific Functional Subdomains on Ocular VHL Disease

The results of the analysis indicated that the position of missense mutations in the α -domain versus the β -domain had a statistically significant effect on the risk of ocular involvement in VHL disease, as well as on the location of RCHs in patients with ocular VHL disease. We further examined the effect on these parameters of having missense mutations in specific functional subdomains in the VHL protein. Three subdomains

were examined: (1) surface residues in the α -domain that are essential for binding of elongin C, a protein that directly interacts with the VHL protein (residues 155, 158, 159, 161, 162, 165, 166, 169, 178, 180, and 184); (2) hydrophobic residues that are essential for the structural integrity of the β -domain (residues 78, 86, 76, 119, 117, and 130); and (3) residues that bind to another interacting protein, the Tat-binding protein-1 (TBP), that facilitates the degradation of HIF-1 α , the substrate of the VHL protein (residues 136-154).²⁴ The prevalence of ocular VHL disease, ocular phenotypes scored, and visual acuity in either the better- or worse-seeing eye were not significantly correlated with the location of missense mutations in any of the three subdomains, when corrected for interpedigree correlations ($P > 0.05$ for all comparisons, data not shown). These results indicate that the differences in ocular phenotype associated with the location of missense mutations in the α -domain versus the β -domain was unlikely to be related to the function of these three subdomains and may be related to other changes in the structure or function of the VHL protein.

DISCUSSION

In the present study, we analyzed the distribution of missense mutations in a population of 412 participants with clinically definite VHL disease in whom a complete ophthalmic examination and clinical scoring for ocular VHL phenotype were performed. In this large population, missense mutations were almost always (>98%) located in one of two primary structural domains: the α - and the β -domains, as have been found in other VHL studies.^{16,27} These observations indicated the importance

TABLE 2. Correlation of Ocular Phenotype of Participants with Ocular VHL Disease with the Presence of Missense Mutations in the β -Domain versus α -Domain

Ocular Phenotype	Mutations in the α -Domain	Mutations in the β -Domain	OR (95% CI) α -Domain vs. β -Domain	P
All Participants with RCHs ($n = 157$)				
Bilateral RCHs	38/71 (53.5)	55/86 (64.0)	0.71 (0.33–1.50)	0.3661
Severe structural involvement in at least 1 eye	16/71 (22.5)	14/86 (16.3)	1.71 (0.66–4.40)	0.2673
Juxtapapillary RCH in at least 1 eye	13/86 (15.1)	26/71 (36.6)	4.56 (1.77–11.76)	0.0017
Peripheral RCH in at least 1 eye	78/86 (90.7)	55/71 (77.4)	0.24 (0.08–0.71)	0.0104
All Participants with RCHs in the Peripheral Retina ($n = 133$)				
At least 1 eye with ≥ 3 peripheral RCH	22/55 (40.0)	38/78 (48.7)	0.72 (0.32–1.62)	0.4244
At least 1 eye with ≥ 5 peripheral RCH	13/55 (23.6)	17/78 (21.8)	1.28 (0.51–3.21)	0.5959
>1 Quadrant involved in periphery	22/55 (40.0)	20/78 (25.6)	1.45 (0.62–3.42)	0.3921

Data are the proportion of participants with ocular phenotype (% of total). Age- and sex-adjusted odds ratios (ORs). Numbers in bold indicate ORs and P values for which $P < 0.05$. RCH, retinal capillary hemangioblastoma related to VHL disease; 95% CI, 95% confidence interval.

TABLE 3. Correlation of Visual Acuity of Participants with Ocular VHL Disease with the Position of Missense Mutations in the β -Domain versus the α -Domain

Eye	Mutation in α -Domain	Mutation in β -Domain	α -Domain – β -Domain* (95% CI)	P
Better-seeing	82.85	82.45	–0.52 (–6.02–4.98)	0.8525
Worse-seeing	51.23	58.67	–9.75 (–23.36–3.86)	0.1602

n = 157. Data are expressed as mean visual acuity in letters.

* Difference in mean visual acuity in letters.

of these two domains in pathologically relevant functions of the VHL gene. However, how molecular changes in different VHL protein domains that result from genetic mutations give rise to various types of VHL tumors is still incompletely understood.²⁸ In particular, how aberrant VHL protein functions arising from mutations in different domains influence RCH formation and growth in the retina is also largely unknown.

Previous genotype–phenotype correlations of *VHL* mutations have related the prevalence of VHL organ system manifestations to different mutation types—particularly the risk of pheochromocytoma.^{18,29,30} With regard to VHL-associated manifestations in the eye, the type of gene mutation has also been correlated with the prevalence of ocular manifestations and the severity of their effects. Webster et al.,³ in a study of 183 patients, had failed to detect an association between the type or position of mutation and severity of disease. However, a separate study by Dollfus et al.,³¹ of 211 patients reported a greater number of VHL-related RCH tumors in patients with a gene deletion compared with those with a truncating mutation. In another study of 873 patients with VHL disease, we found that gene deletions resulted in a significantly lower prevalence of RCHs and better visual function compared with either truncating mutations or missense mutations,²⁰ suggesting that the nature of VHL protein dysfunction indeed influences the phenotype of ocular disease.

In the present study, we focused specifically on the question of whether different missense mutations occurring in different domains can influence ocular phenotype. The ocular phenotype of VHL disease, apart from rare exceptions,³² is composed primarily of a single lesion type, the RCH. However, the RCH phenotype can vary significantly between patients. They may appear unilaterally or in both eyes, may be located in the peripheral or juxtapapillary positions, and can range in severity from causing no symptoms to the complete disruption of the globe and complete blindness.^{2–4} The nature of the RCH phenotype is thus related to a patient's overall visual prognosis,⁴ and influences the treatment of a particular RCH.^{26,33,34} As such, with the establishment of additional genotypic correlations to ocular phenotypic features, a patient's mutational genotype may contribute useful information for counseling and monitoring and may help provide information on the molecular etiology of ocular RCHs in general.

We analyzed missense mutations according to their position in either the α -domain, which is responsible for nucleating the ubiquitin-ligase complex with its molecular partners, or the β -domain, which is involved in the direct binding of the primary substrate HIF.²⁴ In our study population, although we found that participants with RCHs (*n* = 159/412) and those without RCHs (*n* = 253/412) had missense mutations that were distributed in both domains, there was a significantly higher prevalence of RCHs among participants with mutations in the α -domain, indicating the possible importance of the proper formation of the ubiquitin-ligase complex in RCH formation.

We did not detect a significant relationship between the domain position of missense mutations and the severity of ocular VHL disease, including the laterality of the disease (i.e.,

one or both eyes with RCHs), the number of peripheral RCHs, and the prevalence of severe, structurally disruptive disease. However, the position of missense mutations correlated significantly with RCH location, with a greater prevalence of juxtapapillary RCHs associated with α -domain missense mutations and a greater prevalence of peripheral retinas RCHs associated with β -domain missense mutations. These correlations did not appear to arise solely from particular missense mutations from within the α - or β domain. Analyses performed after adjustment for the most frequently represented missense mutations (codon 98 in the β domain, and codon 167 in the α -domain) did not alter the statistical significance of the relationships between the domain position of missense mutations and the phenotypic characteristics of ocular VHL. The spatial location of RCHs in the retina has an intriguing bimodal distribution in which most lesions are found either in the peripheral retina or in the juxtapapillary region, but seldom in the extrapapillary areas in the posterior pole.^{2,4} The position of an RCH is of prognostic and therapeutic significance, with juxtapapillary lesions being associated with a worse visual prognosis,^{4,26} which is likely attributable to their proximity to the optic nerve, making them less approachable by ablative treatments.³⁵ It is possible that depending on the central-to-peripheral retinal oxygenation gradients that exist in different parts of the eye³⁶ and depending on the degree that a particular *VHL* mutation increases HIF levels,^{37,38} a retinal cell with a mutated *VHL* gene in a particular retinal locus may differ in its ability to grow and give rise to a clinically evident RCH.

Last, we speculated that differences in RCH prevalence and phenotype that correlate with missense mutation position in the α -domain versus the β -domain are conferred by smaller subdomains—in particular to specific amino acid sequences that are essential in maintaining the structure of the domain or that directly interact with different molecules that make up the proteasome. We examined whether the phenotypes that correlate with the domain position of the missense mutation are more specifically conferred by a subset of residues in that domain, which possesses a specific structural or binding function. Specifically, we examined (1) the residues in the α -domain that bind Elongin C,²⁴ (2) residues in the β -domain that are essential for its structural integrity, and (3) residues in the α -domain that are involved in the binding of TBP-1, a component of the proteasome essential for in HIF degradation.³⁹ In all three cases, we did not find a statistical correlation of additional phenotypic differences with the subset of participants with missense differences in those subdomains. As a result, differences in the phenotype of VHL ocular disease may not arise directly from these particular molecular interactions but may arise from more complex interactions involving multiple parts of the proteasome.

In summary, we have identified additional genotype–phenotype correlations that show a relationship of the position of missense mutations to differences in the prevalence of ocular VHL disease and clinically significant aspects of the RCH phenotype. With the availability of genetic testing for the VHL gene, affected kindred in VHL families have access to early preclinical diagnosis and can be monitored with modified sur-

veillance protocols.¹⁵ The findings in this and other genotype-phenotype correlations can help provide the prognostic information that is useful for guiding ophthalmologists and geneticists in counseling and following up patients. In addition, these findings may be helpful in future studies of the molecular pathogenesis of ocular VHL disease and may influence the development of therapies to prevent and treat associated ocular tumors.

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