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Screening for von Hippel-Lindau Disease by DNA Polymorphism Analysis

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Objective.—Von Hippel-Lindau (VHL) disease is a rare, inherited multisystem neoplastic disorder. There is no biochemical test available to distinguish VHL disease gene carriers from their healthy siblings. We evaluated DNA polymorphism analysis as a method for identifying disease gene carriers.

Design.—Prospective comparison of the results of DNA analysis with a comprehensive clinical screening examination.

Setting.—The Clinical Center of the National Institutes of Health.

Patients.—Blood was collected from 182 members of 16 families with VHL disease. Forty-eight asymptomatic individuals, at risk of developing this hereditary illness (with an affected parent or sibling), were examined for occult disease at the Clinical Center of the National Institutes of Health and tested by DNA polymorphism analysis.

Results.—DNA polymorphism analysis predicted nine disease gene carriers and 33 individuals with the wild-type (normal) allele among the 48 individuals at risk of developing VHL disease; the test was not informative in six individuals. All nine individuals predicted to carry the VHL gene had evidence of occult disease on clinical examination. There was no clinical evidence of VHL disease in 32 of 33 individuals predicted to carry the wild-type allele.

Conclusions.—DNA polymorphism analysis can identify individuals likely to carry the VHL disease gene among asymptomatic members of disease families. This technique serves to focus attention on those individuals who require periodic medical examination and may help to alleviate the morbidity and mortality associated with this disease.

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VON HIPPEL-LINDAU (VHL) disease is a rare (one in 36 000 live births) autosomal dominant trait characterized by a predisposition to develop retinal angiomas, hemangioblastomas of the brain and spinal cord, renal cell carcinomas, pheochromocytomas, cystadenomas of the pancreas and epididymis, and islet cell carcinomas of the pancreas.[1-9] Individuals who inherit the disease gene may be free of manifestations of the illness throughout their lifetimes or may develop tumors in one or more of the target organs. The multisystem character of the illness, combined with the fact that multiple tumors may form in each target organ, produces considerable morbidity and mortality. Ocular lesions may lead to impaired vision and blindness. Central nervous system tumors may produce weakness, paralysis, and death. Renal cell carcinomas may metastasize and be fatal. The usual treatments for VHL disease include laser treatment of retinal angiomas and surgical resection of tumors of the kidney, the adrenal gland, and central nervous system.

Early diagnosis might prevent death from hemangioblastomas and renal cell carcinomas. At present, the only approach to early diagnosis is periodic examination of the eye, brain, and abdomen in all asymptomatic members of VHL families. Magnetic resonance imaging with gadolinium enhancement is

Table 1.—New DNA Polymorphisms Associated With the Locus for von Hippel-Lindau Disease

DNA Probe (Locus)	Restriction Endonuclease	Allele	Allele Size, kb	Allele Frequency	Heterozygosity*
LIB 2-42 (D3S571)	*Msp* I	1	2.9	0.75	
		2	2.7	0.25	0.38
LIB 51-10 (D3S627)	*Msp* I	1	7.3	0.46	
		2	7.1	0.25	
		3	5.4	0.29	0.64
c-LIB-3 (D3S18)	*Hinf*I	1	1.95	0.50	
		2	1.85	0.01	
		3	1.75	0.26	
		4	1.70	0.23	0.62

*Estimation of the frequency of each allele was based on examination of the parents in 56 families provided by the Centre d'Etude du Polymorphisme Humaine.

recommended for detection of hemangioblastomas of the brain and spinal cord; computed tomography is recommended for detection of tumors of the kidneys, adrenal glands, and pancreas.[10,11] Examination of all target organs is required to ensure detection of disease that may be limited to a single organ. The examinations are costly, inconvenient, and may not yield definitive diagnostic information. If it were possible to identify disease gene carriers, medical attention could be directed to those individuals at high risk of tumor development.

There has been considerable progress in the molecular genetics of VHL disease. The VHL disease gene belongs to the family of tumor suppressor genes.[12] Seizinger et al[13] reported linkage of the VHL disease gene to *RAF1*, a proto-oncogene located at 3p25.[13,14] This observation has been confirmed.[15-18] The disease gene has been located in a 6- to 8-centimorgan interval between *RAF1* and D3S18, an anonymous DNA marker located at 3p26.[17] Other workers have recently demonstrated that probe 64E2 is a distal flanking marker.[18] The identification of probes that flank the disease gene means that it should be possible to identify carriers of the disease gene by DNA polymorphism analysis. We tested the feasibility of this approach by prospectively comparing the results of restriction fragment length polymorphism (RFLP) analysis with a comprehensive clinical examination in asymptomatic, at-risk members of families with VHL disease. We found that RFLP analysis can distinguish VHL disease gene carriers from their healthy siblings.

METHODS

Patients

Families with a history of VHL disease were identified from the literature, from other families with this health problem, and through interested ophthalmologists, urologists, and neurosurgeons. For our study, families with two or more affected members were included. Asymptomatic family members and family members in whom there was uncertainty about the diagnosis were examined (after informed consent) at the Clinical Center of the National Institutes of Health, Bethesda, Md. (This project was approved by the Clinical Research Subpanel of the National Cancer Institute.) The procedure consisted of a history and physical examination, ophthalmologic examination, magnetic resonance imaging of the brain and spinal cord with gadolinium enhancement, computed tomography of the abdomen (with and without contrast), 24-hour urine collection to test for catecholamines, and for men, ultrasound examination of the scrotum. Screening examinations were performed on individuals 11 of age and older; for individuals aged 11 through 20 years, ultrasound examination of the abdomen was substituted for computed tomography. (In three children under the age of 11 years, examination was limited to the eyes.) A family member was considered affected if one or more of the following disease manifestations was present: retinal angioma(s), spinal or cerebellar hemangioblastoma(s), pheochromocytoma(s), pancreatic cysts, and renal cell carcinoma before the age of 60 years. Disease diagnosis was made without knowledge of RFLP status.

Blood samples were obtained from members of 16 consecutive VHL disease families. Families were numbered sequentially beginning with VHL-26 (VHL families 1 through 25 were the subject of a previous report). The members of the families resided in Pennsylvania, Virginia, New Jersey, North Dakota, South Dakota, Minnesota, New Hampshire, Wisconsin, and Iceland. Some families were described previously.[5,19,20] Lymphoblastoid cell lines were established from isolated blood lymphocytes to provide a source of additional DNA.

DNA Analysis

DNA was extracted from peripheral blood leukocytes or lymphoblastoid cell lines with the phenol-chloroform method. DNA samples were digested with the restriction endonucleases *Taq* I, *Bgl* I, *Dra* I, *Hinf*I, *Bam*HI, or *Msp* I (Bethesda Research Laboratories, Gaithersburg, Md), separated by agarose-gel electrophoresis, transferred by Magnagraph (MSI, Honeoye Falls, NY) filters with 1.5 mol/L sodium chloride and 0.15 mol/L sodium citrate and prehybridized for 3 hours at 65°C in 0.75 mol/L sodium chloride, 0.075 mol/L sodium citrate, 0.05 mol/L sodium phosphate (pH 6.7), 0.5 mg of boiled sonicated salmon-sperm DNA per milliliter, 0.2% Ficoll 400, 0.2% polyvinyl-pyrrolidone, and 0.2% bovine serum albumin. Probes were radiolabeled to a specific activity of 1 to 5×10^9 counts per minute by random priming with deoxycytidine triphosphate labeled with radioactive phosphorus (^{32}P).[21] The filters were hybridized at 65°C for 16 hours. Filters were washed twice at room temperature in 0.45 mol/L sodium chloride, 0.045 mol/L sodium citrate, and 1% sodium dodecyl sulfate. The filters were washed at 60°C for 60 minutes in 0.015 mol/L sodium chloride, 0.0015 mol/L sodium citrate, and 1% sodium dodecyl sulfate. Washed filters were placed in plastic bags and exposed to Kodak X-OMAT AR film for 1 to 3 days.

A panel of five loci (D3S571,[22] *RAF1*,[13,14] D3S18,[17,23] D3S191,[22,24] and D3S627) were used to predict the risk of being a VHL disease gene carrier. New DNA polymorphisms located close to the locus for VHL disease are listed in Table 1. On the basis of analyses of 56 families provided by the Centre d'Etude du Polymorphisme Humane, Paris, France, the order and approximate recombination distances of the markers are shown in Fig 1. DNA samples from VHL families were initially tested with *RAF1/Taq* I, *RAF1/Bgl* I, and D3S18/*Bam*HI. If these probe-enzyme combinations proved to be informative, no further testing was done. If these probe-enzyme combinations were not informative, the families were tested with additional proximal or distal genetic markers. Haplotypes were created for the *RAF1* and D3S18/*Bam*HI systems. A genetic marker was considered to be informative when it was possible to predict the risk of an asymptomatic individual being a disease gene carrier based on the inheritance of the genetic marker.

Linkage Analysis

We estimated the presence of linkage in each family using the Lod-score method, calculated with the LINKAGE (Howard Hughes Medical Institute, University of Utah School of Medicine, Salt Lake City) computer program.[25] All anal-

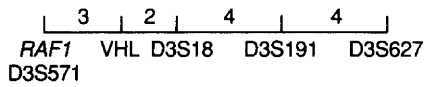

Fig 1.—Genetic map of loci around the von Hippel-Lindau (VHL) disease gene. The distance between adjacent loci is shown in centimorgans. It was not possible to determine the relative order of *RAF1* and D3S571.

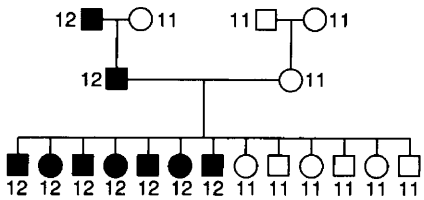

Fig 2.—Coinheritance of a disease gene and a genetic marker. A three-generation family is shown with an autosomal dominant illness. The family has been typed with a polymorphic probe whose alleles are designated 1 and 2. Affected individuals are indicated by the solid symbols. Note the complete concordance between the inheritance of the disease gene and allele 2. The frequency of recombination (θ) between the disease and the genetic marker is 0.

yses were performed under the assumption that the allele for VHL disease had dominant expression; 11 age-dependent categories were used for age-dependent penetrance with a frequency of 0.0001 for the disease gene.[13] Age-dependent penetrance (for selected ages) was 14% for ages 1 through 14 years; 50% for ages 20 through 24 years; 78% for ages 30 through 34 years; and 92% for ages 40 through 44 years. Calculations to estimate the risk of inheritance of the mutant allele at the VHL locus were generated by programs within LINKAGE version 5.03; risk calculations were performed on a Dell system (Austin, Tex) 200 personal computer. HOMOG version 2.51 (Jurg Ott, Columbia University, New York City, NY) was used to test for genetic heterogeneity.[26]

RESULTS

Linkage Analysis

To predict the risk of inheritance of a disease gene, it is necessary to identify polymorphic markers that are inherited along with the disease gene and to determine the frequency of recombination between these markers and the disease gene. Figure 2 depicts the concept of coinheritance or linkage. The pedigree of an imaginary three-generation family has been typed with a polymorphic probe whose alleles are designated 1 and 2. There is complete concordance between inheritance of the disease gene and allele 2. The inheritance of the disease and this genetic locus is said to be linked. Results of linkage analysis are expressed as recombination frequencies. A recombination frequency of 1% means that one recombination occurred in 100 opportunities for recombination (meiosis). In Fig 2 the frequency of recombination between the marker and the disease gene is 0.

Table 2.—Families With von Hippel-Lindau Disease Tested by Restriction Fragment Length Polymorphism Analysis

Family	No. of Members Tested	Lod Score* 2-42 (D3S571)	p628 (*RAF1*)	c-LIB-1† (D3S18)	L162† (D3S18)	c-LIB-3† (D3S18)	38-96 (D3S191)	51-10 (D3S627)
26	8		0.24	0.29			0.94	0.50
27	8		1.08	1.06				−0.01
28	21		0.92	−0.72	−0.13	−0.30	1.46	0.50
29	4		−0.93	−0.01			0.00	
30	11		0.66	0.94			−0.59	−0.14
31	11		1.31	0.03	1.11		0.00	−0.08
32	7	0.35	0.07	0.61		0.09		0.22
33	6		−0.23	0.03			0.02	0.19
34‡	18	0.17	1.22	0.76	2.32		0.05	0.01
35	13	0.37	0.33	0.59		0.42		0.47
36‡	19	−0.06	1.03		0.11	0.89		1.33
37	11	−0.02	−0.03	0.19	−0.03	0.86	1.41	−0.35
38	12	0.63	0.05	0.22	−0.16	0.53	0.24	−0.11
39	8	−0.69	0.02	0.58	0.29		0.00	0.23
40	15	2.30	0.00		2.47		0.29	1.54
41	10		0.37		−0.09	0.03		
Totals	**182**	**3.05**	**6.11**	**4.57**	**5.89**	**2.52**	**3.82**	**4.30**
Estimate of Θ	...	.05	.03	.04	.03	.11	.05	.08
95% Confidence limits	...	.01-.17	.01-.13	.01-.16	.01-.14	...	.01-.15	.01-.21

*The Lod score is the logarithm (to the base 10) of the likelihood of observing the data if the loci are separated by the distance Θ, divided by the likelihood of observing the data if the two loci are completely unlinked (defined by $\Theta = 0.5$). A Lod score of 3.0 or greater is generally considered conclusive evidence of linkage. The total Lod scores are those for which the sum of all families combined is maximal. An empty space indicates that the family was not tested with that particular probe because another proximal or distal probe was informative in that family.

†Probes c-LIB-1, L162, and c-LIB-3 all detect the D3S18 locus.

‡Linkage between VHL and *RAF1* in VHL-34 and VHL-36 was previously reported by Seizinger et al[13] and for VHL-34 by Vance et al.[15]

Previously, the inheritance of VHL disease was shown to be linked to polymorphic genetic markers *RAF1*, D3S18, and D3S191.[13,15-18] Before predicting the risk of inheriting the VHL gene, we wanted to determine whether the mutant alleles occurring in affected members in these 16 VHL families were coinherited with the previously identified genetic markers (Table 2). We also used D3S571 and D3S627, genetic markers predicted by genetic mapping studies, to be located near the VHL gene. We found that in this group of 16 VHL families, the inheritance of the VHL gene was linked to *RAF1*, D3S18, and D3S191. The recombination fraction between the VHL gene and *RAF1* was 3%. To increase the number of individuals in whom it was possible to predict risk, we used three probes to detect the D3S18 locus. The recombination fraction between the VHL gene and D3S18 was 3% to 11%. The recombination fraction between the VHL gene and D3S191 was 5%. These values agreed with previously reported results. As predicted from mapping studies, D3S571 and D3S627 were found to be linked to the VHL gene. Two recombination events were detected between D3S18 and the VHL gene. No evidence for genetic heterogeneity was detected with the HOMOG program [$\chi^2(1) = 0$].

Risk Assessment

Once genetic markers have been identified that are linked to the disease gene, it is possible to predict the risk of an asymptomatic individual being a disease gene carrier. The family in Fig 3 illustrates some principles of risk prediction. Predictive information is desired for the individual indicated by the arrow. Without any additional information, the risk of the individual's inheriting the disease gene from her affected parent is 0.5. Using the individual's age and the known age-dependent onset of the illness, it is possible to calculate an age-dependent risk. If the individual is 12 years old and the age-dependent penetrance is 14%, then the age-dependent risk is 0.46 (legend, Fig 3).[27] If a genetic marker has been identified that recombines with the disease with a frequency of 0.1, it is possible to predict risk with greater precision. In the example shown,

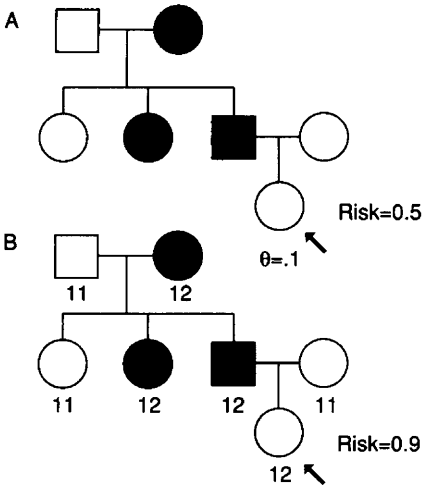

Fig 3.—Estimation of risk. A three-generation family is depicted that is affected with an autosomal dominant illness. In panel A, the risk of the individual indicated by the arrow inheriting the disease gene from her affected parent (without any additional information) is 0.5. If the age of the individual and the age-dependent onset of the illness are known, it is possible to calculate age-dependent risk according to the following formula: the risk is equal to (1 − chance of being affected at present age)/1+(1 − chance of being affected at present age).[35] For a 12-year-old child with a parent with von Hippel-Lindau disease, the age-dependent risk is 0.46. In panel B, the results of genetic typing are shown for a marker located close to the disease gene; this marker recombines with the disease gene in 10% of informative meioses (θ = 0.1). Based on the results of the genetic typing, the risk of the individual's inheriting the disease gene is calculated to be 0.9.

the child inherits allele 2 from his or her affected parent; the risk of the child's carrying the disease gene is 0.90.

Forty-eight asymptomatic individuals, at risk of developing VHL disease, were screened at the Clinical Center of the National Institutes of Health and tested by DNA polymorphism analysis. The DNA test was informative in 42 of 48 individuals (Tables 3 and 4). Nine of 42 individuals in whom the DNA test was informative were predicted to carry the VHL gene. All nine individuals predicted to carry the disease gene by DNA analysis (predicted risk, 0.926 to 0.997) showed evidence of VHL disease on the comprehensive screening examination (Tables 3 through 5). Thirty-two of 33 individuals predicted to carry the normal counterpart of the disease gene had no evidence of the disease on screening examination.

One individual (No. 2905), predicted to carry the normal counterpart of the VHL gene, was found to have a single renal cell carcinoma. The clinical and pathologic findings in this 37-year-old individual were atypical for VHL disease. All 11 individuals examined at the Clinical Center who had renal cell carcinoma associated with VHL disease had at least one additional manifestation of VHL disease. Individual 2905 had a renal tumor without other manifestations of VHL disease; renal cysts, a common finding in VHL-associated renal cell carcinoma, were not present in the resected kidney specimen. These atypical clinical and pathologic findings raise the possibility that the tumor that developed in individual 2905 was a phenocopy, a tumor of sporadic origin masquerading as an inherited tumor.[28] For the purpose of this analysis, individual 2905 was classified as affected.

Markers were evaluated for ability to predict the risk of an asymptomatic individual being a VHL gene carrier. Marker D3S571 was informative in 12 of 38 (32%), *RAF1* in 12 of 48 (25%), D3S18 (c-LIB-1) in 11 of 48 (23%), D3S18 (CRI-LI62) in 16 of 35 (46%), D3S18 (c-LIB-3) in 10 of 26 (38%), D3S191 in 16 of 35

Table 3.—Risk Assessment in von Hippel-Lindau Disease

At-Risk Subject Within Each Family	Age, y	Age-Dependent Risk*	Probe-Derived Risk†	Clinical Status
26-1	10	0.46	0.998	Affected
26-2	31	0.18	0.042	Not affected
27-1	7	0.49	0.002	Not affected
27-2	31	0.18	0.002	Not affected
27-3	32	0.18	0.040	Not affected
28-1	24	0.33	0.711	Not affected
28-2	28	0.25	0.939	Affected
28-3	30	0.18	0.060	Not affected
28-4	34	0.18	0.060	Not affected
28-5	42	0.07	0.061	Not affected
29-1	31	0.18	0.579	Not affected
30-1	36	0.11	0.002	Not affected
30-2	38	0.11	0.002	Not affected
31-1	18	0.40	0.129	Not affected
31-2	29	0.25	0.002	Not affected
31-3	34	0.18	0.002	Not affected
31-4	37	0.11	0.002	Not affected
32-1	17	0.40	0.083	Not affected
32-2	18	0.40	0.926	Affected
34-1	14	0.46	0.040	Not affected
34-2	18	0.40	0.040	Not affected
34-3	24	0.33	0.040	Not affected
34-4	44	0.07	0.040	Not affected
35-1	10	0.46	0.007	Not affected
35-2	14	0.46	0.997	Affected
35-3	22	0.33	0.934	Affected
35-4	32	0.18	0.043	Not affected
35-5	33	0.18	0.067	Not affected
35-6	37	0.11	0.500	Not affected
36-1	24	0.33	0.500	Not affected
36-2	26	0.25	0.070	Not affected
36-3	28	0.25	0.070	Not affected
36-4	30	0.18	0.928	Affected
36-5	33	0.18	0.070	Not affected
36-6	34	0.18	0.498	Not affected
36-7	37	0.11	0.100	Affected
36-8	40	0.07	0.070	Not affected
37-1	23	0.33	0.044	Not affected
38-1	46	0.04	0.500	Not affected
38-2	49	0.04	0.101	Not affected
38-3	52	0.03	0.101	Not affected
38-4	53	0.03	0.500	Not affected
39-1	26	0.25	0.958	Affected
40-1	12	0.46	0.940	Affected
40-2	15	0.40	0.060	Not affected
40-3	27	0.25	0.940	Affected
40-4	37	0.11	0.060	Not affected
40-5	67	0.00	0.066	Not affected

*The age-dependent risk was calculated using Bayesian methods and the age-dependent penetrance of VHL. For a sample calculation of age-dependent risk, see Fig 2.

†The risk of inheritance of the VHL gene was calculated using programs within LINKAGE. Information on probe-derived risk was based entirely on DNA studies.

Table 4.—Comparison of DNA Polymorphism Analysis for von Hippel-Lindau Disease With a Comprehensive Clinical Screening Examination*

Clinical Examination	Results of RFLP Analysis†‡		
	Positive	Negative	Not Informative
Positive	9	1	0
Negative	0	32	6

*RFLP indicates restriction fragment length polymorphism. Clinical manifestations of the nine individuals who were positive by RFLP analysis and clinical examination are in Table 5.

†A positive RFLP test for von Hippel-Lindau disease was defined as a risk of 0.90 or greater; a negative RFLP test was defined as a risk of 0.10 or less. For examples, see Table 3. An uninformative test result was a risk greater than 0.10 and less than 0.90.

‡The sensitivity of the DNA polymorphism test for von Hippel-Lindau disease was 100%, the specificity was 97%, the positive predictive value was 90%, and the negative predictive value was 100%.

Table 5.—Results of Clinical Examination in Asymptomatic Individuals Predicted by DNA Analysis to be Carriers of the VHL Gene

Subject	Age, y	Risk of Being Carrier	Results of Clinical Examinations
2302	10	0.997	Retinal angiomas
2734	13	0.960	Pancreatic cysts
2655	14	0.997	Pancreatic cysts
2832	20	0.913	Hemangioblastomas of the spinal cord, pancreatic cysts
2651	22	0.956	Retinal angiomas, hemangioblastomas of the cerebellum and spinal cord, renal-cell carcinoma, pancreatic cysts
2707	26	0.958	Retinal angioma, hemangioblastoma of the cerebellum and spinal cord, renal cyst
2760	27	0.960	Retinal angioma, pancreatic cysts
2399	28	0.929	Retinal angiomas, hemangioblastomas of the spinal cord, renal-cell carcinoma, pancreatic cysts
2672	30	0.897	Retinal angiomas, hemangioblastomas of the cerebellum and spinal cord, renal tumor and renal cysts, pancreatic cysts

(46%), and D3S627 in 18 of 46 (39%) of individuals tested. Genetic markers were informative at either a proximal locus *or* distal locus in 42 of 48 (88%) of individuals. Genetic markers were informative at a locus both proximal *and* distal to the disease gene in 21 of 48 (44%) individuals.

Renal Cysts

Seven of 52 asymptomatic individuals had renal cysts as the only abnormality detected by the imaging studies. The ages of these individuals were 24, 31, 31, 37, 39, 44, and 52 years; the number of renal cysts detected varied from one to three. Because of the age-dependent increase in the frequency of simple renal cysts in the general population,[29] and because of the difficulty in distinguishing renal cysts associated with VHL disease from sporadic renal cysts, we did not consider renal cysts to be diagnostic of the disease. Individuals with renal cysts detected by computed tomography were studied by ultrasound examinations to further delineate structural characteristics of the renal cysts. The detection of an irregular renal cyst wall in a 37-year-old individual led to surgical exploration and the removal of a renal cell carcinoma. The predictions of risk for the seven individuals with renal cysts were 0.040, 0.042, 0.060, 0.100, 0.101, 0.50, and 0.579.

COMMENT

We evaluated DNA polymorphism analysis as a method to detect carriers of the VHL gene. This method has been used to detect carriers of the retinoblastoma, multiple endocrine neoplasia type 2a, and familial adenomatous polyposis genes (cancer susceptibility genes),[30-32] as well as carriers of the autosomal dominant polycystic kidney disease gene and Huntington's disease gene (degenerative diseases of the kidneys and brain).[33,34] We prospectively compared genetic testing with a comprehensive clinical screening examination in 48 individuals at risk of developing VHL disease. We found agreement between the results of the DNA test and the clinical screening examination in 41 of 42 individuals. All nine asymptomatic individuals predicted by genetic testing to be VHL gene carriers had characteristic manifestations of VHL disease detected by the clinical screening examination. Thirty-two of 33 individuals predicted to carry the normal counterpart of the VHL gene did not have manifestations of VHL disease. In six individuals the DNA test was not informative. It was not possible to predict risk in one family because we were unable to identify VHL disease in the proband's ancestors. These results support the notion that the genetic markers *RAF1* and D3S18, together with D3S191, D3S571, and D3S627, can distinguish VHL gene carriers from their siblings who carry the normal counterpart of the disease gene.

Several issues must be addressed in evaluating this DNA test for the VHL gene carrier state. Genetic heterogeneity (the existence of distinct genetic loci producing the same disease phenotype) would complicate the prediction of VHL disease carrier status. No evidence for genetic heterogeneity was found in the 41 VHL families we examined and in the 30 VHL families examined by Maher et al.[35] No evidence for genetic heterogeneity was found by Seizinger et al[18] in 39 of 40 VHL families studied. In one VHL family, the disease locus had an atypical spacial relationship to genetic markers on chromosome 3p, suggesting the possibility of an inversion. This unusual VHL family was not considered to represent true genetic heterogeneity.[18] The genetic markers described in this article should prove useful in predicting carrier status in all VHL disease families that have been studied to date by linkage analysis.

One measure of the usefulness of a panel of genetic markers is the proportion of individuals for whom it is possible to predict the gene carrier status. Using this panel of seven probes we were able to predict carrier status in 42 of 48 (88%) of at-risk individuals. It would be desirable to isolate additional highly polymorphic markers to simplify the task of risk prediction.

There may be uncertainty about the diagnosis of VHL disease in a family member with a *single* neoplastic lesion. This was the case in individual 2905 who was predicted not to carry the VHL gene. There are no pathologic or radiologic characteristics of individual neoplastic lesions that unequivocally distinguish sporadic from hereditary lesions. Only ancillary features point toward a diagnosis of VHL disease: multiplicity of tumors in one organ, bilateral involvement of a paired organ, or multisystem involvement. When the disease gene is isolated, it will be possible to test individuals like 2905 for VHL gene mutations and provide unequivocal information on carrier status.

There are several practical matters to be considered in applying DNA-based predictive testing to VHL disease. To successfully perform DNA-based predictive testing, it is necessary to obtain DNA samples from a minimum of two affected family members, and information on paternity must be accurate. It may be necessary to test multiple probes because, by chance, a given probe may not provide useful information in a particular family. At present no research

or commercial laboratory is performing DNA-based predictive testing for VHL disease on a routine basis. Laboratories with skill and experience in molecular genetic diagnosis can obtain the probes described in this report and can readily perform the necessary tests.

Because the test does not measure the disease gene itself, errors in risk prediction can occur. When risk predictions are based on typing with a marker on one side of the disease gene, the error rate is approximately equal to the recombination fraction between the marker and the disease. When predictions of disease gene status are based on informative markers on both sides of the disease gene and there is no recombination between the flanking markers, the error rate will be less than one in 100.

We envision that an optimal program for management of asymptomatic members of families with VHL disease would make use of the results of both the DNA test and the clinical screening examination. The treatment of individuals found to have a positive DNA test and a positive clinical examination will be dictated by the lesions found on clinical examination. Individuals found to have a negative DNA test and a negative clinical examination are unlikely to be VHL gene carriers and consequently do not require frequent monitoring. Individuals found to have a positive DNA test and a negative clinical examination are likely to be VHL gene carriers and should receive annual examinations for manifestations of the illness. When it is not possible to predict whether an asymptomatic individual is a disease gene carrier, these individuals must be treated as though they were carriers of the VHL gene. Otherwise, individuals who have occult manifestations of the disease gene may go undetected.

The ability to recognize gene carriers in a cancer susceptibility syndrome like VHL disease provides an avenue for a number of useful future lines of investigation. It should be possible to determine whether early detection of tumors confers a survival benefit to VHL gene carriers. Bilateral nephrectomy followed by renal transplantation is an option for VHL patients with bilateral renal cell carcinoma. Siblings who do not carry the VHL gene could be identified by DNA polymorphism analysis and considered as possible kidney donors. Populations at high risk of developing tumors of the kidney and brain might be useful in evaluating the efficacy of new methods of chemoprevention.

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