Title

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Journal

Proceedings of the National Academy of Sciences of the United States of America, 88(7)

ISSN

0027-8424

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Publication Date

1991-04-01

DOI

10.1073/pnas.88.7.2864

Peer reviewed

Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 2864–2868, April 1991
Medical Sciences

Genetic flanking markers refine diagnostic criteria and provide insights into the genetics of Von Hippel Lindau disease

(hereditary tumor syndrome/renal cell carcinoma/pheochromocytoma/brain tumors/"tumor suppressor" gene)

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Communicated by Kurt J. Isselbacher, December 10, 1990

ABSTRACT **Von Hippel Lindau disease (VHL) is a hereditary syndrome, associated with tumors and cysts in multiple organ systems, whose expression and age of onset are highly variable. The availability of a genetic test for the early and reliable detection of individuals carrying the defective gene would be beneficial for VHL patients and their relatives, since many of the manifestations of VHL can be successfully treated if detected in their early stages, while the complications of undetected disease can be devastating. We have previously shown that the VHL gene maps to chromosome 3p. To provide genetic markers for the development of a reliable diagnostic test, and to further narrow and eventually clone the VHL defect, we have generated DNA markers for chromosome 3p. With these markers, we have performed a multipoint genetic linkage analysis in 28 VHL pedigrees, comprising 470 individuals, 164 of whom were affected with VHL. Here we report the identification of tightly linked markers, including flanking markers that bracket the VHL gene to a small region on chromosome 3p25–p26. This finding has several major implications. While visceral cysts of the kidney, pancreas, and epididymis are commonly found in VHL and are considered diagnostic criteria for this disorder, they also occur in the general population. The presence of cysts, unaccompanied by other more typical lesions such as retinal and cerebellar hemangioblastoma, may therefore represent a major diagnostic problem, leading to errors in the assessment of disease status. The application of flanking markers for the VHL gene for presymptomatic diagnostic testing confirms that epididymal cysts are indeed not suitable as a diagnostic criterion in this disorder. Pheochromocytomas occur nonuniformly in VHL families and may also be associated with other hereditary tumor syndromes; our genetic studies imply that the phenotype in VHL families with and without pheochromocytomas is caused by defects within the same gene. The absence or presence of this tumor type is therefore due to the pleiotropic expression of a single gene rather than to the existence of several different genes for VHL. The region on chromosome 3p13–p14 known to contain several chromosomal translocation breakpoints in families with "pure familial renal cell carcinoma" is quite proximal to the VHL locus in 3p25–p26 we have identified. Chromosome 3p may therefore contain two loci for renal cell carcinoma: one gene (or genes) in 3p13–p14 and the VHL gene in 3p25–p26, whose aberration is also associated with other typical manifestations of VHL. Since renal cell carcinoma, pheochromocytoma, and visceral cysts can occur sporadically even in young people and may also be associated with other tumor syndromes, the availability of flanking markers for the VHL gene will be useful in identifying VHL gene carriers, particularly among those individuals at risk in whom these are the only manifestations of disease. The isolation and characterization of the VHL gene, based on the identification of flanking markers, will have important implications for diagnosis and treatment of patients with VHL, as well as for a much larger number of individuals having the sporadic counterparts of VHL-associated tumor types.**

Von Hippel Lindau disease (VHL) is an autosomal dominant disorder associated with neoplasms of the central nervous system (hemangioblastomas in the cerebellum, retina, and spinal cord), pheochromocytomas, bilateral and multifocal renal cell carcinomas, pancreatic tumors, and cysts of the kidney, pancreas, epididymis, and other organs (1–4). The clinical diagnostic criteria most frequently used by Melmon and Rosen (1) state that a single lesion of the Lindau complex (retinal hemangioblastoma, other central nervous system hemangioblastoma, pancreatic cyst, renal and epididymal abnormalities) is sufficient for diagnosis of VHL, provided clear documentation for VHL exists in at least one other family member. However, while certain features such as retinal and cerebellar hemangioblastomas are most typically associated with VHL, other lesions, including renal cell carcinomas, renal cysts, pheochromocytomas, pancreatic cysts, islet cell tumors, and epididymal cysts, are less specific for the diagnosis of VHL, since they often occur in the general population (5–7).

Current diagnostic techniques such as computed tomography, magnetic resonance imaging, and ultrasound are sensitive to the detection of even small, asymptomatic lesions.

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Abbreviations: VHL, Von Hippel Lindau disease; RFLP, restriction fragment length polymorphism; cM, centimorgan(s).

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Thus, they may also lead to an increased number of false-positive diagnoses. However, if one stringently restricts the diagnostic criteria to only highly characteristic tumors, one risks falsely excluding the diagnosis in individuals with early or atypical manifestations. This is important since a number of VHL-associated lesions (such as retinal, cerebellar, and spinal hemangioblastoma) are treatable when recognized early, while delayed diagnosis may lead to serious complications or death (1–4).

Based on our previous finding that the VHL gene maps to chromosome 3p (8), we have now identified genetic markers that flank the VHL defect and narrow the gene location to a small chromosomal region near the tip of the short arm of chromosome 3, in 3p25–p26. This provides an important prerequisite for the development of a reliable diagnostic test and for cloning the VHL gene itself.

METHODS AND MATERIALS

Approximately 40 ml of blood was obtained from each individual of 28 VHL pedigrees. Twenty milliliters of each sample was used to establish Epstein–Barr virus-transformed lymphoblastoid cell lines as a permanent source of DNA (9). The remaining portion of each sample was used for direct DNA extraction (10, 11). DNA was also prepared from lymphoblastoid cell lines as described (12).

The diagnosis of VHL was made in members of pedigrees, each with a clearly established family history of VHL, using the presence of at least one central nervous system hemangioblastoma (retinal, cerebellar, and spinal hemangioblastoma), or at least two of the following tumors: renal cell carcinoma, pheochromocytoma, pancreatic islet cell carcinoma, pancreatic adenocarcinoma, pathologically confirmed epididymal cystadenoma, or a combination of one of the above tumors, together with either pancreatic cysts or renal cysts.

We have recently generated a large number of sequence hybridization probes for chromosome 3 (13), a subset of which has been mapped to 3p14–pter, by using a hybrid cell deletion mapping panel (14). Probes mapping to the region 3p14–pter were screened for restriction fragment length polymorphism (RFLP) as described (15, 16). Markers displaying RFLP were analyzed in the "Venezuelan reference pedigree" (16) to prove their Mendelian inheritance, to determine their chromosomal position in relation to other markers whose relative location on chromosome 3p is known, and to contribute to the construction of a fine structure linkage map for chromosome 3p.

Two cosmid probes, 233E2 (D3S720) and 479H4 (D3S719), were characterized. These markers were used for linkage studies in both reference pedigrees and VHL families. 479H4 displays a *Bgl* I RFLP, with allelic fragments of 8.1 and 6.9 kilobases (kb), having frequencies of 57% and 43%, respectively ($n > 100$). 233E2 has a *Stu* I RFLP; the allelic fragments of 3.4 and 2.8 kb exhibit frequencies of 54% and 46%, respectively ($n > 100$).

In addition to these markers, the following previously characterized probes on chromosome 3p were used: THRB (thryoid hormone receptor B; erbA-β) in 3p22–p24 (17), c-RAF1 in 3p25 (17), and 64E2 (D3S95) in 3p25–p26 (17, 18).

For RFLP typing, ≈ 5 μg of each DNA sample was digested to completion with appropriate restriction enzymes, and the resulting fragments were resolved by agarose gel electrophoresis, transferred to nylon membranes by the Southern blot technique, and hybridized with ^{32}P-labeled probe DNA as described (10–12).

Two-point and multipoint analyses between the markers and the construction of a reference genetic linkage map for chromosome 3p were carried out in the Venezuelan reference pedigree (16), using the computer program MAPMAKER (version 1.0; ref. 19) on a VAX 8700 computer. The Venezuela reference pedigree currently contains >1100 potentially informative meioses and has been described in detail elsewhere (20).

Two-point and multipoint linkage analyses between chromosome 3p markers and the VHL gene were carried out using the computer program LINKMAP of the linkage package (version 4.9; ref. 21). For this analysis, an age-of-onset correction was used, consisting of 10 age-dependent penetrance classes based on the age of onset curve of 363 VHL cases by Go *et al.* (2). A penetrance of 95% was assumed for all ages >44 years, with a frequency of 0.0001 for the disease gene (8). Altering this penetrance to 90% or 100% did not significantly change the overall logarithm of odds (lod) scores.

Homogeneity of linkage was tested by using the admixture test as embodied in the computer program HOMOG (22). Briefly, this program compares the maximum likelihood of two models: model 1 assumes that all families are linked to one locus, and model 2 assumes that only a subset of families is not linked to the locus. Significance may be tested by the likelihood ratio test where the -2 log-likelihood difference approximates a χ^2 with degrees of freedom determined by the difference in the number of parameters estimated.

RESULTS

For this study, blood samples were collected and lymphoblastoid cell lines were established from 470 individuals of 28 VHL families, including 164 individuals affected with VHL. Families were from different parts of the continental United States (pedigrees 2, 3, 5, 6, 8–10, 18, and 21–28), Hawaii (pedigree 1, of Spanish descent; described in detail in refs. 2 and 4), Canada (pedigree 7, described in detail in ref. 23), The Netherlands (pedigree 4), and Federal Republic of Germany (pedigrees 11–17, 19, and 20) (24). Affected individuals from all 28 families exhibited typical VHL lesions, in accordance with the diagnostic criteria described in *Methods and Materials*. The most typical lesions included cerebellar and retinal hemangioblastomas (diagnosed in each of the 28 VHL pedigrees) and renal cell carcinomas (diagnosed in 23 of the 28 VHL pedigrees). Eleven pedigrees were also associated with pheochromocytomas. The VHL pedigrees in this study thus represent a wide range of phenotypic expression of VHL. For example, in pedigree 7, almost 60% of the affected individuals were afflicted with pheochromocytomas (23), while not a single member of the >40 affected individuals in the Hawaiian pedigree 1, and none of the 11 affected individuals of VHL pedigree 2 have thus far displayed this particular tumor type.

As a prerequisite for the precise chromosomal localization of the VHL gene, we constructed a genetic linkage map with a number of polymorphic chromosome 3p markers by using the computer program MAPMAKER (version 1.0; ref. 19). For the markers used in this analysis, two orders were possible: 3centromere–THRB–cRAF1–233E2–64E2–479H4–3pter (see Fig. 1), followed by the order THRB–cRAF1–233E2–479H4–64E2. Odds favoring the first over the second order were only 1:3. However, all other orders were found to be significantly less likely (odds >500:1 against).

The maximum-likelihood estimates for the distance between the markers, based on multipoint linkage analysis in reference pedigrees, are as follows: cRAF1–233E2, 3 centimorgans (cM); 233E2–64E2, 5 cM; 64E2–479H4, 2 cM.

When all 28 VHL pedigrees were included in the analysis, a multipoint linkage analysis between the VHL gene and chromosome 3p markers unambiguously placed the VHL locus telomeric to THRB (odds >120:1) but did not allow the definite placement of the VHL locus in relation to cRAF1, 64E2, and 479H4 (data not shown).

The inability to localize a disease gene among a set of mapped markers may be caused by underlying genetic heterogeneity for the disease. To test for this possibility, we used the admixture test in the HOMOG (22) computer program. Analysis of two-point lod scores for cRAF1, 233E2, 64E2, and 479H4 did not significantly support heterogeneity [χ^2 (1) = 0.00, 1.37, 0.92, and 0.00; P = 0.50, 0.12, 0.16, and 0.50, respectively], although the results for 233E2 and 64E2 were suggestive. To further test the possibility of heterogeneity using maximal information, we examined the lod scores of a multipoint analysis, which included the markers cRAF1, 233E2, 64E2, and 479H4. The homogeneity test clearly indicated heterogeneity [χ^2 (2) = 15.31; $P < 0.001$]. Examination of the posterior probabilities of linkage indicated that the sole source of heterogeneity was pedigree 28 and suggested that family 28 could and should be formally separated from the other families. It should be noted that family 28 is a clinically typical VHL pedigree, which has been described in detail (25, 26) (see *Discussion* for further details).

Table 1 lists the two-point analyses between the VHL gene and various polymorphic markers for chromosome 3p25–p26 in pedigrees 1–27. There was no recombination between 233E2 and VHL in any of pedigrees 1–27, suggesting that 233E2 is the most tightly linked marker thus far identified for the VHL gene. The combined lod score was $\hat{z}$ = 8.59 at $\hat{\theta}$ = 0.00, with a 1-lod unit confidence interval (approximating a 95% confidence interval) with a θ value of 0.00–0.06.

It is noteworthy that VHL families with and without pheochromocytomas significantly contributed to the positive linkage between VHL and the most closely linked markers cRAF1, 64E2, 479H4, and 233E2 when analyzed independently. For example, pedigree 2, a large VHL pedigree without pheochromocytomas, showed linkage between VHL and 233E2 with a maximum lod score of $\hat{z}$ = 1.46 at $\hat{\theta}$ = 0.00. Similarly, pedigree 7, a VHL pedigree in which ≈60% of the affected individuals exhibit pheochromocytomas, was genetically linked to 233E2 with a maximum lod score of $\hat{z}$ 1.32 at $\hat{\theta}$ = 0.00. Similar results were obtained in the multipoint linkage analysis. This suggests that the phenotype in VHL families with and without pheochromocytomas is caused by a defect in the same gene.

Fig. 1 shows a multipoint linkage analysis between the VHL gene and the markers cRAF1, 233E2, 64E2, and 479H4, based on VHL pedigrees 1–27. By far the most likely position of the VHL gene was found to be between cRAF1 (the centromeric flanking marker) and 64E2 and 479H4 (the telomeric flanking markers). The maximum lod score in favor of this position is $\hat{z}$ = 20.35 (z is the $\log_{10}$ of the odds in favor of linkage). This location is 7080 times more likely than the next likely position of the VHL gene [centromeric to cRAF1 ($\hat{z}$ = 16.50)]. The region between the flanking markers spans ≈8 cM, based on the recombination frequencies between cRAF1 and 64E2 generated from the reference map (see above). A similar analysis with the order of 479H4 vs. 64E2 reversed provided similar results.

In contrast to pedigrees 1–27 in which cRAF1 and 64E2 were found to be flanking markers for the VHL gene, in VHL pedigree 28 the maximum lod score for the region between

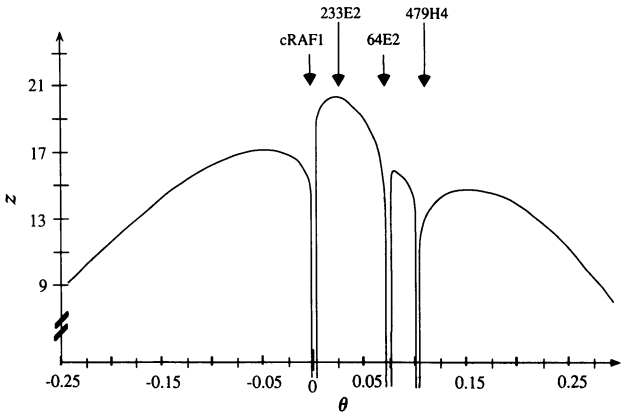

FIG. 1. Multipoint linkage analysis between the VHL gene and DNA markers for chromosome 3p. The multipoint analysis utilized the computer program LINKMAP of the linkage package (version 4.9; ref. 21) and is based on VHL pedigrees 1–27 (see Table 1). The lod score (z) is the $\log_{10}$ of the odds in favor of linkage at a given recombination fraction (θ) compared to the unlinked state. Arrows indicate the relative location of DNA markers on chromosome 3p. For markers see text.

cRAF1 and 64E2 was negative ($\hat{z}$ = −2.60). Recombinations between the VHL gene and the markers cRAF1, 233E2, 479H4, and 64E2 were observed in 4 of the 22 clearly affected (for diagnostic criteria, see *Methods and Materials*) living individuals of VHL pedigree 28. cRAF1 showed two recombinations, 233E2 had three recombinations, and 479H4 and 64E2 exhibited one recombination each with the disease gene.

However, the multipoint linkage analysis in this pedigree revealed considerably positive lod scores both centromeric and telomeric to the linkage group cRAF1–233E2–64E2–479H4 [$\hat{z}$ = 1.88 at $\hat{\theta}$ = −0.15 (15 cM centromeric to cRAF1), and $\hat{z}$ = 1.69 at $\hat{\theta}$ = 0.25 (15 cM telomeric to 479H4)]. Although pedigree 28 had to be formally separated from the other 27 VHL pedigrees based on the result of the HOMOG heterogeneity test, several lines of evidence suggest that this result is not due to genetic heterogeneity in VHL (see *Discussion*).

To clarify whether two at-risk individuals with diagnostically ambiguous clinical findings in pedigree 25 are indeed VHL gene carriers, we studied the segregation between the VHL gene and the flanking markers cRAF1 and 64E2 in this pedigree (Fig. 2). Pedigree 25 is a typical VHL family with respect to its clinical manifestations. Of the living individuals in this family, a, b, and c are clearly affected with VHL, displaying retinal and cerebellar hemangioblastomas and renal cell carcinomas. However, even after the use of current screening techniques (25, 26), the disease status in individuals d and e was not clear by clinical criteria. Individual d displayed multiple (more than five) ovarian cysts but no tumors. Similarly, her 12-year-old son e had bilateral epididymal cysts detected by scrotal ultrasound imaging but no tumors. Patients such as individuals d and e, who have a clearly established family history of VHL but show nothing

Table 1. Two-point linkage analysis between the VHL gene and markers for chromosome 3p25–p26

	θ								
Markers	0.00	0.05	0.10	0.15	0.20	0.30	0.40	$\hat{\theta}$	$\hat{z}$
233E2 (D3S720)	8.59	7.70	6.73	5.71	4.68	2.67	0.98	0.00	8.59
cRAF1	−∞	5.22	5.74	5.51	4.95	3.27	1.34	0.10	5.74
64E2 (D3S95)	−∞	3.81	3.85	3.48	2.94	1.66	0.56	0.07	3.91
479H4 (D3S719)	−∞	1.32	1.88	1.94	1.78	1.28	0.53	0.13	1.95

The lod score (z) is the $\log_{10}$ of the odds in favor of linkage at a given recombination fraction (θ), compared to the unlinked state. z is the maximum lod score at the given recombination fraction θ.

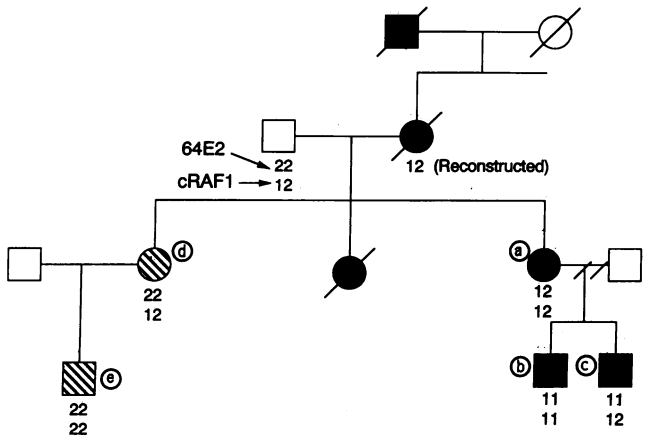

FIG. 2. Determination of the VHL gene carrier status in at-risk individuals with diagnostically ambiguous clinical findings by using the genetic flanking markers 64E2 and cRAF1. Solid symbols represent individuals from VHL pedigree 25 who are clearly affected with VHL, based on the occurrence of cerebellar and retinal hemangioblastomas and renal cell carcinomas. Hatched symbols represent family members at risk for VHL whose only VHL-related lesions are visceral cysts, including the 12-year-old family member e who has bilateral epididymal cysts.

else than visceral cysts as their only VHL-related lesions, represent a diagnostic dilemma. In fact, individual e's bilateral epididymal cysts, together with the clear family history, would fulfill the diagnostic criteria for VHL, according to the classification by Melmon and Rosen (1), the most commonly used classification in clinical practice. [Individual d's ovarian cysts, which are particularly common in the general population, may not be sufficient for the diagnosis of VHL, despite the positive family history. If she carries the gene, she would represent a case of incomplete penetrance or nonpenetrance of the VHL gene, which has been observed occasionally (2–4, 24).] However, in both cases, a definitive diagnosis is impossible by current criteria.

A genetic analysis between the VHL gene and the flanking markers 64E2 and cRAF1 in this family revealed that individuals d and e have not inherited the defective VHL gene (233E2 was not informative in this pedigree). While the clearly affected VHL patient b is homozygous for the "1" allele of both flanking markers, individual e is homozygous for the "2" allele of both markers (see Fig. 2). The error rate of this analysis is <1% for individual e, since both flanking markers were informative, both of which exhibit individual recombination rates with the VHL gene of <10%. Thus, the sole occurrence of epididymal cysts proves not to be suitable as a diagnostic criterion for VHL, even in the context of a clearly established family history for this disorder.

DISCUSSION

With the aid of newly generated DNA markers for chromosome 3 and their multipoint linkage analysis in a number of VHL families from different parts of America and Europe, we have established closely linked flanking markers for the VHL gene, providing the prerequisite for a reliable diagnostic test. The application of these new markers for presymptomatic diagnostic testing in VHL implies the need for a modification of the currently used diagnostic criteria (1–4, 23, 24). This genetic study confirms our previous empiric clinical observation that epididymal cysts, although very common and quite characteristic for VHL (26), are indeed not a reliable criterion for the diagnosis of this disorder.

The precise mapping of the VHL gene to chromosome 3p25–p26 has several additional implications that are of relevance for both clinicians and cancer scientists interested in the genetics and biology of this disorder.

Pheochromocytomas, although a typical feature of VHL, occur only in some VHL families (1–4). Moreover, this tumor type may also be associated with other hereditary cancer syndromes, including multiple endocrine neoplasia type 2, which maps to chromosome 10, "pure familial pheochromocytoma," and von Recklinghausen neurofibromatosis, whose gene defect resides on chromosome 17 (for review, see ref. 27). It had not yet been established whether VHL families with and without pheochromocytomas share the same gene defect. This study provides strong evidence that VHL in pedigrees with and without pheochromocytomas is caused by defects in the same gene, the VHL gene in chromosome 3p25–p26. However, it is still conceivable that distinct mutations within different regions of the VHL gene itself account for the clinical variability of symptoms observed in VHL. This question can be addressed once the VHL gene is cloned.

In contrast to VHL pedigrees 1–27, the gene defect in VHL pedigree 28 did not map between the flanking markers cRAF1 and 64E2/479H4. However, several observations suggest that this is not a reflection of "true" genetic heterogeneity but may be caused by other factors:

(*i*) Although the lod scores for pedigree 28 are negative between the flanking markers cRAF1 and 64E2/479H4, they are positive on both ends outside these markers ($\hat{z} = 1.88$ at $\hat{\theta} = 0.15$ for the region centromeric to cRAF1 and $\hat{z} = 1.69$ at $\hat{\theta} = 0.25$ for the region telomeric to 479H4). Such a result may be caused by cases of nonpaternity, chromosomal inversions, mix-ups in the collection and processing of the blood and DNA samples, or misdiagnosis. However, a reexamination of the clinical data, as well as a recollection and reanalysis of blood samples from those individuals who represented crossovers in the linkage analysis, and therefore contributed to the negative lod scores in pedigree 28, confirmed the previously obtained diagnoses and genotypes, thus largely excluding possible misdiagnoses and/or mixups. Typing of pedigree 28 with two VNTR (variable number of tandem repeats) markers for chromosome 17p in an attempt to uncover possible cases of nonpaternity has so far not revealed any discrepancies (B.R.S., unpublished observation); however, to effectively exclude this possibility, further studies using additional highly polymorphic markers are planned.

Alternatively, a constitutional inversion in this pedigree may explain the negative lod scores between the flanking markers, together with positive lod scores on both sides outside of these markers. Although an inversion has not been detected in high-resolution karyotypes from this pedigree (M.R.F.-K., unpublished observation), the chromosomal rearrangements may well be beyond the resolution of cytogenetic analysis. We are presently addressing the possibility of a chromosomal inversion with fluorescence *in situ* hybridization studies. In fact, the possible detection of a chromosomal inversion may provide an important short cut to the isolation of the VHL gene through the cloning of the inversion breakpoints that may directly disrupt the VHL locus.

(*ii*) The clinical manifestations of family 28 are quite typical of VHL and include all common lesions, such as cerebellar, retinal, and spinal hemangioblastomas, renal cell carcinomas, pancreatic tumors, epididymal cystadenomas, and pheochromocytomas (see refs. 25 and 26). To our knowledge, this pedigree, which is like several other VHL families in this study from the eastern United States and like most of the VHL families in this study of Caucasian origin, does not belong to any particular isolated gene pool.

(*iii*) We have recently shown that a number of different tumor types obtained from affected individuals of VHL pedigree 28 are associated with specific loss or deletions on chromosome 3p, including the VHL region (28). In all infor-

mative cases, the copy of chromosome 3 containing the normal VHL gene from the unaffected parent was lost, whereas the mutant allele from the affected parent was retained in the tumor tissue (28). This not only suggests that the VHL gene is a recessive tumor suppressor gene similar to the retinoblastoma gene but also confirms that the gene defect in pedigree 28 maps to chromosome 3p.

(*iv*) Several other investigators have recently confirmed our previous linkage between the VHL defect and cRAF1 in chromosome 3p25 (29, 30). They have not observed any evidence for genetic heterogeneity, based on more than 30 different VHL pedigrees from the United States and Europe (29, 30).

(*v*) The 27 VHL families in our study whose gene defect maps to the region 3p25–p26 belong to different ethnic groups and are from geographically diverse regions. Furthermore, they represent a wide spectrum of variable expression of VHL, including families with no pheochromocytomas, as well as VHL pedigrees in which almost 60% of the affected individuals are afflicted with this particular tumor type. This is in agreement with the existence of only a single gene causing VHL.

However, despite the high likelihood of genetic homogeneity in VHL (see above), we recommend a cautious application of the genetic markers for diagnostic testing in VHL until we have gained a better insight into the nature of the unusual genetic recombinations in VHL pedigree 28, thereby unambiguously excluding any genetic heterogeneity.

Even before we mapped the VHL gene to chromosome 3p (8), this chromosome was thought to harbor a locus associated with renal cell carcinoma. This was based on the discovery of several pedigrees with "pure familial renal cell carcinoma," all of which showed translocations involving the region 3p13–p14, including t(3;8) (31), t(3;11) (32), and t(3;6) (33) translocations. In contrast, our study maps the VHL gene to the region 3p25–p26 of chromosome 3, which is distant from the translocation region. Based on our preliminary genetic mapping data, the distance between the two regions is ≈50 cM, which may roughly correspond to 50 million bases. Consequently, chromosome 3p may contain two genes associated with renal cell carcinoma: one gene (or even several loci) in 3p13–p14, and the VHL tumor suppressor gene in 3p25–p26 whose deletion, aberration, or loss of function is also associated with other typical VHL manifestations.

We are grateful to Dr. M. Raff for providing cRAF1 probe and to Helene Lyne for editing the manuscript. B.R.S. is the recipient of a Faculty Research Award from the American Cancer Society. Funding for this work was provided by Grant ROI-CA49455 from the National Cancer Institute (B.R.S.) and by Grant ROI NS22224 from the National Institute for Neurological and Communicative Disorders and Stroke (J.F.G.).

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