Detailed genetic mapping of the von Hippel-Lindau disease tumour suppressor gene

F M Richards, E R Maher, F Latif, M E Phipps, K Tory, M Lush, P A Crossey, B Oostra, K H Gustavson, J Green, G Turner, J R W Yates, W M Linehan, N A Affara, M Lerman, B Zbar, M A Ferguson-Smith

Cambridge University
Department of
Pathology,
Cambridge.
F M Richards
E R Maher
M E Phipps
M Lush
P A Crossey
J R W Yates
N A Affara
M A Ferguson-Smith

Laboratory of
Immunobiology,
National Cancer
Institute-Frederick
Cancer Research
Facility, Frederick,
USA.
F Latif
K Tory
M Lerman
B Zbar

Erasmus University,
Rotterdam, The
Netherlands.
B Oostra

University of Uppsala,
Sweden.
K H Gustavson

Division of
Community Medicine,
Memorial University
of Newfoundland,
Canada.
J Green

Yorkshire Regional
Genetics Service and
ICRF Genetic
Epidemiology
Laboratory, Leeds.
G Turner

Surgery Branch,
National Cancer
Institute, USA.
W M Linehan

Correspondence to
Dr Maher, Department
of Clinical Genetics,
Addenbrooke's Hospital,
Hills Road, Cambridge
CB2 2QQ.

Received 9 August 1992.
Revised version accepted
29 September 1992.

Abstract

Von Hippel-Lindau (VHL) disease is an autosomal dominant inherited familial cancer syndrome characterised by a predisposition to the development of retinal, cerebellar, and spinal haemangioblastomas, renal cell carcinoma, and pheochromocytoma. The gene for VHL disease has been mapped to chromosome 3p25-p26 and flanking markers identified. We report the detailed genetic mapping of the VHL disease locus in 38 families. Significant linkage was detected between VHL disease and *D3S601* ($Z_{\max}=18.86$ at $\theta=0.0$, CI 0.0-0.025), *D3S18* ($Z_{\max}=11.42$ at $\theta=0.03$, CI 0.005-0.08), *RAF1* ($Z_{\max}=11.02$ at $\theta=0.04$, CI 0.007-0.01), and *D3S1250* ($Z_{\max}=4.73$ at $\theta=0.05$, CI 0.005-0.15). Multipoint linkage analysis mapped the VHL disease locus between *D3S1250* and *D3S18* close to *D3S601*. There was no evidence of locus heterogeneity. This study has (1) confirmed the tight linkage between VHL disease and *D3S601*, (2) identified *D3S1250* as the first marker telomeric to *RAF1* which maps centromeric to the VHL disease gene, and (3) narrowed the target region for isolation of the VHL disease gene by positional cloning techniques to a 4 cM interval between *D3S1250* and *D3S18*. These findings will improve the clinical management of families with VHL disease by improving the accuracy of presymptomatic diagnosis using linked DNA markers, and will enhance progress towards isolating the VHL disease gene.

(J Med Genet 1993;30:104-7)

Von Hippel-Lindau (VHL) disease is a dominantly inherited familial cancer syndrome with variable expression. The minimum birth incidence is 1/36 000 and the most frequent complications are haemangioblastomas of the central nervous system and retina, renal cell carcinoma, and pheochromocytoma.^{1,2} However, many different tumour types have been reported in this disorder.³ Clinical heterogeneity has been noted with some families showing a high incidence of pheochromocytoma and others a very low frequency.^{2,4-6} The variable expression and age dependent penetrance (mean age at diagnosis is 26.3 years) make the follow up of affected patients and their relatives problematical, so that although the morbidity and mortality of VHL disease

can be reduced by regular ophthalmological and systemic screening,⁷ long term compliance with the complicated screening protocol may be difficult to achieve.

The gene for VHL disease was mapped to the short arm of chromosome 3 by Seizinger *et al*,⁸ and subsequently we and others have localised the VHL locus telomeric to the *RAF1* oncogene in chromosome 3p25-p26.⁹⁻¹¹ In a previous genetic linkage study of 22 VHL disease families, we localised the VHL gene to a 10 cM interval between *RAF1* and *D3S225*, but were unable to orientate the VHL disease gene with respect to *D3S18* which lies within this interval.¹⁰ However, Hosoe *et al*⁹ mapped the VHL disease gene between *RAF1* and *D3S18*. We now report a genetic linkage study of 38 VHL disease families using chromosome 3p25-p26 markers which refines further the localisation of the VHL disease gene.

Methods

PATIENTS

Subjects from 38 families with VHL disease were investigated using a panel of polymorphic DNA markers from chromosome 3p25 to p26. A total of 167 affected patients (at least two from each family) and 171 relatives and spouses were genotyped. VHL disease was diagnosed using standard criteria.^{2,12} All affected patients had proven retinal angioma, central nervous system haemangioblastoma, renal cell carcinoma, or pheochromocytoma. Six families contained patients with pheochromocytoma.

DNA ANALYSIS

High molecular weight DNA was isolated from peripheral blood or lymphoblastoid cell lines by conventional methods. After digestion with the appropriate restriction endonuclease, electrophoresis, Southern analysis, and autoradiography were performed as described previously.^{10,13} Details of the DNA probes used are shown in table 1. Families were initially typed with the *TaqI* RFLP at *D3S601* and then the two other RFLPs if this was uninformative. *D3S225* was found to be poorly informative and was not typed in all families.

GENETIC LINKAGE ANALYSIS

LIPED and LINKAGE computer programs were used for two point and multipoint linkage analysis in VHL disease families as described previously.^{10,13} Age dependent penetrance

Table 1 Details of restriction fragment length polymorphisms used in this study.

Locus	Probe	Restriction enzyme	Allele sizes (kb) (allele frequency)	Frequency of heterozygosity	Ref
RAF1	p627	TaqI	6.8(0.74)/6.3(0.26)	0.34	9 10 27
RAF1	p627	BglII	4.0(0.54)/3.3(0.46)	0.49	9 10 27
D3S732	LIB 4A-52"	HindIII	9.0(0.65)/7.5 + 1.5(0.35)	0.46	14-16
D3S1250	c-LIB-56	EcoRI	2.8(0.2)/1.2(0.8)	0.32	This paper
D3S601	LIB 19-63'	TaqI	4.3(0.47)/3.9(0.53)	0.5	15 16
D3S601	c-LIB-11.8	BamHI	11.0(0.85)/9.6(0.05)/7.3(0.10)	0.27	16
D3S601	c-LIB-7.1	BglII	3.7(0.7)/2.0 + 1.7(0.3)	0.42	16
D3S18	c-LIB-I	BamHI	8.7(0.69)/4.7(0.31)	0.43	9 15
D3S225	42-26'	HindIII	9.8(0.72)/5.5(0.28)	0.40	9 15

values were as follows: age 10 years = 0.08, 15 = 0.19, 20 = 0.37, 25 = 0.52, 30 = 0.67, 35 = 0.78, 40 = 0.86, 45 = 0.91, 50 = 0.94, 60 = 0.98.¹¹⁴ The multipoint linkage maps were constructed with the LINKMAP program of the LINKAGE package using five point analyses. Recombination fractions were assumed to be equal in males and females.^{15 16} Genetic distances were calculated using Haldane's function and confidence intervals were determined by taking values of the recombination fractions corresponding to a lod score one unit less than the maximum. Formal heterogeneity testing was performed with the HOMOG program.¹⁷

Genetic linkage studies in 60 CEPH families have established the marker order as: (RAF1, D3S732)-D3S601-D3S18-D3S225.^{9 15 16} The position of a new marker, D3S1250, within this framework map was established by genetic linkage studies in 40 CEPH families. The CRI-MAP computer program was used to localise D3S1250 within the existing genetic map.¹⁸

Results

BACKGROUND MAP: GENETIC AND PHYSICAL MAPPING OF MARKER LOCI

The most likely background map is shown in fig 1. The order (RAF1, D3S732)-D3S1250-D3S601-D3S18-D3S225 was 26 times more

likely than D3S1250-(RAF1, D3S732)-D3S601-D3S18-D3S225. The localisation of D3S1250 between (RAF1, D3S732) and D3S601 is consistent with the results of physical mapping experiments using chromosome suppression in situ hybridisation studies and pulse field gel electrophoresis (unpublished observations).

VHL DISEASE FAMILY ANALYSIS

The two point linkage results between VHL disease and the six loci studied are shown in table 2. Significant lod scores (> 3) were found with RAF1, D3S1250, D3S601, and D3S18. The closest marker was D3S601 (Zmax = 18.86 at $\theta = 0.0$, CI 0.0-0.025), followed by D3S18 (Zmax = 11.42 at $\theta = 0.03$, CI 0.005-0.08), RAF1 (Zmax = 11.02 at $\theta = 0.04$, CI 0.007-0.01), and D3S1250 (Zmax = 4.73 at $\theta = 0.05$, CI 0.005-0.15).

Multipoint linkage analysis showed that the VHL locus mapped to the D3S1250-D3S18 interval (fig 2). The probability of the VHL gene mapping between D3S1250 and D3S18 was 5.12×10^4 times greater than that of the next most likely location telomeric to D3S18, and 9.23×10^8 and 1.52×10^9 times more likely than in a location centromeric to (RAF1, D3S732) and in the (RAF1, D3S732)-D3S1250 interval respectively.

Three affected subjects were recombinant at the RAF1 locus. Two of these were also recombinant at D3S1250, placing the VHL disease gene telomeric to D3S1250. The third RAF1 recombinant subject was not recombinant at D3S1250. All three RAF1 recombinant subjects were informative and non-recombinant at D3S601. Two D3S18 recombinant subjects were not recombinant at D3S601 or more centromeric markers.

Homogeneity testing with the HOMOG program provided no evidence for locus heterogeneity; the most likely proportion of linked families (alpha) was 1.0 (95% confidence interval 0.85-1.0). The maximum lod score in a multipoint analysis for the six families containing patients with pheochromocytoma was 10.0 in the interval between D3S1250 and D3S601 (fig 3).

Discussion

The improved localisation of the VHL disease gene presented in this paper will (1) accelerate progress towards the identification and characterisation of the VHL disease gene by a positional cloning strategy, (2) aid the investigation of the possible role of the VHL tumour

Figure 1 Background map of chromosome 3p25-p26 markers.

Table 2 Combined linkage analysis of 39 families with VHL disease: pairwise lod scores for linkage between VHL disease and chromosome 3 markers.

Locus	Recombination fraction						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
RAF1	—∞	10.32	10.95	10.01	7.26	4.28	1.56
D3S732	—∞	0.52	1.52	1.62	1.22	0.65	0.18
D3S1250	—∞	4.08	4.73	4.38	3.11	1.73	0.56
D3S601	18.86	18.43	16.67	14.44	9.95	5.62	1.93
D3S18	—∞	11.13	11.17	9.99	6.94	3.80	1.23
D3S225	—∞	—2.01	—0.56	—0.03	0.26	0.23	0.12

Figure 2 Multipoint genetic linkage analysis of 38 families with VHL disease. The most likely location of the VHL disease gene is between D3S1250 and D3S18 (see text for details).

Figure 3 Multipoint genetic linkage analysis for six VHL disease families containing patients affected by pheochromocytoma (broken line) and 32 families not containing a subject with pheochromocytoma (unbroken line).

suppressor gene in human cancers, and (3) increase the accuracy and availability of presymptomatic diagnosis of VHL disease using linked DNA markers.

We have confirmed the findings of Latif *et al*¹⁶ in an independent set of families that the VHL gene maps close to D3S601 in the RAF1–D3S18 interval. So far no recombinants have been identified between D3S601 and VHL disease and the total lod score is in excess of 40 with an upper confidence interval for θ of less than 0.02. Seizinger *et al*¹¹ mapped the VHL disease locus to a 10 cM interval between RAF1 and D3S719, and did not identify any recombinants with another marker, D3S720, which maps within the RAF1–D3S719 interval. The relative positions of D3S601 and D3S720 are not known, but Liu *et al*¹⁹ have reported that D3S18 and D3S719 are contained in a single cosmid. Thus, there is broad agreement that the VHL disease gene is localised centromeric to (D3S18, D3S719). In previous studies the proximal limit for the region containing the VHL disease gene has been defined by RAF1.^{9–11} We have now shown that the VHL disease gene is contained within a region bounded by D3S1250 and D3S18. Estimates of the genetic distances between markers flanking the VHL disease gene are variable, such that Latif *et al*¹⁶ estimated the RAF1–D3S18 interval as 6 to 8 cM and Seizinger *et al*¹¹ estimated the RAF1–D3S719 interval as 10 cM. We have estimated the RAF1–D3S18 interval as about 6 cM, and the genetic distance between D3S1250 and D3S18 to be approximately 4 cM.

The availability of presymptomatic diagnosis of VHL disease using linked DNA markers^{14,20} has improved the management of VHL disease families by enabling those relatives predicted to be at low risk to be screened less frequently. The finding that D3S1250 is the closest centromeric polymorphic DNA marker to the VHL disease gene will increase the accuracy and informativeness of presymptomatic diagnosis of VHL disease by genetic linkage analysis. We did not find any evidence of locus heterogeneity, and it appears that the well defined clinical heterogeneity seen in VHL disease reflects allelic rather than locus heterogeneity. Glenn *et al*⁶ found that one family in which 27 of 47 affected subjects developed pheochromocytoma showed similar linkage to D3S18 and RAF1 as 19 VHL disease families in which only four of 209 affected patients had a pheochromocytoma. Our findings are similar except that in the 'pheochromocytoma VHL disease family' reported by Glenn *et al*⁶ renal cell carcinoma had not been detected. However, in our six 'pheochromocytoma VHL disease family', four families contained patients with renal cell carcinoma. Therefore, it appears that all VHL disease families, regardless of variations in predisposition to pheochromocytoma and renal cell carcinoma, are linked to chromosome 3p25–p26 markers. Nevertheless, the possibility of locus heterogeneity in VHL disease in which only a small number of families would

be unlinked cannot yet be excluded and relatives who are predicted to be at low risk on DNA analysis should not be discharged from follow up. Presymptomatic diagnosis of VHL disease patients with flanking DNA markers has shown that many asymptomatic gene carriers have subclinical renal or pancreatic cysts.²⁰ The presence of only one of these features or of epididymal cysts alone does not provide an unequivocal diagnosis of VHL disease in subjects with a positive family history¹¹ (unpublished observations).

Many different tumour types have been associated with VHL disease and chromosome 3p allele loss has been shown in at least five types of VHL disease tumours, including haemangioblastoma, renal cell carcinoma, pheochromocytoma, pancreatic tumour, and choroid plexus papilloma²¹ (Maher *et al.*, unpublished observations). This suggests a common mechanism of tumorigenesis in the diverse tumours associated with VHL disease. The extent to which the VHL disease gene is involved in the pathogenesis of sporadic tumours has not yet been well defined. Chromosome 3p allele loss has not been shown in sporadic cerebellar haemangioblastomas (although this may be because of technical difficulties), but chromosome 3p allele loss is a common finding in renal cell carcinoma.²²⁻²⁴ However, chromosome 3p allele loss in sporadic renal cell carcinoma is not restricted to the region of the VHL disease locus, and there is evidence that at least three tumour suppressor genes on chromosome 3p may contribute to the pathogenesis of renal cell carcinoma.^{25,26} The isolation and characterisation of the VHL disease gene will enable (1) the function of the gene to be elucidated, (2) the reliable detection of gene carriers by direct mutation detection, and (3) the role of VHL disease mutations in the pathogenesis of non-familial cancers to be defined.

We thank Action Research, the Cancer Research Campaign, and the Iris Fund for the Prevention of Blindness for financial support. We are grateful to the many colleagues who have given us access to families, to the patients and relatives who took part in this study, and to Dr U Rapp for the p627 probe.

1 Maher ER, Iselius L, Yates JRW, *et al.* Von Hippel-Lindau disease: a genetic study. *J Med Genet* 1991;28:443-7.

2 Maher ER, Yates JR, Harries R, *et al.* Clinical features and

- natural history of von Hippel-Lindau disease. *Q J Med* 1990;77:1151-63.
- 3 Horton WA, Wong V, Eldridge R. Von Hippel-Lindau disease: clinical and pathological manifestations in nine families with 50 affected members. *Arch Intern Med* 1976;136:769-77.
 - 4 Green JS, Bowmer MI, Johnson GJ. Von Hippel-Lindau disease in a Newfoundland kindred. *Can Med Assoc J* 1986;134:133-8.
 - 5 Lamiell JM, Salazar FG, Hsia YE. Von Hippel-Lindau disease affecting 43 members of a single kindred. *Medicine (Baltimore)* 1989;68:1-29.
 - 6 Glenn GM, Daniel LN, Choyke P, *et al.* Von Hippel-Lindau (VHL) disease: distinct phenotypes suggest more than one mutant allele at the VHL locus. *Hum Genet* 1991;87:207-10.
 - 7 Moore AT, Maher ER, Rosen P, Gregor Z, Bird AC. Ophthalmological screening for von Hippel-Lindau disease. *Eye* 1991;5:723-8.
 - 8 Seizinger BR, Rouleau GA, Ozelius LJ, *et al.* Von Hippel-Lindau disease maps to the region of chromosome 3 associated with renal cell carcinoma. *Nature* 1988;332:268-9.
 - 9 Hosoe S, Brauch H, Latif F, *et al.* Localization of the von Hippel-Lindau disease gene to a small region of chromosome 3. *Genomics* 1990;8:634-40.
 - 10 Maher ER, Bentley E, Yates JRW, *et al.* Mapping of the von Hippel-Lindau disease locus to a small region of chromosome 3p by genetic linkage analysis. *Genomics* 1991;10:957-60.
 - 11 Seizinger BR, Smith DI, Filling-Katz MR, *et al.* Genetic flanking markers refine diagnostic criteria and provide insights into the genetics of Von Hippel-Lindau disease. *Proc Natl Acad Sci USA* 1991;88:2864-8.
 - 12 Melmon KL, Rosen SW. Lindau's disease. *Am J Med* 1964;36:595-617.
 - 13 Maher ER, Bentley E, Yates JR, *et al.* Mapping of von Hippel-Lindau disease to chromosome 3p confirmed by genetic linkage analysis. *J Neurol Sci* 1990;100:27-30.
 - 14 Maher ER, Bentley E, Payne SJ, *et al.* Presymptomatic diagnosis of von Hippel-Lindau disease with flanking DNA markers. *J Med Genet* 1992;29:902-5.
 - 15 Tory K, Latif F, Modi W, *et al.* A genetic map of 96 loci on the short arm of human chromosome 3. *Genomics* 1992;13:275-86.
 - 16 Latif F, Yao M, Tory K, *et al.* D3S601 is located between RAF1 and D3S18 close to VHL. *Am J Hum Genet* 1992;51(suppl):A63.
 - 17 Ott J. *Analysis of human genetic linkage*. Baltimore: Johns Hopkins University Press, 1985:105-9.
 - 18 Lander ES, Green P. Construction of multilocus genetic linkage maps in humans. *Proc Natl Acad Sci USA* 1987;84:2363-7.
 - 19 Liu W, Vance J, Smith DI. cA479 (D3S719): a cosmid mapped telomeric of the von Hippel-Lindau disease gene contains the D3S18 locus. *Hum Mol Genet* 1992;1:201.
 - 20 Glenn GM, Linehan M, Hosoe S, *et al.* Screening for von Hippel-Lindau disease by DNA-polymorphism analysis. *JAMA* 1992;267:1226-31.
 - 21 Tory K, Brauch H, Linehan M, *et al.* Specific genetic change in tumors associated with von Hippel-Lindau disease. *J Natl Cancer Inst* 1989;81:1097-101.
 - 22 Zbar B, Brauch H, Talmadge C, Linehan M. Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma. *Nature* 1987;327:721-4.
 - 23 Kovacs G, Szücs S, De Riese W, Baumgärtel H. Specific chromosome aberration in human renal cell carcinoma. *Int J Cancer* 1987;40:171-8.
 - 24 Anglard P, Tory K, Brauch H, *et al.* Molecular analysis of genetic changes in the origin and development of renal cell carcinoma. *Cancer Res* 1991;51:1071-7.
 - 25 Maher ER, Yates JR. Familial renal cell carcinoma: clinical and molecular genetic aspects. *Br J Cancer* 1991;63:176-9.
 - 26 Yamakawa K, Morita R, Takahashi E, Hori T, Ishikawa J, Nakamura Y. A detailed deletion mapping of the short arm of chromosome 3 in sporadic renal cell carcinoma. *Cancer Res* 1991;51:4707-11.
 - 27 Bonner T, O'Brien SJ, Nash WG, Rapp UR, Morton CC, Leder P. The human homologues of the raf (mil) oncogene are located on human chromosomes 3 and 4. *Science* 1984;223:71-4.

For The Blind to support gene mapping of autosomal dominant nystagmus.

- 1 Hemmes GC. *Over hereditaire nystagmus*. Wageningen: H Veerman & Zonen, 1924.
- 2 Forssman B, Ringner B. Prevalence and inheritance of congenital nystagmus in a Swedish population. *Ann Hum Genet* 1971;35:139-47.
- 3 Forssman B. Hereditary studies of congenital nystagmus in a Swedish population. *Ann Hum Genet* 1971;35:119-39.
- 4 Forsythe WI. Congenital hereditary vertical nystagmus. *J Neurol Neurosurg Psychiatry* 1955;18:196-8.
- 5 Von Noorden GK, Preziosi TJ. Eye movement recordings in neurological disorders. *Arch Ophthalmol* 1966;76:162-71.
- 6 Dell'Osso LF, Flynn JT, Daroff RB. Hereditary congenital nystagmus: an intrafamilial study. *Arch Ophthalmol* 1974;92:366-74.
- 7 Spooner SN, Bateman JB, Yee RD. Congenital nystagmus in identical twins: discordant features. *J Pediatr Ophthalmol Strabism* 1986;23:115-9.
- 8 Creel D, O'Donnell FF, Witkop CJ. Visual system anomalies in human ocular albinos. *Science* 1978;201:931-3.
- 9 McKusick VA. *Mendelian inheritance in man*. 9th ed. Baltimore: Johns Hopkins University Press, 1990.
- 10 Fisher T. Congenital nystagmus in father and child. *BMJ* 1902;ii:694.
- 11 Hawthorne CO. Nystagmus in three generations. *BMJ* 1903;i:425.
- 12 Fattowich G. Sul nistagmo congenito familiare. *G Psichiat Neuropatol* 1936;64:393.
- 13 Allen M. Three pedigrees of eye defects: primary hereditary nystagmus. Case study with genealogy. *J Hered* 1942;33:454-6.
- 14 Gresty M, Page N, Barrett H. The differential diagnosis of congenital nystagmus. *J Neurol Neurosurg Psychiatry* 1984;47:936-42.
- 15 Hammerstein W, Gebauer HJ. Familiarer nystagmus und hypoplastische der makula bei reziproker, balanzierter translocation 5/16. *Fortschr Ophthalmol* 1989;36:718-21.

Corrections

In the paper by Richards *et al* on 'Detailed genetic mapping of the von Hippel-Lindau disease tumour suppressor gene' (*J Med Genet* 1993;30:104-7), an important collaborator, Dr Per Enblad, was inadvertently omitted from the authorship. The correct authorship is as follows.

Richards FM, Maher ER, Latif F*, Phipps ME, Tory K*, Lush M, Crossey PA, Oostra B†, Enblad P‡, Gustavson KH‡, Green J§, Turner G||, Yates JRW, Linehan M¶, Affara NA, Lerman M*, Zbar B*, Ferguson-Smith MA.

Cambridge University Department of Pathology, Cambridge, UK; *Laboratory of Immunobiology, National Cancer Institute, Frederick Cancer Research Facility, Frederick, USA; †Erasmus University, Rotterdam, The Netherlands; ‡University of Uppsala, Sweden; §Division of Community Medicine, Memorial University of Newfoundland, Canada; ||Yorkshire Regional Genetics Service and ICRF Genetic Epidemiology Laboratory, Leeds, UK; ¶Surgery Branch, National Cancer Institute, USA.

In the paper by Padayachee *et al* on 'Mapping of the X linked form of hyper IgM syndrome (HIGM1)' (*J Med Genet* 1992;30:202-5), the primer sequence for DXS102¹³ under the heading OLIGONUCLEOTIDE PRIMERS was referenced Luty *et al*. This is incorrect and should be:

Gedeon AK, Holmon K, *et al*. Characterization of new pcr based markers for mapping and diagnosis: AC dinucleotide repeat markers at the DXS237 (GMGX9) and DXS102 (cX38.1) loci. *Am J Med Genet* 1992;43:255-60.